

Unsecure Access and Lack of Safety Disposal Procedures of Ethidium Bromide in Laboratory of Molecular Biology in Faculty Of Science/Kufa University

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Abstract:

Ethidium bromide (EtBr) is a mutagen and a possible carcinogen. However Ethidium bromide is the most commonly utilized dye throughout the Faculty of Science in Electrophoresis gels for the identification of DNA. These gels will be agarose-based. Ethidium bromide is a highly toxic and potentially mutagenic chemical which may be fatal if swallowed, inhaled, or absorbed through the skin. The waste by-products of the DNA identification process must be managed and disposed in a manner to protect public health and the environment. Unfortunately there is no an appropriate way to discard the agarose gel that containing EtBr. Usually EtBr gels are discarded using the normal trash and aqueous ethidium bromide (EtBr) stock solutions discharge to a sink drain. Even though EtBr is known as mutagen it is still being kept in unsecured storage cabinet with unlimited access by any person who can inters the molecular laboratory in Faculty of Science.

Key word: Ethidium bromide, Safety Disposal, mutagen, Carcinogenic dyes.

Introduction

Ethidium bromide (3, 8-diamino-5-ethyl-6-phenyl phenanthridinium bromide) intercalates between the bases of native DNA, resulting in several biological anomalies (1). Ethidium bromide was used as homidium (Homidium bromide) in a strategic chemoprophylactic regime to control trypanosomiasis in Boran cattle in Kenya in veterinary medicine since the middle of last century (2). Ethidium bromide has long been known to promote loss of the trypanosome's mitochondrial genome (3, 4).

Many researchers objective to re-evaluate the efficacy and to investigate the toxicity of homidium bromide in goats infected with trypanosomiasis and concluded that the use of this drug should be restricted. (5).

A study by Youssif *et al.* (6) found Single i.m. dose of homidium bromide at 5 mg/kg and 1 mg/kg repeated daily for 8 days was toxic and fatal preceded by severe clinical signs which accompanied by varying degrees of congestion and hemorrhages in the different body organs, hepatorenal fatty changes or necrosis, cardiomyositis, neuropathy, pneumonia and bloody enteritis and decrease in Hb, RBC, PCV as well as increase in AST and SDH activities.

Ethidium bromide is carcinogenic and the effect depends on the organism and the conditions of exposure. It is thought to act as a mutagen because it intercalates double stranded DNA deforming the DNA. (7)

The carbon and hydrogen in Ethidium bromide have high electron densities and will interaction with nucleobase pairs (i.e., ethidium bromide inserts itself between the strands intercalates in double stranded DNA at every 2.5 base pair) (8, 9)

The partial positive charge on ethidium exocyclic amines (Figure 1) is important for mediating electrostatic attraction and hydrogen bonding interactions with DNA phosphate groups and may strengthen their hydrogen bonding with DNA phosphate groups. The phosphate and amino groups are within hydrogen bonding distances in a crystal structure of ethidium intercalated into nucleotide diphosphates (10)

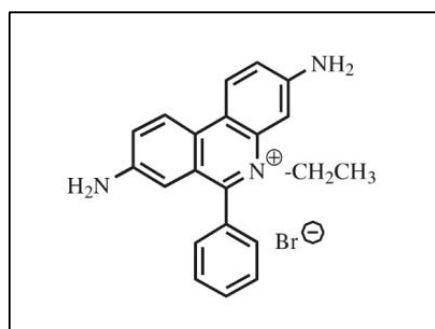


Figure 1: The Structural formula of ethidium bromide (11)

This could affect DNA biological processes, like DNA replication and transcription. Ethidium bromide has also been used extensively to reduce mitochondrial DNA copy number in proliferating cells (12)

Even though EtBr is known as an inhibitor and mutagen it is still being used in the molecular biological laboratories as intercalators to visualize DNA (13)

After usage they are disposing aqueous solutions containing < 5 mg/L of EtBr in the sanitary sewer without any neutralizing treatment, considering such concentrations as nonhazardous. In order to study the impact of trace amounts of EtBr, the Walter *et al.*, 2013 study used EtBr as toxicant and E.coli KL226 as the test organism. It was evident from that research that evens the subpicomolar (10^{-13} M) concentration of EtBr

to have the ability to cause mutation in E.coli KL226. Therefore, no trace amounts of EtBr should ever be thought as non-hazardous in day to day laboratory practices (14).

National handling and disposal of ethidium bromide

We should follow the instructions of environmental health and safety and lab safety guidelines for handling and disposal the *hazardous* substances. According to the Guidelines for safe work practices in human and animal medical diagnostic laboratories (15), the WHO Health Topics Biosafety (16), VCHRI Safety Orientation Guide (17), and Hazardous Laboratory Chemicals Disposal Guide (18), the handling and disposal of ethidium bromide include:

1. Handling and storage requirements of ethidium bromide

- Handle pure EtBr in a chemical fume hood because the powder can easily contaminate the entire laboratory.
- Designate an area where EtBr work is going to be performed, and use EtBr solutions only in that area.
- Cover surfaces within the designated area with a plasticlined absorbent pad. Replace the pad on a scheduled basis or when it becomes contaminated.
- Use PPE, including laboratory coat, eye protection and gloves when handling EtBr solutions and gels. Note: Latex gloves provide little protection against EtBr. Nitrile gloves provide an effective short-term barrier. Double-gloving provides increased protection.
- Wash hands thoroughly after removing gloves.
- Application of sodium hypochlorite solutions to spent solutions of EtBr will deactivate the ethidium bromide, but the reaction products are mutagenic, according to the Ames test (19). Use an alternative deactivation method, use or a permitted hazardous waste treatment facility to dispose of these spent solutions.

Also the Guidelines for the Safe Use & Disposal of Ethidium Bromide of Towson University (20) mentioned that bleaching with hypochlorite is not recommended. Such treatment reduces the mutagenic activity of EtBr but it converts the dye into a compound that is also mutagenic.

2. Disposal of ethidium bromide

Ethidium bromide waste is considered a mutagen. All items contaminated with ethidium bromide must be disposed according to the following instructions:

- Do NOT put into garbage or down sink.
- Collect and manage even small volumes or concentrations of EtBr waste as hazardous waste or follow local regulations.
- Bag materials coming into contact with EtBr, and dispose of as hazardous chemical waste.
- Minimize EtBr solution volumes by adding activated charcoal. The charcoal can be collected by filtration and placed into leak-resistant containers for hazardous waste disposal.

- Place agarose gels containing EtBr into a leak-resistant plastic container and dispose as hazardous waste.

Handling and disposal in our faculty:

In the molecular laboratory in Faculty of Science, there is no an appropriate methodology to discard the agarose gel that containing EtBr (Figure 2). Usually EtBr gels are discarded using the normal trash with other regular biohazardous material like gloves and debris (e.g. pipette tips) that are visibly contaminated with ethidium bromide and aqueous Ethidium Bromide (EtBr) Stock Solutions discharge to a sink drain. Even though EtBr is known as mutagen it is still being kept in unsecured storage cabinet with unlimited access by any person who can inters the molecular laboratory in Faculty of Science as shown in Figure 3.

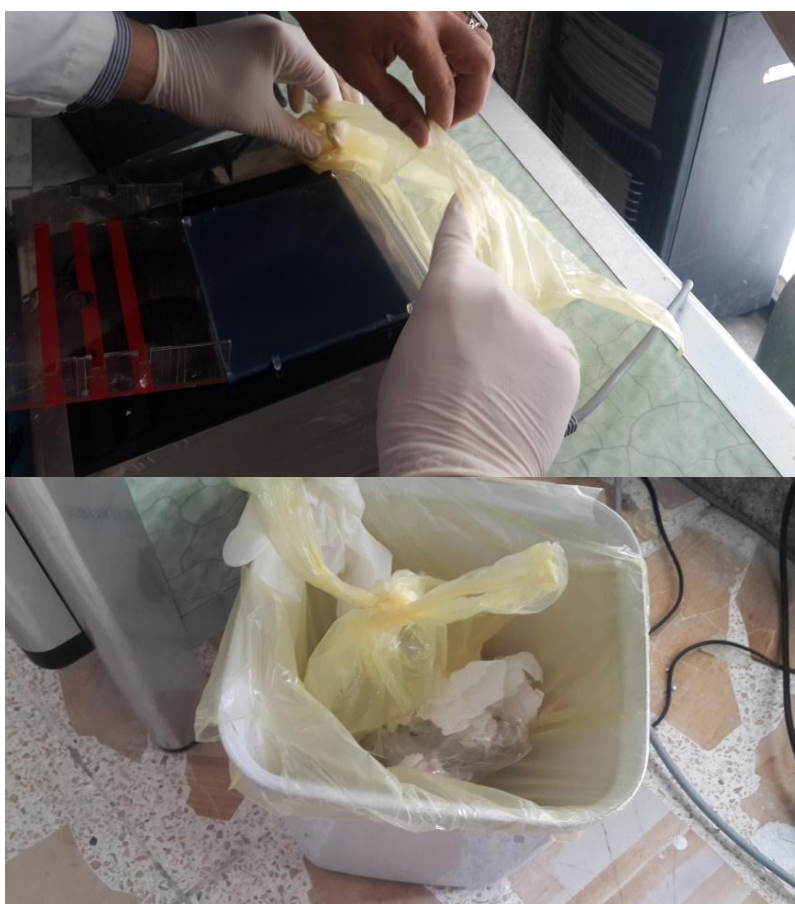


Figure 2: Discard the agarose gel that containing of ethidium bromide.



Figure 3: unsecured storage of ethidium bromide.

Recommendations:

The Control of Substances *Hazardous* to Health and environment is very important so our universities should prepare *Guidelines for Ethidium Bromide Waste Management & Disposal*, which provide general guidance to researchers and labs on how to work safely with Ethidium bromide. These *Guidelines* will describe the minimum requirements for the safe storage, use, handling, and disposal of particularly hazardous substances, including spill and accident response procedures.

وصول غير آمن وعدم وجود إجراءات سليمة للتخلص من بروميد الإيثيديوم في مختبر الأحياء الجزيئي في كلية العلوم/ جامعة الكوفة

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الخلاصة:

الأثيديوم برومايد هي مادة كيميائية مطفرة و مسرطنة محتملة. مع ذلك فان الأثيديوم برومايد هي من اكثر الصبغات المستخدمة شيوعا خلال الترحيل الكهربائي لجل الاكاروز لتشخيص ال DNA في كلية العلوم. ان الأثيديوم برومايد هي مادة عالية السمية و مطفرة و التي قد تكون قاتلة اذا ما ابتلعت او استنشقت او تم امتصاصها عبر الجلد. يجب ان تعامل مخلفات تشخيص DNA و يتم التخلص منها بطريقة ملائمة تضمن حماية الصحة العامة و البيئة. لسوء الحظ لا توجد طريقة ملائمة للتخلص من الاكاروز جل المحتوي على

الاثيديوم برومايد. حيث بالعادة يتم رميها بسلة المهملات العادية أما المحاليل الحاوية على الاثيديوم برومايد فيتم سكبها في مغسلة المختبر. و بالرغم من معرفة خطورة مادة الاثيديوم برومايد كمادة مطفرة الا انها في الوقت الحاضر تحفظ في خزانة غير مؤمنة يمكن الوصول اليها من قبل اي شخص يدخل المختبر في كلية العلوم. الكلمات المفتاحية: الأثيديوم برومايد، التخلص الآمن، مادة مطفرة، الصبغات المسرطنة.

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