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A Review on Toxicity and Degradation of Ethidium Bromide (EtBr)

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ABSTRACT

Ethidium Bromide (EtBr) serves as an interpolating agent utilized for its fluorescent properties in gel electrophoresis, aiding in the detection of various DNA fragments. However, its usage has been associated with DNA mutations, inhibition of mitochondrial protein synthesis and other alterations, particularly at high concentrations. Due to its remarkable stability in the environment and propensity to degrade into mutagenic compounds, especially when exposed to bleaching agents, there are significant concerns regarding its widespread use. Given the potential toxic and mutagenic effects of EtBr and the possibility of its entry into the food chain, it becomes crucial to monitor its concentration at emission sources across different ecosystems, including aquatic, terrestrial, and plant environments. Improper disposal of EtBr poses substantial risks, including the induction of mutations and cancer. Various disposal methods have been explored, such as phytoremediation and employing bacteria capable of degrading this chemical. Proper management of EtBr disposal is paramount to mitigate its harmful impact on the environment and living organisms.

Keywords Carcinogenicity, DNA fragment, Ethidium Bromide, Phytoremediation.

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INTRODUCTION

Ethidium Bromide (EtBr) is an odorless, dark red, crystalline, amorphous powder with a bitter taste, and solubility in water, chloroform, and ethanol (Zhang *et al.* 2013) (Fig. 1). It has found widespread application in depleting mitochondrial DNA and generating cell lines with reduced mitochondrial DNA content. Additionally, it has been extensively utilized in rat models for demyelination to evaluate endogenous remyelination (Kuypers *et al.* 2013). Advancements in molecular techniques have elucidated the mechanism of action of EtBr on DNA structure and function. Multiple studies have highlighted its role in swiftly inhibiting DNA synthesis by forming a planar ring system between base pairs of the DNA (Amirijavid and Mohammadi 2014, Del Giudice and Wolf 2021). Over the past decades, it has been uncovered that EtBr induces helix distortion of free minicircles, hampers replication initiation, and the cumulative effects of these actions lead to DNA loss and eventual cell death (Fogg *et al.* 2021).

Improper handling of Ethidium Bromide (EtBr) waste poses a significant risk of contaminating water

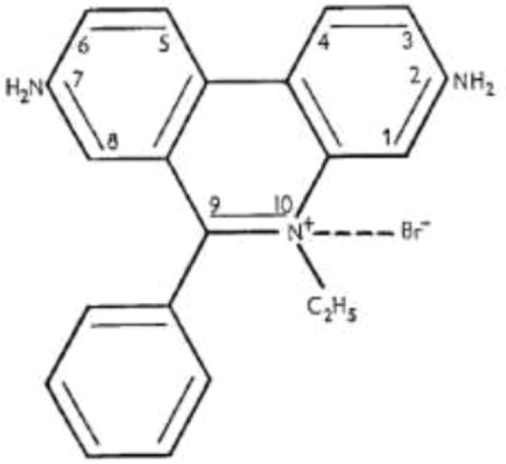

Fig.1. Ethidium Bromide (C21H20BrN3, MW 394.31). Source: Zhang *et al.* (2013).

supplies. If not properly treated or disposed of, EtBr waste may seep into waterways via sewer systems, potentially infiltrating underground water sources. These contaminated waters could then be utilized for various purposes such as irrigation in gardens or agricultural fields, as well as for washing or drinking. Additionally, there's a concerning correlation between the presence of EtBr waste and an increased incidence of certain carcinogenic cases in humans when its pathway is traced. Studies, such as one by Zhang *et al.* (2013), have highlighted the severe environmental consequences of EtBr release into ecosystems, including water pollution, eutrophication and disruptions in aquatic life. Hence, it is imperative to ensure the proper treatment and management of this waste before disposal to mitigate these risks. While the human body possesses mechanisms to tolerate certain levels of chemicals, it actively works to eliminate harmful substances. Organs like the kidneys filter toxins from the blood, excreting them through urine, while the liver detoxifies and converts chemicals into less harmful forms, eliminating them through feces, sweat and inhalation (Pizzorno 2015, Sarwar *et al.* 2017, Limaye *et al.* 2018).

Toxic and mutagenic effects of EtBr : It once deemed beneficial for treating trypanosomiasis, is now extensively utilized in laboratory settings. However, its role has shifted from medicinal to potentially hazardous due to its mutagenic properties, attributed to its carcinogenic nature and its interference with vital biological processes like DNA replication and transcription (Kuypers *et al.* 2013). Studies have demonstrated its irreversible impact on organisms in their growth phase, causing a significant decline in DNA content, indicating interference with nucleic acid synthesis. Research published in Nature Science elucidated EtBr ability to induce chromosomal abnormalities, impeding normal cell division in sea urchin eggs. Moreover, investigations into its effects on mitochondrial DNA breakdown revealed that varying concentrations of EtBr in cultured cells led to a decrease in mitochondrial DNA content, resulting in cellular demise, particularly at higher doses (25 microM). Even at minute concentrations, EtBr exhibits toxic effects on *Drosophila melanogaster*, affecting productivity, morphology and biochemical parameters, likely due to its genotoxic properties (Singh and Singh 2018).

Degradation of EtBr : Ethidium bromide liquid waste is often disposed of conventionally, such as by pouring it down a drain, especially if its concentration falls below mandated levels. However, this long-standing practice poses a risk of escalating ethidium bromide levels in sewage water (municipal/ground/surface), leading to its accumulation in nearby soils and plants. Crops irrigated with such contaminated wastewater may introduce this mutagenic dye into the food chain, posing potential health hazards to both humans and animals. To address this concern, researchers have developed various physical techniques including the utilization of Boron-doped diamond electrodes (Zhang *et al.* 2013), customized pumices (Heibati *et al.* 2015), carbon nanotubes (Moradi *et al.* 2014) among others, for the degradation of EtBr. Additionally, scientists have explored numerous biological methods, such as employing specific plant species like tomatoes, alfalfa and vetiver grass (Amirijavid and Mohammadi 2014) for phytoremediation of EtBr.

Sources of pollution : EtBr is commonly utilized in molecular biology labs as a fluorescent dye for staining nucleic acids. Your depiction suggests that EtBr pollution predominantly arises from urban and industrial sources, such as manufacturing plants, industries, and potentially veterinary facilities (Singh

and Singh 2018). Pollution can manifest through the direct discharge of wastewater containing EtBr into bodies of water like ponds, lakes and rivers, which serve as common receptacles. The transportation of EtBr from these sources to the environment can be influenced by factors like volatility, polarity and adsorption characteristics. Once in the environment, it may undergo alterations like surface modification, degradation and dissolution. Understanding these processes is pivotal in determining the destiny and dissemination of EtBr pollutants. Further exploration is imperative to fully grasp. EtBr's environmental dynamics, including its transformation pathways and potential ramifications on ecosystems and human health. Such research can guide the formulation of strategies aimed at mitigating this pollution and the associated risks (Singh and Singh 2018).

Environment and ecological risk assessment of EtBr : Detecting, identifying and quantifying Ethidium Bromide (EtBr) and its transformation products across various environmental compartments is imperative for comprehending its presence and destiny. This endeavor proves highly challenging due to several reasons. Firstly, there is a scarcity of data concerning the environmental impacts of EtBr across different mediums of life such as soil, water, air, plants and animals. Additionally, the absence of analytical methods with sufficiently low detection limits exacerbates the challenge. Consequently, the development of innovative techniques for monitoring EtBr contamination, tracing its waste and facilitating its remediation remains an ongoing hurdle. Given the toxic or mutagenic nature of EtBr and its potential transfer within the food chain, it is paramount to monitor its concentration at emission sources across all environmental mediums, including plants and other organisms (Singh and Singh 2018). While advanced ultra-sensitive instrumental techniques like UV-Vis spectrophotometry and LC-MS are available for EtBr detection, they are not yet employed routinely, likely due to their higher costs. However, their utilization could significantly enhance the quantitative determination of EtBr in water, suspended matter, soil, and biota. Furthermore, the absence of ecological risk assessment indices for this contamination complicates efforts to understand its environmental impact and the resulting effects on various components. Comprehensive comprehension necessitates innovative approaches involving studies on EtBr-induced biological and biochemical effects on plants and human health (Singh and Singh 2018). Therefore, addressing these challenges requires a concerted effort to develop novel methodologies and expand the scope of monitoring and assessment techniques.

Substitute of EtBr : Ethidium Bromide (EtBr) has long been the standard DNA stain used in gel electrophoresis, but its toxicity and mutagenicity have raised concerns among researchers. As a result, several alternative dyes have been developed to address these issues while still providing efficient DNA visualization. SYBR Safe™, GelRed™, Nancy 520, Red Safe™, GelStar™, Novel Juice, and other dyes you mentioned are among the alternatives to EtBr. These dyes offer various advantages such as reduced toxicity, non-mutagenicity and improved safety for users and the environment. SYBR Safe™, for example, as you mentioned, is developed by invitrogen and is marketed as non-genotoxic, non-mutagenic, and non-hazardous. It's important for researchers to carefully consider the properties and performance of these alternative dyes when choosing the appropriate one for their experiments. Additionally, it's essential to follow proper handling and disposal procedures for any DNA staining dyes to minimize risks to health and the environment (Singh and Singh 2018). This stain interacts with the DNA grooves instead of intercalating the DNA double stranded (Haines *et al.* 2015).

Uses of Ethidium Bromide : EtBr has demonstrated its efficacy in depleting mitochondrial DNA, thereby enabling the generation of cell lines devoid of mitochondrial DNA. Additionally, its application as a demyelinating agent in rats has facilitated the evaluation of endogenous remyelination (Kuypers *et al.* 2013). This compound has also been utilized in modeling mouse ventral spinal cord demyelination, allowing for the comprehensive assessment of behavioral function, inflammation, myelin status and axonal viability (Kuypers *et al.* 2013). Its distinctive molecular structure enables it to intercalate between stacked nucleotide bases of nucleic acids, leading to carcinogenic and mutagenic effects by disrupting crucial biological processes such as DNA replication, transcription, and cellular structure and function

(Kuypers *et al.* 2013).

CONCLUSION

The unregulated usage and improper disposal of EtBr can pose risks of toxicity and carcinogenicity across various levels of the biological hierarchy. Its presence in water bodies and soil can result in the transfer of EtBr through plants, potentially leading to bioaccumulation and subsequent exposure to higher organisms. Further research is necessary to fully understand the toxic, mutagenic and carcinogenic properties of EtBr and its metabolites, as well as their residence time in soil, water and other environmental components. Additionally, investigating its interactions with other contaminants in aquatic environments and soil, along with assessing associated human health hazards and ecological risks, is imperative for a comprehensive understanding of the potential impacts.

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