

Ethidium Bromide: Disposal, Decontamination, and Destruction

Procedure: 8.03
Version: 2.1

Created: 2/28/2012
Revised: 7/19/2018

A. Purpose

1. This policy provides general guidance to researchers and labs on how to work safely with Ethidium bromide. This mix has been designated as “particularly hazardous” by OSHA. This Policy will describe the minimum requirements for the safe storage, use, handling, and disposal of particularly hazardous substances, including spill and accident response procedures.
2. Ethidium bromide is mutagenic and moderately toxic and must be handled with care. The powder form is considered an irritant to the upper respiratory tract, eyes, and skin. Ethidium bromide is thought to act as a mutagen because it bonds in double stranded DNA, thereby deforming the molecule. This is believed to block or trip biological processes occurring on DNA. Preparation of stock solutions and any operations capable of generating Ethidium bromide dust or aerosols should be conducted in a fume hood to prevent inhalation. Nitrile gloves, a lab coat, and eye protection should be worn at all times, as with working with any hazardous material.

B. Applicability/Scope

1. This Policy is applicable to, and must be adhered to by, all Columbia University laboratory workers (i.e., Principal Investigators, laboratory personnel, students, visiting researchers, etc.) who use or work with Ethidium bromide. Careful handling and stringent controls of Ethidium bromide is essential in order to protect workers and the environment, and to comply with OSHA regulations.
2. Additional safety requirements may apply, depending on if Ethidium bromide is mixed/used with a specific chemical. For example, carcinogens that are also highly flammable require both particularly hazardous substance controls as well as fire safety controls. Contact the Office of Environment, Health & Safety (212-305-6780) for guidance on use of chemicals that may require further controls. Information and guidance on handling of particularly hazardous substances can also be found in the Lab Specific Chemical Hygiene Plan.

C. Definitions

1. EPA: The United States Environmental Protection Agency. This agency is responsible for the creation and enforcement of regulations governing the management and disposal of hazardous materials and wastes
2. NYCDEP: The New York City agency responsible for the delivery of fresh water and treatment of sewage discharge within Brooklyn, Queens, Manhattan, The Bronx, and Staten Island. This agency sets the guidelines for appropriate waste water discharge into sanitary sewer systems.
3. NYSDEC: The New York State Department of Environmental Compliance. This agency is the State Level counterpart of the USEPA and acts to enforce state and federal laws regarding the protection of the environment.
4. Hazardous Chemicals/Wastes: For the purposes of this policy, a hazardous waste or chemical is any material that is characteristically flammable, corrosive, reactive, toxic, infectious, or radioactive.

D. Responsibilities

1. All employees working for or on behalf of Columbia University and handling chemicals must:
 - a. Be adequately trained to handle hazardous chemicals and the subsequently generated waste

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- i. Supervisors must ensure that their employees are trained to an adequate standard for his or her assigned tasks and duties. Guidance and assistance in these matters can be obtained by contacting Environmental Health and Safety.
 - b. Be familiar with the particular hazards of his or her assigned research area, be it a laboratory or other space where hazardous chemicals are used.
 - c. Contact Environmental Health and Safety with any and all questions regarding appropriate disposal of potentially hazardous chemicals wastes.

- 2. EH&S
 - a. Develop, comply with and enforce this policy.
 - b. Pick up from laboratories and maintain waste in proper storage facilities.
 - c. Supply containers and labels for waste storage.
 - d. Provide proper waste disposal.

- 3. Contractors, Facilities Operations, Project Managers, Laboratory Personnel, and Students
 - a. Comply with this policy.
 - b. Correctly store waste containers in compliance with this policy.
 - c. Request containers and labels needed from EH&S.

E. Procedures

- 1. When working with Ethidium bromide, minimize the potential for spills. Where practical, purchase pre-mixed stock solutions from chemical manufacturers in lieu of preparing solutions. If solutions of Ethidium bromide must be prepared, consider performing this process in a fume hood. Perform all processes that generate Ethidium bromide dusts or mists inside the fume hood to minimize inhalation exposures. Prevent accidents by transporting small quantities of Ethidium bromide in secondary containment.
 - a. When an ultraviolet light source is used in work with Ethidium bromide, added caution is required. As a general rule, avoid exposing unprotected skin and eyes to intense UV sources.
 - b. Based on these considerations, Environmental Health & Safety (EH&S) recommends the following disposal, destruction, and decontamination procedures for ethidium bromide.
 - c. Electrophoresis gels or agarose gels containing ethidium bromide are a common waste stream at Columbia University. All gels containing ethidium bromide are collected and disposed of through EH&S as chemical waste. The containers are provided by EH&S, must be labeled as “Non-hazardous Waste – Ethidium Bromide Gels & Debris” When the container is approximately 75 % full, submit an online Chemical Waste Pickup Request. Ethidium bromide solutions and buffer solutions containing ethidium bromide should be filtered, decontaminated, or destroyed using one of the methods below or collected as chemical waste.
 - d. Aqueous buffer solutions containing ethidium bromide can be released down the drain after decontamination or destruction. Any solution containing heavy metals, organic solvents,

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cyanides, sulfides, acids (pH<2), or bases (pH>12) must be disposed of as hazardous chemical waste. As with all hazardous waste, waste solutions cannot be diluted or neutralized for drain disposal.

- e. Methods of Decontamination/Destruction Using Commercially Available Filtration Kits
 - i. Commercially Available Filtration Kits are designed to filter buffer solutions through a bed of activated carbon is a simple and effective method of removing ethidium bromide. The filtrate can then be drain disposed. One such kit is available from Q-Biogene, who offers the EtBr GreenBag Disposal Kit. The Green Bag is essentially a charcoal “teabag” which is placed in the ethidium bromide solution overnight and removes up to 10 mg EtBr/bag. One kit (50 bags) has the capacity to remove 500 mg of ethidium bromide from solutions. After the solution has been treated, it may be poured down the drain and the bag can be disposed of as hazardous waste through EH&S.
- f. Methods of Decontamination of Up to 100 ug/ml of Dilute Solutions
 - i. Method One, (Lunn and Sansone, 1987): Add 2.9 g of Amberlite XAD-16 for each 100 ml of solution up to 100 ug/ml ethidium bromide. Amberlite XAD-16, a nonionic, polymeric absorbent, is available from Rohm and Haas and sold by Sigma Chemical Company. Store the solution for 12 hours at room temperature, shaking it intermittently. Filter the solution through a Whatman No. 1 filter. Discard the filtrate to the drain. Seal the filter and Amberlite in a plastic bag, apply a completed Chemical/hazardous Waste label, and dispose of the bag as hazardous waste through EH&S.
 - ii. Method Two, (Bensaude, 1988): Add 300mg of powdered activated charcoal for each 100 ml of up to 10 ug/ml solution of ethidium bromide. Store the solution for 1 hour at room temperature, shaking it intermittently. Filter the solution through a Whatman No. 1 filter. Discard the filtrate to the drain. Seal the filter and activated charcoal in a plastic bag, apply a completed Chemical/hazardous Waste label and dispose of the bag as hazardous waste through EH&S.
- g. Methods for Destruction of Concentrated Solutions (0.5 mg/ml)
 - i. Method, (Lunn and Sansone 1987): Add sufficient water to reduce the concentration of ethidium bromide to 0.5mg/ml or less. Prepare a fresh 5% hypophosphorous acid solution. The solution must be freshly diluted before use. Pour 10 ml of hypophosphorous acid into 90 ml of water, stirred briefly. Use a chemical fume hood to prepare this solution. Important: Hypophosphorous acid is usually supplied as a 50% solution and is corrosive. In addition to the above, a sodium nitrate solution must be prepared as follows: 0.5 molar should be freshly prepared by dissolving 3.45 grams of sodium nitrite in water to a final volume of 100 ml. Use a chemical fume hood to prepare this solution. For each 100 ml of ethidium bromide solution, add 20 ml of fresh 5% hypophosphorous acid and 12 ml of fresh 0.5 molar sodium nitrite.
- h. Note: Decontamination solution must be stirred for at least 20 hours at room temperature. Bring the pH to neutral (pH = 7) with sodium bicarbonate. Discard the solution to the

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drain. As per RCNY Title 15, Chapter 19 Section 3, no solution with a pH lower than 5 or greater than 11 can be drain disposed. Note: When working with or diluting any hazardous chemical, the appropriate personal protective equipment (eyewear, gloves, lab coat) must be worn.

- i. Note: Do Not Use hypochlorite (bleach) to treat ethidium bromide. Bleach treatment can produce mutagenic products and leave behind up to 20% of the original ethidium bromide.
- j. Method for Surface Decontamination of Ethidium Bromide
 - i. Method, (Bensaude, 1988): Wipe up excess liquid with paper towels. Wet surface with ethanol and sprinkle activated charcoal on the surface.
 - ii. Wipe up the charcoal/ethanol mixture with paper towels. Seal the wastes in a plastic bag, label, and dispose of the bag through EH&S.
 - iii. Unless visibly contaminated, PPE and materials with Ethidium Bromide must be disposed of as red bag waste.

F. Emergency Contacts

N/A

G. Medical Surveillance

N/A

H. Record Keeping

N/A

I. Appendices

- 1. Non-Hazardous Substances Permitted for Drain Disposal
- 2. Examples of Hazardous Materials Not Suitable For Disposal
 - a. Ethanol: an ignitable/flammable chemical that meets the ignitability characteristic of hazardous waste as defined by EPA and NYSDEC, thus must be collected for hazardous waste disposal. As a flammable liquid, it is also forbidden from entering the public sewer (even with copious amount of water).
 - b. Acetone: see Ethanol
 - c. Methanol, Propanol and Butanol: see Ethanol
 - d. Chromerge: a sulfuric acid (e.g., corrosive) and chromium trioxide (e.g., toxic) solution use for cleaning laboratory glassware. This mixture is a corrosive, toxic hazardous waste. EH&S always recommends laboratories try alternative glass cleaning products (such as Alconox or NoChromix).
 - e. Dyes and Stains: the exact chemical contents of dyes and stains will determine whether they may be drain disposed. For example, Coomassie Blue and “Destain”, which contain methanol and acetic acid, would both be considered hazardous wastes and prohibited from drain disposal. EH&S recommends all dyes and stains be collected for proper waste disposal.
 - f. Ethidium Bromide Solution: due to its mutagenic properties it must be collected for hazardous waste disposal, unless filtered, decontaminated or destroyed, using methods

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approved by EH&S (see [Ethidium Bromide: Safe Handling and Proper Disposal Policy for accepted methods](#))

- g. Any material that meets and EPA definition of Ignitable, Corrosive, Reactive, or toxic is prohibited from sink disposal. Please note, the EPA, NYSDEC, and NYCDEP do not generally consider quantity or volume limits regarding the drain disposal of chemicals. Due to this, even very small quantities of chemicals (ex. Liquid Scintillation Vials) must be collected for proper disposal by EH&S personnel.

J. Forms

N/A

K. References

N/A

L. Acknowledgements

N/A