

## **Chemical Risk Management Protocol**

### **Safe Methods of Use (SMOU)**

# **Disposal and Decontamination of Ethidium Bromide**

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# 1 Purpose

This Safe Method of Use applies to principal investigators (PIs), laboratory managers, designated laboratory person (DLPs), and all staff and students who direct or participate in the use of ethidium bromide at the University of Auckland.

Note: the word 'shall' denotes a mandatory requirement and the word 'should' denotes a recommendation.

# 2 Disclaimer

**Please read this SMOU in conjunction with the Chemical Risk Management Guidelines and the safety data sheet for ethidium bromide.**

Note: 'Shall' denotes a mandatory requirement and 'should' denotes a recommendation.

# 3 Ethidium bromide

Ethidium bromide (EtBr; 2,7-diamino-10-ethyl-phenylphenanthridinium bromide) is widely employed for the rapid visualisation of nucleic acids in electrophoretic gels. It is a mutagen, a probable carcinogen and poses a fatal risk of toxicity via inhalation. It additionally may be harmful by ingestion and skin absorption.

## Safer Alternatives

**In the first instance, please consider using a safer alternative to ethidium bromide.** There are many different options, for example, SYBR Safe marketed by Thermo Fisher. These should still be used with care but pose a significantly reduced risk compared to ethidium bromide (EtBr). Treatment of DAB with hypochlorite solution (bleach) also produces a mutagenic product in solution and is not a suitable method of deactivation.

# 4 PPE

1. Ethidium bromide (EtBr) should be handled only when wearing nitrile gloves.
2. It is strongly recommended that EtBr is purchased in a liquid form to reduce handling risk.
3. If solid EtBr is to be weighed, the operation **shall** be carried in a fume hood or a ventilated area. Use of appropriate PPE such as a dust or respirator mask **should** be considered.
4. If the handling process involves pouring or splashing a large volume of EtBr liquid, a face shield can further reduce the exposure risk

## 5 Decontamination and disposal

Correct procedures for the disposal of EtBr depend on the nature of the waste materials and the concentration of EtBr that they contain. Much of the following advice is based on Maniatis et al. (1) and Lunn & Sansone (2, 3). Although Maniatis et al. offer a choice of protocols, the sodium nitrite method is preferred for surface decontamination as the reaction products retain very little mutagenic activity. However, a small amount of nitrogen dioxide is given off when the components of the decontamination solution are mixed. Hence the procedure is best carried out in the fume hood.

### 5.1 Solid Waste

Solid waste contaminated with EtBr, such as tissues, gloves or stained electrophoresis gels, **shall** be placed in appropriate packaging and sent for disposal via an approved waste contractor.

Contaminated sharps can be disposed of in sharps bins.

Bulk EtBr **shall** be placed in a labelled container and disposed of via an approved waste contractor

### 5.2 Liquid Waste

The following are suggest methods of dealing with liquid ethidium bromide waste.

#### A. Adsorption Method

##### ***Activated Charcoal – for solutions of EtBr < 0.5 mg/ml***

- Dilute to <0.5mg EtBr/ml
- Add 100mg powdered active charcoal to each 100ml solution.
- Keep at room temperature for 1 hour, shaking intermittently.
- Filter through a Whatman No. 1 filter. Discard the filtrate.
- Wrap the filter and charcoal in a plastic bag. Dispose via an approved chemical waste contractor.

Dilute waste can also be decontaminated by adsorption onto proprietary adsorbents which include a column marketed by Merck that changes colour when exhausted, or the Green Bag (cat #2350-200) marketed by MP biomedical. Persons wishing to use

these alternatives should satisfy themselves that the products produce the desired effect when the manufacturers' instructions are followed.

***Adsorption on Amberlite XAD-16 Ion Exchange Resin – for solutions of EtBr < 0.1 mg/ml***

- Dilute the aqueous ethidium bromide solution such that the total concentration of ethidium bromide does not exceed 0.1 mg/mL.
- For each 100 mL aliquot of ethidium bromide solution, add approximately 3.0 grams of Amberlite XAD-16 ion exchange resin and stir the resulting mixture for 20 hours.
- Filter the Amberlite resin from the aqueous solution and dispose via an approved chemical waste contractor.

**B. Bleach Decontamination Procedure (Ref 4) Armour Method**

This procedure is somewhat more complicated and is therefore only recommended if extraction is not possible.

Perform the following in a fume hood:

- Dilute solutions of EtBr to a final concentration of less than or equal to 0.034% w/v (34 mg EtBr /100 ml solution).
- Add 10 ml fresh bleach for every 1 mg EtBr (bleach deteriorates upon exposure to air).
- Stir the mix continuously for 4 hours or overnight.
- Test the final solution with a UV light to ascertain that the EtBr is destroyed.
- Dispose final solution to sewer diluting 1 part solution with 20 parts tap water.

**Please Note: this procedure is only for the decontamination of solutions containing ethidium bromide and not for surfaces as it relies on a 4 hour reaction time to ensure complete oxidation and conversion of all ethidium bromide to non-mutagenic 2-carboxybenzophenone.**

### C. Sodium Nitrite Procedure

This procedure is somewhat more complicated and is therefore only recommended if extraction is not possible.

- Dilute solutions with water to reduce the EtBr concentration to <0.5mg/ml.
- To the diluted solution, **IN A FUME HOOD** add 0.2 volume of fresh 5% hypophosphorous acid and 0.12 volume of fresh 0.5M sodium nitrite. Mix carefully. Important: check with indicator paper that the pH of the solution is <3.0 (if substantial amounts of buffers are present, it might be necessary to add more hypophosphorous acid. For mixtures containing alcohols, e.g isopropanol, 1-butanol, consult Ref No 2)
- Incubate 24 hours at room temperature. (A check for loss of fluorescence can be used to monitor completion of the inactivation process.) Add a large excess of 1M sodium bicarbonate before discarding.

#### **NB:**

Hypophosphorous Acid - is usually supplied as a 50% solution which is corrosive and must be handled with care. Dilute freshly before use.

Sodium Nitrite - dissolve 34.5g NaNO<sub>2</sub> in water and dilute to 1000ml. Note: there is a 2-fold discrepancy between the intended Molar concentration and the instructions for making up the solution. The present instructions accord with the original paper on EtBr inactivation by Lunn & Sansone (3)

## 5.3 Cleaning of Equipment and Laboratory Surfaces Contaminated with Ethidium Bromide

Glass, stainless steel, Formica, floor tiles, benches, fume hoods and the filters of transilluminators can be successfully decontaminated using the following technique. (No change in the optical properties of the transilluminator filter could be detected even after a number of treatments with the decontamination solution.)

- Unplug all electrical equipment before decontamination and wear appropriate protective equipment, including rubber gloves, lab coat, and chemical goggles.
- Make up the decontamination solution just prior to use. Dissolve 4.2 g of sodium nitrite in 250 ml water, **IN A FUME HOOD** slowly add 20 ml hypophosphorous acid (50%) and make up to a final volume of 300 ml with water.

- Wash the contaminated surface once with a paper towel soaked in the decontamination solution, taking care to avoid wetting electrical components. Then wash five times with water-soaked paper towels using a fresh towel each time.
- Soak all the towels in decontamination solution for 1 hour before disposal by incineration.
- Use a portable UV lamp to check that decontamination is complete. EtBr absorbs a broad range of UV light, so short (254nm), medium (300-315nm) or long (365-6nm) wavelength lamps can be used. Appropriate eye protection must be worn to guard the user against UV light while the lamp is switched on.
- Neutralise the used decontamination solution with sodium bicarbonate and discard as aqueous waste.
- Dry off the decontaminated surface or equipment. Arrange for electrical equipment to be checked by a competent electrician before plugging in for the first time unless you are absolutely certain that none of the electrical components have been wetted.

If the decontamination solution (pH 1.8) is considered to be too corrosive for the surface to be decontaminated, then use six H<sub>2</sub>O washes. Again, soak all towels in decontamination solution for 1 hour before disposal.

## 6 Emergency Exposure Procedures

- If EtBr contacts the eyes, immediately flush them with copious amounts of cold water for at least 15 minutes. (If it is available, an emergency eyewash is the best and safest way to do this.)
- For skin contact, immediately wash the affected area with soap and copious amounts of cold or cool water.
- If a person inhales EtBr dust, move them to an area where they can breathe fresh air.
- After any exposure to EtBr (via skin, inhalation, or eye contact), the affected person should immediately seek medical attention.

## References

1. Molecular Cloning, A Laboratory Manual; T. Maniatis, E.F. Fritsch & J. Sambrook (Cold Spring Harbor Laboratory, 2nd Edition, 1989, pages E8 – E9)
2. Destruction of Hazardous Chemicals in the Laboratory; G. Lunn & E.B. Sansone (Wiley Interscience, 1990, pp. 117-122, ISBN 0-471-51063-7).
3. G. Lunn & E.B. Sansone; Analytical Biochemistry, 1987, 162, 453-458.
4. Tested laboratory procedures for disposal of small quantities of hazardous chemicals in Waste Disposal in Academic Institutions, James A. Kaufman, ed., Lewis Publishers, 1990, p. 127.