New Guidance Governing Ethidium Bromide Disposal – with revisions

Updated on 16th March 2007 (Revisions highlighted by blue text)

Introduction

Ethidium Bromide is a potent mutagen used as a nucleic acid stain for many years. It fluoresces under ultraviolet light and produces an increased fluorescence when bound to double-stranded DNA. Typically, Ethidium Bromide is purchased in solution form and is soluble in water. Historically, the crystal or powder form has been purchased. Although Ethidium Bromide is an effective tool it possesses hazard properties that dictate that commensurate high level control measures be implemented to mitigate health and safety risk. In addition to the Health & Safety considerations of Ethidium Bromide, special disposal measures are often required. This document clarifies what these measures are for different forms of Ethidium Bromide waste. These measures should be followed by all University of Edinburgh members and other users working in University-managed laboratories.

Special Waste

The Special Waste Regulations 1996 and subsequent Special Waste Amendment (Scotland) Regulations 2004 require that Special Waste be identified, labelled, segregated from other (non-Special) waste types and disposed of in an appropriate and authorised manner. The hazardous (or Special) nature of a waste is determined using Risk Phrases (available through the Approved Supplier List or Material Safety Data Sheets) and where necessary, by assessesing the concentration of the hazardardous component within that waste. Only waste containing Ethidium Bromide above the hazardous threshold is classified as a Special Waste. Where this is the case, the waste MUST be consigned to an authorised contractor for treatment in an appropriate facility.

COSHH

The UK Control of Substances Hazardous to Health (COSHH) Regulations require that where ever possible, exposure to hazardous substances be avoided. Where it is not possible to avoid exposure, the Regulations require that exposure controls be implemented in a hierarchical order, the first of which is substitution with a substance that is non-hazardous, or less hazardous, before moving further down the hierarchy to mechanical controls and lastly Personal Protective equipment (PPE). Consequently the routine use of Ethidium Bromide is to be discouraged and should only be used where product evaluation and risk assessment have concluded that there is no viable safer alternative. Should the latter be the case serious consideration should be given to purchasing ONLY ready-made solutions so as to avoid the higher-risk inhalation hazard created by using this chemical in its powdered form. For more information, please refer to the University of Edinburgh Health & Safety website: http://www.safety.ed.ac.uk/resources/General/EthBrHazards.shtm.

Hazard Overview

Ethidium Bromide is harmful if swallowed, it is very toxic by inhalation and, in powder form is irritating to the upper respiratory tract, eyes, mucous membranes and skin. Although there is no evidence, at this time, for the carcinogenicity or teratogenicity of this substance in humans, Ethidium Bromide is so strongly mutagenic, causing living cell mutations, that it should be regarded as a possible carcinogen and teratogen.

Risk Phrases

Ethidium Bromide has been assigned the following EU Risk Phrases:

- *R 22 Harmful if swallowed*
- *R 26 Very toxic by inhalation*
- *R 36/37/38 Irritating to eyes, respiratory system and skin*
- *R 68 Possible risk of irreversible effects.*

Special Waste Threshold

Waste containing Ethidium Bromide is classified as hazardous (and therefore designated as **Special Waste**) where the Ethidium Bromide component of the waste is present at a concentration of **at least (>/=) 0.1%**.

New Waste Disposal Procedures

There have been variations across the University around procedures for purchase and use of Ethidium Bromide. For the sake of clarity and in order to take this into account, this section outlines specific procedures for of the known forms of Ethidium Bromide waste.

Powder / Crystal Form

ALL waste Ethidium Bromide powder / crystals (*including*: where it has spilt and been cleared up and any materials contaminated with powder; Ethidium Bromide containers with solid residue in them) is **Special Waste**. This type of waste must be contained securely in a yellow rigid container with a blue lid and disposed of to an authorised Cytotoxic Waste incinerator via the Clinical Waste Contractor. **Due to the increased hazard of this form of the chemical and the expense / difficulty in disposing of it appropriately, it is strongly recommended that Ethidium Bromide powder is NOT used.**

Solutions with a concentration of ATLEAST (>/=) 0.1%

Any waste solution of 0.1% (e.g. 1mg/ml) or higher is **Special Waste** (*including*: unwanted 5mg/ml or 10mg/ml stock solutions). This type of waste must be contained securely in a yellow rigid container with a blue lid and disposed of to an authorised Cytotoxic Waste incinerator via the Clinical Waste Contractor.

Solutions with a concentration of less than (<) 0.1%

Any waste solutions of less than 1mg/ml (*including:* e.g. working solutions of 5µg/ml or 10µg/ml or staining solutions of 20µg/ml) are not Special Waste however, they are potentially still a Health & Safety risk. This type of waste solution must be decontaminated prior to disposal to drain.

Gels with a concentration of less than (<) 0.1%

Normal gels contain far less than 0.1% (1mg/ml) Ethidium Bromide and are therefore not Special Waste. Due to a small but potential Health & Safety risk, these gels must be disposed of via the Clinical Waste stream. Waste gels should therefore be put into **ORANGE Clinical Waste sacks** with other Clinical Waste and disposed of via the University's Authorised Contractor. All sacks containing this kind of waste must be labelled with both a Barcode Label (circle "Lab Plastics") AND an "*Ethidium Bromide waste - Below Hazardous Threshold*" label.

Activated Carbon Filters and "De-staining" Bags

The preferred method of decontaminating / deactivating dilute solutions of Ethidium Bromide is to use appropriate Activated Carbon Filters or De-staining Bags. Spent filters and de-staining that have been used to decontaminate solutions of Ethidium Bromide are **Special Waste**. This type of waste must be contained securely in a yellow rigid container with a blue lid and disposed of to an authorised Cytotoxic Waste incinerator via the Clinical Waste Contractor.

Final Notes

These new procedures will come into effect immediately. Heads of School via Laboratory Technicians, Waste Coordinators and Responsible Persons must ensure that these new procedures are circulated and followed in all locations where Ethidium Bromide is being used.

As mentioned previously, in its powdered form and at stock concentration in liquid form, Ethidium Bromide is classified as a Special Waste and as such, its disposal is more onerous and expensive. In these forms, it also presents a higher Health & Safety hazard and as such special care must be taken during its use.

Serious consideration should also be given to replacing Ethidium Bromide with safer alternatives. Where this is not possible, consideration should be given to updating laboratory procedures (e.g. by not adding the chemical to gels, using low concentrations of Ethidium Bromide in staining solutions, etc). As well as presenting less of a hazard to users, there is some evidence that suggests that reducing the concentration of Ethidium Bromide can produce gels of higher clarity.

Please contact:

- ***Waste & Environment Manager*** to arrange for an uplift of Cytotoxic (Special) Waste, for label templates and further information (Tel - 0131 651 4287. Email - Fleur.Rothwell@ed.ac.uk).