Scope

The following procedure describes waste disposal and treatment for solid ethidium bromide in gels and ethidium bromide solutions.

Purpose

Ethidium bromide is considered a mutagen and must be neutralized or disposed of properly.

Background

Ethidium Bromide Gel Waste (gels and gloves, gauze, etc.)

The procedure for disposal of these materials has not changed since the university has recently obtained an extension from the GVRD to continue incinerating ethidium bromide contaminated solid waste at the Environmental Services Facility. Landfill options are being investigated for future disposal purposes. If you have any comments or suggestions please contact the Environmental Programs Officer at 822-9280.

Ethidium Bromide Solutions

This material is mutagenic and must be handled with care. One of the following methods listed below must be followed to treat the waste prior to safe sewer disposal.

Procedure

1.0 Contaminated Solid Waste

Collect solid waste in a thick, plastic garbage bag, ensuring that there is no liquid waste present. Once bag is full, package in a cardboard box, affix an Environmental Services Waste Generator Tag with the generator bar code sticker attached, and check off the appropriate box (Appendix A), and call 822-6306 for pick-up.

2.0 Contaminated Liquid Waste

Method 1[1,2]

An aqueous solution is diluted to less than 0.5 mg/mL ethidium bromide. For each 100 mL, add 20 mL of 5 % hypophosphorous acid (made by diluting commercially available 50%, 1:10) and 12 mL of 0.5 M sodium nitrate. Stir briefly to mix and leave it to stand for 20 hours. Finally, neutralize with sodium bicarbonate before discarding.

If the ethidium bromide is in an organic solvent like isopropanol, for each volume of ethidium bromide add 4 volumes of a decontaminating

[i] **Lund & Sansone, Anal. Biochem. 162:453-458, 1987.**

[2] Dr. G. Spiegelman, Waste Watchers (HSE at UBC), vol 1, #1, p.3, June 1994

solution (e.g. 4.2g sodium nitrate, 20 mL hypophosphorous acid (50%) in 300 mL H_20). Stir for 20 hours. Neutralize with sodium bicarbonate before discarding.

If the ethidium bromide is in butanol the same decontaminating solution which was used for isopropanol can also be used, but this time stir for 72 hr. Then add 2 g activated charcoal for each 100 mL, stir for 30 min, and filter. Again neutralize with sodium bicarbonate; separate the layers and discard.
Remember that the activated charcoal is now a hazardous waste, see Section 1.0, Contaminated Solid Waste.

Method 2
Very dilute solutions of ethidium bromide can be destroyed by treatment with household bleach. To convert ethidium bromide to the physiologically inactive product 2-carboxybenzophenone stir the ethidium bromide solution with household bleach for 2 hours. For a solution containing 0.5 mg/mL of ethidium bromide use 440 mL of bleach for each 100 mL of solution. The solution is now ready for safe sewer disposal.

Method 3
Filters can be used to extract ethidium bromide and other fluorescent dyes from aqueous solutions. Pour dilute solutions of ethidium bromide into the filter system, and turn the vacumn on. The ethidium bromide solution filters through the cartridge and the hazardous molecules are permanently trapped in the reusable cartridge. The filtered liquid can then be safely disposed of down the drain. When saturated, cap and dispose of the used cartridge and place a new filter into the system.

The used cartridge is still highly **contaminated** and will need to be further treated as contaminated solid waste. Refer to, Section 1.0 Contaminated Solid Waste, for steps on how to properly dispose of used cartridges.

One type of filter is BondEx Ethidium Bromide & SYBR Green Detoxification Cartridges, for more information about this particular filter, contact Clontech Laboratories at 1-800-662-2566

It has been shown[3] that when ethidium bromide solutions of these dilute concentrations are used, the product solution does not show excess

mutagenicity over standards in the Ames test.

[3] Hazardous Waste Minimization in the Academic Laboratory, Dr.M. Armour, Networking News, ACS, Sept 94, vol.8 #2, p.5.

For further information, please contact the Environmental Programs Officer (822-9280), Department of Health, Safety and Environment.

Attachment(s)-

Appendix A-UBC Environmental Services Waste Generator Tag-Biohazardous (Red)

Revisions

R02:
Revision in Contaminated Liquid Waste procedure due to the addition of method 3, which incorporates the use of a filter system.

Appendix A

UBC Environmental Services Waste Generator Tag- Biohazardous (Red)