Ethidium Bromide Hazards and Precautions

Ethidium bromide (EtBr) is commonly used as a non-radioactive marker for identifying and visualizing nucleic acid bands in electrophoresis and in other methods of nucleic acid separation. EtBr is a dark red, crystalline, non-volatile solid, moderately soluble in water, which fluoresces readily with a reddish-brown color when exposed to ultraviolet (UV) light. Although it is an effective tool, its hazardous properties require special safe handling and disposal procedures.

Health Hazards

EtBr is a potent mutagen (may cause genetic damage), and moderately toxic after an acute exposure. EtBr can be absorbed through skin, so it is important to avoid any direct contact with the chemical. EtBr is an irritant to the skin, eyes, mouth, and upper respiratory tract. It should be stored away from strong oxidizing agents in a cool, dry place, and the container must be kept undamaged and tightly closed.

Safety Precautions

- Good work practices can help reduce hazardous exposures.
- To prevent inhalation exposure, work with EtBr powder or crystals in a fume hood, or work with premixed EtBr solutions or tablets to avoid handling the powder directly.
- To prevent skin contact when working with liquid solutions, wear protective gloves, a laboratory coat, and chemical goggles. Change gloves frequently.
- Provide EtBr users with safety training on EtBr hazards, use, and proper cleanup procedures. Include this fact sheet in the CHP as a standard operating procedure (SOP).
- Review an EtBr Material Safety Data Sheet (MSDS) and this EH&S fact sheet before handling the material. • Wear eye protection and ensure that there is unobstructed access to an eyewash/shower unit in the work area.
- As with any chemical, to avoid ingestion do not eat or drink where EtBr is handled, processed, or stored.
- Always wash hands thoroughly after handling EtBr, even if gloves are used.

Emergency exposure procedures

- **Eye care**: If EtBr comes in contact with the eyes, immediately flush them with copious amounts of cold or cool water for at least 15 minutes, preferably in an emergency eyewash.
- **Skin care**: In the event of skin exposure, remove contaminated clothing and immediately wash the affected area with soap and copious amounts of cold or cool water for 15 minutes.
- **If swallowed or inhaled**: In the case of EtBr ingestion, obtain medical attention immediately. If EtBr dust is inhaled, move the victim to a source of fresh air.

Spill Procedures

If a spill of any size of EtBr enters a sink or floor drain, immediately notify EH&S (683-4495) during normal business hours or ODU Police (683-4000) after hours.

Spills (small, low hazard) of EtBr solutions may be cleaned by laboratory staff. Wear appropriate PPE at all times. Use paper towels to absorb any liquid. For solids (crystal or powder), place wet paper towels over the top to prevent generation of dust, wipe up, and properly dispose. Once liquid or solid material is cleaned up, the area must be decontaminated and surveyed. Use a UV light to locate any remaining EtBr and then follow one of the decontamination procedures below or an equivalent method:

Method 1

- Scrub the area with clean wet paper towels and soap and water.
- Repeat this step six times to remove contamination.
- Properly dispose of all paper towels and contaminated PPE.
- Use a UV lamp to check the area for any remaining contamination. If contamination remains, repeat the process until the area is free of detectable contamination.
- double-bag along with contaminated gloves and other items and properly dispose of through EH&S

Method 2

- Just prior to using EtBr, prepare a decontamination solution of 4.2 g of sodium nitrite and 20 ml of hypophosphorous acid (50 percent) in 300 ml of water.
- Wash the area with a paper towel soaked in decontamination solution. Rinse the area five times with paper towels soaked in tap water, using a fresh towel each time.
- Use a UV lamp to check the area for any remaining contamination. If contamination remains, repeat the process until the area is free of detectable contamination.
- Soak all the towels in decontamination solution for one hour; double-bag along with contaminated gloves and other items and properly dispose of through EH&S

Disposal

DISPOSAL GUIDELINES FOR ETHIDIUM BROMIDE

Waste Stream	Description	Waste Disposal Procedure
Buffer Solutions	Typically contain very small concentrations of EtBr (<0.5 mg/L)	EH&S strongly recommends that buffer solutions be run through a filter or treated with tea bags prior to drain disposal. *(See below for approved Filter Methods)*
Stock solutions, cesium chloride solutions	Typically contain higher concentrations of EtBr (1–10 mg/ml)	The high concentration of EtBr in most of these solutions makes filtration/absorption impractical. Dispose of them through EH&S.
Gels	Typically contain lower concentrations of EtBr (3–5 mg/L)	**Allow gels to dry out**, then place in bags. Use a 5 gallon bucket, labeled *"Ethidium Bromide Waste"* with double bag liner. (No Biohazard Bags). Dried gels may be bagged with EtBr-contaminated debris. Dispose of gels through EH&S.
Contaminated Debris	Gloves, spill cleanup materials, and other lab supplies contaminated with EtBr	Broken glassware and sharps must be placed in puncture-resistant containers. Other debris may be placed in labeled bags and mixed with Gel waste. Dispose of contaminated sharps and debris through EH&S.
Crystals and powders	Typically pure or concentrated EtBr	Dispose of EtBr crystals and powders through EH&S.

Approved Filter Methods for Buffer solutions.

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.

1. **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.

   - 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.

2. **Greenbag Kit**:
   Another simple charcoal filtration method is the Green Bag, manufactured by BIO 101; The Green Bag® 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.

Alternatives to Ethidium Bromide

GelRed and GelGreen are nucleic acid gel stains from the company Biotium which offer cell membrane impermeability, high sensitivity, instrument compatibility, stability, and compatibility with all downstream manipulations. Concentrations less than 750 mg/L (750 ppm) may be disposed of in the sink if they have been neutralized with sodium bicarbonate first. Biotium, the manufacturer of GelRed and GelGreen produced a safety report and an overview of the dye, which can be viewed here. Visit Biotium's website to learn more http://www.biotium.com/product/product_info/Newproduct/GelStains.asp

SYBR Safe comes from the company Invitrogen and claims that Sybr safe is less mutagenic, non- genotoxic and non-hazardous for waste disposal. Full details of this product including a downloadable version of a report, compiled by Molecular Probes, on the mutagenicity and environmental safety of the product, from the test results of two independent organizations, can be accessed at: http://probes.invitrogen.com/products/sybrsafe/. . SYBR Safe in particular might be less mutagenic than ethidium bromide, but should be treated like ethidium bromide before disposal.