

GUIDANCE NOTE

Using Ethidium Bromide

1.0 Scope

This document identifies the requirements for using Ethidium Bromide (EtBr) in laboratories in Swansea University. This includes risk assessment, substitution of EtBr, control, safe use, the use of appropriate personal protective equipment (PPE), training and competence, disposal, and reporting incidents.

2.0 Ethidium Bromide

EtBr is a Category 2 potent mutagen most often used in molecular biology where its ability to intercalate nucleic acids is used in agarose gel electrophoresis of both DNA and RNA.

EtBr fluoresces a red-orange colour when exposed to ultraviolet (UV) light, and with increased fluorescence when bound to double-stranded DNA. Due to the high hazard properties of EtBr high-level controls must be implemented to mitigate the health and safety risk and appropriate waste disposal methods in place.

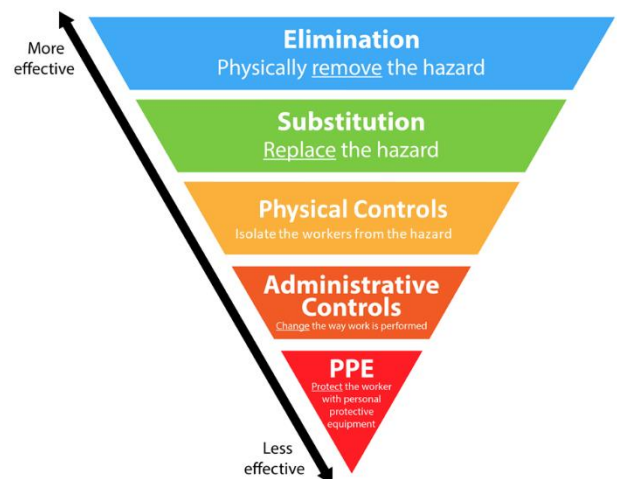
Before commencing any work with EtBr, a full and comprehensive chemical risk assessment in accordance with Control of Substances Hazardous to Health regulations (COSHH) should be carried out (see section 3), and a specific activity safe operating procedure (SOP) produced in accordance with the university guidance.

Please see the [Staff H&S Intranet pages](#) and [PG H&S Intranet pages](#) for the university chemical risk assessment forms, SOP and guidance.

3.0 Risk Assessment

The COSHH regulations state a risk assessment should identify the hazards, who might be harmed and how they might be harmed, and that wherever possible exposure to hazardous substances should be avoided. Where it is not possible to avoid exposure, the regulations require that the hierarchy of control (shown right) is used to implement controls.

The hierarchy of control identifies elimination of the hazard at the top (most effective) and where this is not possible, it is followed by substitution i.e. using a less hazardous chemical. Measures including administration/ management controls



and PPE are at the bottom of the control hierarchy as they are least effective/ most likely to fail to danger.

4.0 Hazard Breakdown

EtBr is so strongly mutagenic that it should be considered carcinogenic and teratogen. Specific control of inhalation and prevention of contact with skin must be observed.

EtBr has been assigned the following EU hazard statements:

Hazard statements:

- H302 Harmful if swallowed.
- H331 Toxic if inhaled.
- H341 suspected of causing genetic defects.

4.1 Routes of Entry/ potential health effects

- **Ingestion** - EtBr is harmful if swallowed and if ingested it may cause gastrointestinal irritation with nausea, vomiting and diarrhoea.
- **Inhalation** - Toxic by inhalation and in powder form is considered an irritant to the upper respiratory tract, eyes, mucous membranes, and skin. Inhalation is a major hazard that may result in death, lesser causal effects are respiratory tract irritation and methemoglobinemia, which is characterised by dizziness, drowsiness, headache, shortness of breath, cyanosis (bluish discoloration of skin due to deficient oxygenation of the blood), rapid heart rate and chocolate-brown blood.
- **Skin Absorption** - EtBr may cause extreme eye and skin irritation. It can be absorbed through the skin and contribute to other chronic health effects.

As EtBr is so strongly mutagenic with the high likelihood of carcinogenic and teratogenic effects, **pregnant workers should not work with EtBr.**

Please see the [Staff H&S Intranet pages](#) and [PG H&S Intranet pages](#) for University Pregnant, New and Breastfeeding Persons Policy arrangements.

5.0 Substitution

Where there is a practical non/ less hazardous alternative which will achieve the same or similar results to using EtBr, it **must** be used. The reasons for not using a non or less hazardous alternative must be clearly presented as part of the risk assessment and increased cost alone, cannot be the reason.

There are alternatives for EtBr available, and examples are listed below.

Manufacturers claim these may be less toxic than EtBr and may not require UV sources. However, the alternatives listed still bind nucleic acids so are likely to have the same health implications.

It must be noted that the alternatives are also hazardous and substitute one risk for another rather than eliminating the risk. A full chemical risk assessment must be carried out to ensure that the best substance is used for your research.

Alternatives include but are not limited to:

- MaestroSafe
- SYBR Safe
- GelRED
- SafeView

The routine use of EtBr is discouraged and should only be used and where necessary for scientific reasons and the risk assessment determines there are no viable safer alternatives. In this case consideration should be given to lowering the risk as low as possible such as reducing the inhalation hazard by purchasing ready-made solutions.

Once your risk assessment is completed you must complete a SOP which provides detailed instructions on how to carry out the activities/ operate equipment, any training, and PPE required.

Please see the [Staff H&S Intranet pages](#) and [PG H&S Intranet pages](#) for the university chemical risk assessment forms, SOP and guidance.

6.0 Good Working practices

See below good practice control measures that should be implemented when using EtBr and alternatives:

- Purchase pre-cast gels which are readily available and reduce the risks of spills/ contact/ inhalation when using liquid.
- Minimise the quantity used which will also reduce the risk of exposure and spills.
- Where practicable, purchase ready-made liquid solutions or tablets rather than weighing out powder and mixing your own solutions, although this may be more expensive this will reduce the hazardous inhalation route and as a result will minimise the more serious health effects.
- Activities/ experiments capable of generating EtBr dust or aerosols of EtBr solutions must be conducted in a glove box or fume cupboard with a drip tray which is easy to decontaminate to prevent exposure by inhalation.
- All work on the open bench should be in a specified area of the lab with warning signs indicating the tank has EtBr so others lab users know when someone is using it. Always work on containment trays and if running gels cover the bench with suitable material e.g., benchkote etc.

- Always add the EtBr to the cooled gel immediately prior to pouring the gel (just before setting point) into the casting tray.
- Never reheat gel solutions with EtBr in them.
- When transporting stained gels and EtBr bottles around the lab, always use a rigid box which will prevent the gel slipping off the casting tray onto the floor.
- PPE to be used includes:
 - A Howie lab coat with long enough sleeves to cover all skin. When worn the lab coat should be properly fastened with the sleeves rolled down
 - Double nitrile gloves, to allow, removal of upper pair following contamination (first pair under lab coat cuff, second pair over the cuff). Should there be concerns that the chemical has penetrated the glove (glove breakthrough) monitoring for EtBr contamination can be undertaken by placing the operators' hands beneath a standard UV light, where EtBr will show as a reddish/ brown colour on the skin.
 - Safety goggles or tight-fitting safety glasses are recommended when working with solutions in a fume cupboard, if working on the open bench a full-face visor is recommended.
 - Where appropriate, UV face protection should also be used. When viewing gels using an exposed transilluminator.
- Gloves must be removed immediately after working with EtBr and before touching any other surfaces. Gloves should be disposed of through the clinical or chemical waste routes (see section 8.0 below).
- EtBr should be stored safely in a double containment and locked. If decanted or if making up stock solutions, users should label any decanted solutions with a toxic symbol, respiratory irritant, and concentration of EtBr. Please see [Staff H&S Intranet pages](#) and [PG H&S Intranet pages](#) for safe storage and labelling of chemicals information sheet.

In the event of a spillage, you should:

- Make sure that you are wearing appropriate protective clothing i.e., lab coat, eye/ face protection, appropriate chemical resistant gloves. If the spill is a powder, you should wear a respirator fitted with particulate filters to P3 standard. Any users of powdered EtBr must be face fit tested and have appropriate training in their use.
- Spills of EtBr containing solution should be absorbed onto an inert absorbent material (dedicated spill pad/pillow, paper towels, vermiculite, etc.) and the area decontaminated as below in section 7.
- If the spill is powder avoid raising the powder dust into the air by gently mopping up with wet paper towels and proceed to decontaminate as below.
- Any adverse events which can be a spillage, first aid incidents or potential contamination involving EtBr should be reported to you supervisor/ line manager and via the [University - report it](#) system.

7.0 Decontamination

EtBr may be decontaminated using a solution of 5% hypophosphorous acid and 0.5M sodium nitrite (which is strongly acidic), 70% ethanol or other commercial sprays which are available.

If you are preparing the solution of 5% hypophosphorous acid and 0.5M sodium nitrite, this should be carried out in a fume cupboard because nitrogen dioxide is given off when the solution is initially mixed. The solution should be made immediately prior to use and can be used to decontaminate equipment and work areas. There should be appropriate chemical risk assessment and SOP for the preparation and use of this solution.

It is important to monitor gel areas for contamination with a UV wand after cleaning the areas and on a regular basis (weekly minimum as a guide), pay close attention to door handles, taps, bottle tops and pipette barrels.

There are specific hazards associated with using UV lights sources - When using the wand avoid exposure to the eyes for yourself and others in the lab. Only shine the UV on the area to be checked and ensure all persons in the area are wearing appropriate PPE i.e., UV face shield to protect your eyes, lab coat and long cuffed gloves fitted over the cuff of the lab coat to protect your and skin.

The decontamination process involves:

- Washing the area with a paper towel soaked in the decontamination solution.
- The acid could damage the contaminated surface so you should rinse the area five times with paper towels soaked in water, using a fresh towel each time.
- Check with the UV wand for any visible fluorescence.
- Repeat decontamination procedure, as necessary.

8.0 Waste Disposal

EtBr is classified as hazardous chemical waste and as such anything contaminated with EtBr such as all PPE, gels, filters and other solids are ALL be disposed of in a purple lidded box in accordance with to [WMGN27 Infectious Solid and Sharps Cytotoxic & Cytostatic / Medicine Waste](#)).

Solutions with EtBr must be deactivated prior to disposal to drain. This can be done by:

- Filtration - using charcoal filters to remove the EtBr, other filters are available.
- Absorption - using specifically designed bags to absorb EtBr. The bags are placed into the liquid waste and allowed to sit for the allotted amount of time. Do not exceed the bags capacity of absorption of EtBr. Once inactivation is complete, the bags are disposed of with the other EtBr contaminated items and placed in the purple lidded box.



Once deactivated check the filtrate with a UV wand for fluorescence. The filtrate can be poured down the drain. The charcoal filter or bag should be placed in a sealed container and placed in the purple lidded box.

Be aware that filtration will increase the concentration of the EtBr so filters must be disposed of regularly.

9.0 Training

Training on the safe use and disposal should be carried out prior to using EtBr. The training should be carried out by the PI or delegated to a lab member with sufficient experience who is deemed competent to deliver the training. Training must be recorded and signed off on the lab members training record. A template for an appropriate training record is available on the H&S website here: [Staff H&S Intranet pages](#) and [PG H&S Intranet pages](#).