UNIVERSITY OF VICTORIA
Occupational Health, Safety and Environment

Chemical Safety – Special Hazards

Safe Work Procedure (SWP – 006)

Ethidium Bromide

Last revised: 18 May 2022

REVISION HISTORY

	Revision Date	*Author*	*Position*
1.	18 May 2022	Paraskevi Lagaditis	OHSE consultant

DOCUMENT APPROVAL

Approved by: Laboratory Safety Committee

Jody Spence	May 18, 2022
Chair, Laboratory Safety Committee	*Date Approved*

**This revision replaces all previous versions of this document. If a copy is printed, it is the users' responsibility to verify the copy is the most current version of the document.*

University of Victoria

PURPOSE

To provide guidance and instruction of the safe use in laboratories of ethidium bromide (EthBr). In addition to this general Safe Work Procedure (SWP), each lab must develop a lab-specific work procedure unique to the experiments and activities being performed. The Lab SWP must be reviewed by OHSE (see Procedures, #7).

SCOPE

The SWP applies towards the handling, destruction and disposal of ethidium bromide (EthBr) used in laboratories for analysis of nucleic acids.

TRAINING

The following training is required to be completed prior to being permitted to prepare and work with ethidium bromide:

- WHMIS
- Lab Safety for Lab Workers
- Biosafety for Lab Workers
- Lab SWP with documented signoff by the individual and their supervisor.

Refresher training in the General and Lab SWP must be provided when:

- there has been an extended timeframe of inactivity, or
- there has been an incident or injury, or
- 2 years has elapsed since the original training

REGULATION AND POLICY

The University of Victoria will follow WorkSafeBC Occupational Health and Safety Part 30 and the University of Victoria Occupational Health, Safety & Environment Department.

RESPONSIBILITY

It is the responsibility of personnel undertaking activities with special hazards to complete all required training and adhere to these safe work procedures, including any additional lab or job-specific procedures.

It is the PI's or supervisor's responsibility to ensure that individuals working with special hazards have been trained prior to commencing work and have demonstrated competency in safely performing all duties associated with ethidium bromide in accordance with these procedures.

MATERIALS

There are several types of ethidium bromide (EthBr) decontamination solutions but the most commonly used is a mixture of hypophosphoric acid ($H_4P_2O_6$) and sodium nitrate ($NaNO_2$). Prepare EthBr decontamination solution just prior to use. Typical procedure to prepare in a fume hood is to mix 4.2 g of sodium nitrate and 20 mL hypophosphoric acid (50 %) in 300 mL of water.

HAZARD

Ethidium Bromide (EthBr) is a potent mutagen, carcinogen and possible reproductive toxin. It is a dark red crystalline, odourless, non-volatile solid that forms highly fluorescent complexes viewable under ultraviolet (UV) light when intercalated to nucleic acids. It is commonly used as a non-radioactive visualization agent for gel-based nucleic acid separation. Exposure to EthBr can occur by inhalation, ingestion and skin absorption. When possible, use less mutagenic alternatives to EthBr such as GelRed™ or Crystal Violet.

PROCEDURE

1. Handling

a. Always wear nitrile gloves, lab coat and safety glasses when handling EthBr.
b. Wash hands with soap and water whenever gloves are removed even if the gloves are not punctured or torn.
c. Use EthBr in designated areas only labeled “ethidium bromide use”.
d. Minimize use of powdered EthBr; pre-made solutions and tablets are safer.
e. Cover work space with benchtop disposable mat
f. Designate EthBr-only pipettes and tanks to prevent cross contamination.
g. Perform all aerosol generating procedures involving EthBr preparation and contaminated solutions in a fume hood.
h. Prepare the minimum amount of EthBr solution needed for immediate use.
i. Use a UV lamp to check surfaces where EthBr is used to check for contamination.

2. Storage

a. Keep EthBr container tightly closed in a dry and well-ventilated place.
b. Do not store EthBr with oxidizers.

3. Spills

a. Follow OHSE’s general spill response instructions
b. Do not attempt to clean up any spill if not trained. Seek assistance or call Campus Security (250-721-7599)
c. Specific steps for small solid or liquid EthBr spills
    i. Cover gently with paper towels
        - For solid spills, mist towels with water first then lay down carefully to not generate aerosols.
    ii. Dispose the paper towels into the ethidium bromide solid waste container.
    iii. Confirm areas of contamination using UV light.
    iv. If fluorescence is detected, prepare EthBr decontamination solution
        - Mix 4.2 g of sodium nitrate and 20 mL hypophosphoric acid (50 %) in 300 mL of water.
    v. Soak the paper towel in the EthBr decontamination solution.
    vi. Scrub surface with the soaked paper towel.
    vii. Scrub surface five times with a wet paper towel, using a fresh paper towel each time.
    viii. Dispose all paper towels in the ethidium bromide solid waste container.
    ix. Test fluorescence with a UV lamp; repeat washing steps if fluorescence detected.
    x. If no fluorescence is detected, wash surface with soap and water.
    xi. Dispose EthBr decontamination solution via hazardous waste.

d. Specific steps for large solid or liquid spills of ethidium bromide:
    i. Secure the area and warn others.
    ii. Immediately evacuate the area.
    iii. Post “do not enter” signs on the doors of the lab.
    iv. Contact Campus Security at 250-721-7599.
e. Complete a *Department Incident & Investigation Report* to document and review the spill incident.

4. Decontamination

a. Do not let gel materials harden on glassware; clean glassware as soon as possible.
b. Do not use bleach to decontaminate as it is ineffective.
c. Decontaminate all labware that has been in contact with EthBr solutions with EthBr decontamination solution.
d. Wash equipment contaminated with EthBr:
    i. Prepare EthBr decontamination solution just prior to use in a fume hood.
    ii. Soak a paper towel in the EthBr decontamination solution.
    iii. Wash equipment with the soaked paper towel.
    iv. Wash equipment with a wet (water) paper towel five (5) times with a new paper towel each time.
    v. Soak all the used paper towels in the EthBr decontamination solution for 1h.
    vi. Test fluorescence with a UV lamp; repeat washing steps if fluorescence detected.
    vii. Collect EthBr decontamination solution to dispose via hazardous waste.
    viii. Dispose paper towels in EthBr solid waste (black) container.
e. If EtBr decontamination solution (pH 1.8) is deemed too corrosive for the equipment
    ix. Wash equipment with a wet (water) paper towel six (6) times with a new paper each time
    x. Soak all towels in EthBr decontamination solution for 1h before disposal.

5. First Aid and Emergencies

a. Call 911 to summon an ambulance if there is a medical emergency.
b. Call Campus Security at 250-721-7599 for first aid.
c. If material has contacted the eyes, use emergency eyewash and flush for at least 15-20 minutes.
d. For skin contact, flush affected area with running water for at least 15-20 minutes.
e. For inhalation of aerosols or powders, move immediately to fresh air and seek medical attention immediately.

6. Waste Disposal

a. Dispose EthBr solutions (aqueous or organic) that have not been neutralized in the EthBr liquid waste container.
b. Dispose all contaminated disposable labware into the EthBr solid waste container.
c. Dispose gels and resin solids into the EthBr solid waste container.

7. Lab SWP

In additional to this general SWP, each lab that is using ethidium bromide requires a Lab SWP that includes specific procedures for:

a. Preparation of ethidium bromide solution
b. Maximum quantities permitted
c. Preparation of ethidium bromide decontamination solution.
d. Disposal methods.
e. Spill response.

REFERENCES

1. Lunn, G., & Sansone, E.B. *Ethidium bromide.* Destruction of hazardous chemicals in the laboratory. 3rd Ed. 2012. p. 201-210.
2. Sigma-Aldrich, Ethidium bromide, SDS v. 6.0. October 24, 2019.
3. University of Connecticut, *Protocol for the destruction of ethidium bromide.* Retrieved from http://media.ehs.uconn.edu/Chemical/ProtocolForTheDestructionOfEthidiumBromide.pdf