

Safety Data Sheet

Ethidium bromide

Division of Safety
National Institutes
of Health



WARNING!

THIS COMPOUND MAY BE ABSORBED THROUGH THE INTESTINAL TRACT. IT IS MODERATELY TOXIC AND STRONGLY MUTAGENIC. CARCINOGENICITY HAS NOT BEEN DEMONSTRATED. AVOID FORMATION AND BREATHING OF AEROSOLS.

LABORATORY OPERATIONS SHOULD BE CONDUCTED IN A FUME HOOD, GLOVE BOX, OR VENTILATED CABINET.

AVOID SKIN CONTACT: IF EXPOSED, WASH WITH SOAP AND WATER.

FOR EYE EXPOSURE, IRRIGATE IMMEDIATELY WITH LARGE AMOUNTS OF WATER. FOR INGESTION, DRINK PLENTY OF MILK OR WATER. INDUCE VOMITING. FOR INHALATION, REMOVE VICTIM PROMPTLY TO CLEAN AIR. ADMINISTER RESCUE BREATHING IF NECESSARY. REFER TO PHYSICIAN.

IN CASE OF LABORATORY SPILL, WEAR PROTECTIVE CLOTHING DURING CLEANUP. AVOID SKIN CONTACT OR BREATHING OF DUST. WASH DOWN AREA WITH SOAP AND WATER. DISPOSE OF WASTE SOLUTIONS AND MATERIALS APPROPRIATELY.

A. Background

Ethidium bromide (EB) is a dark red crystalline compound that is soluble in water and moderately toxic to rodents. It is strongly mutagenic but carcinogenicity has not been demonstrated; on the contrary, it acts as an anticarcinogen against several transplantable tumors. Its chief medical use has been in the treatment of trypanosome infections; more recently, because of its strong and

Revised: 3/88

Prepared by the Environmental
Control and Research Program

specific ability to react with nucleic acid, it has become an important tool in the study of the secondary and tertiary structures of DNA.

Chemical and Physical Data

1. Chemical Abstract No.: 1239-45-8

2. Synonyms:

Dromilac

Homidium bromide

2,7-Diamino-9-phenylphenanthridine-ethobromide

3,8-Diamino-5-ethyl-6-phenylphenanthridinium bromide

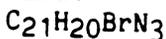
2,7-Diamino-9-phenyl-10-ethylphenanthridinium bromide

2,7-Diamino-10-ethyl-9-phenylphenanthridinium bromide

Phenanthridinium, 3,8-diamino-5-ethyl-6-phenyl, bromide (9CI)

3. Molecular

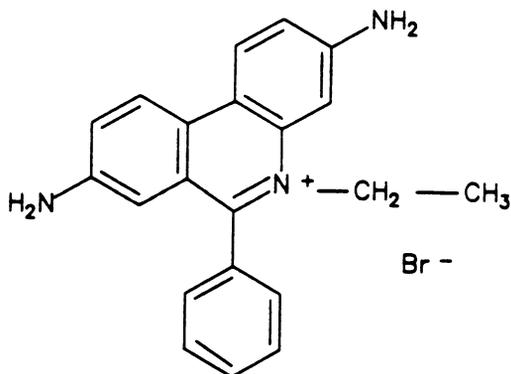
formula:



weight:

394.33

structure:



4. Density: No data.

5. Absorption spectroscopy: In the visible range, EB has a maximum absorption at 480 nm ($\epsilon = 5.30$), which shows a metachromatic shift towards 517 nm on mixing with DNA, the extent depending on DNA concentration, with color change from yellow-orange to bright pink. The isobestic point is at 510 nm (Waring, 1965). The ultraviolet (Kandaswamy and Henderson, 1963; Hudson and Jacobs, 1975), mass (Shabanowitz et al., 1975), and proton magnetic resonance spectra (Thomas and Roques, 1972) have been described. EB shows fluorescence that is strongly enhanced by addition of DNA (LePecq et al., 1964). This has been used in the determination of intracellular DNA (Pauluhn et al., 1980).

6. Volatility: No data; may be expected to be low.

7. Solubility: Soluble in 20 parts of water and 750 parts of chloroform at 20°C.
8. Description: Dark red crystals with bitter taste.
9. Boiling point: No data. Melting point: 248-249°C with decomposition.
10. Stability: Heat stable at 100°C; no data at higher temperatures. Dimerizes in aqueous solution.
11. Chemical reactivity: EB has basic properties with values of $pK_{a1} = 0.713$ and $pK_{a2} = 2.43$. On reaction with acetic anhydride in aqueous solution, it is acetylated nearly exclusively at the 3-amino group (the one closer to the ring nitrogen [Zimmermann and Zimmermann, 1976]). There are no other specific data for EB, but it may be assumed that it exhibits the usual reactivity of primary aromatic amines (alkylation, diazotization, oxidation by permanganate, ring substitution) unless this is modified by the ionic character of EB. The usual preparations retain 1.2% water even after drying at 100°C for 10 hours (Watkins, 1952).
12. Flash point: No data.
13. Autoignition temperature: No data.
14. Explosive limits in air: No data.

Fire, Explosion, and Reactivity Hazard Data

There are no pertinent data on EB or chemically related compounds. What appear below are best estimates.

1. EB does not require special fire-fighting procedures or equipment and does not present unusual fire and explosion hazards.
2. No conditions contributing to instability are known. While aromatic amines in general are slightly flammable, this may not be true for EB because of its salt-like character.
3. No incompatibilities are known.
4. EB does not require nonspark equipment.

Operational Procedures

The NIH Guidelines for the Laboratory Use of Chemical Carcinogens describe operational practices to be followed when potentially carcinogenic chemicals are used in NIH laboratories. The Guidelines

should be consulted to identify the proper use conditions required and specific controls to be implemented during normal and complex operations or manipulations involving EB.

1. Chemical inactivation: Validated methods have been reported (Lunn and Sansone, 1987).
2. Decontamination: Turn off equipment that could be affected by EB or the materials used for cleanup. If more than 10 grams has been spilled or if there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 116) for assistance. Wash surfaces with copious quantities of water. Rinse glassware and animal cages with water.
3. Disposal: It may be possible to decontaminate waste streams containing EB before disposal. For details, see Lunn and Sansone (1987). No waste streams containing EB shall be disposed of in sinks or general refuse. Surplus EB or chemical waste streams contaminated with EB shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (e.g., animal carcasses and bedding) containing EB shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (e.g., tissue cultures) containing EB shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (e.g., absorbent bench top liners) minimally contaminated with EB shall be handled as potentially infectious waste and packaged for incineration, as above. Absorbent materials (e.g., associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing EB shall be handled in accordance with the NIH radioactive waste disposal system.
4. Storage: Store in closed containers.

Monitoring and Measurement Procedures Including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: No published procedures.
2. Analysis: No published procedures. One of two published metabolic studies (Kandaswamy and Henderson, 1963) employed isotope-labeled EB that was extracted from tissue with boiling 95% ethanol, which then was evaporated to dryness and assayed for radio-activity; obviously this method does not distinguish between EB and possible metabolites. In the other study (MacGregor and Clarkson, 1971), EB was determined in bile by an adaptation of the Bratton-Marshall procedure, originally developed for analysis of sulfonamides, which is likewise not specific.

Biological Effects (Animal and Human)

- Absorption: EB is absorbed after parenteral (intravenous, intramuscular, subcutaneous, intraperitoneal) injection. No data have been reported on other routes of administration.
- Distribution: Intraperitoneal EB is rapidly cleared; there is transitory initial general tissue accumulation (with possible small concentration in muscle) followed by rapid urinary excretion. If the renal pedicles are ligated, there is accumulation of EB in the bile (50-55% recovered after 16-18 hours) (MacGregor and Clarkson, 1971).
- Metabolism and excretion: Relatively little is known about this (except for the interaction of EB with nucleic acids to be mentioned later). In normal animals, EB is rapidly excreted in unchanged form in the urine (Kandaswamy and Henderson, 1963). monoacetylated conjugate and unchanged EB are found in the bile after renal ligation (MacGregor and Clarkson, 1971).
- Toxic effects: The only published acute LD50 is 110 mg/kg (mouse, subcutaneous). It should be noted, however, that the intravenous LD50 of ethidium chloride is considerably lower (14 and 21 mg/kg in the mouse and rat, respectively); this may be a function of the different route of administration and/or higher water solubility of the chloride; it is unlikely that this change of anion would significantly alter the toxicity. Toxic effects of EB have been reviewed (Waring, 1975). Acute toxicity is due to cardiovascular or respiratory failure; chronic toxicity is associated with a number of symptoms, including liver necrosis. The main biological (and toxicological?) effect of EB so far has been demonstrated only in vitro. It consists of a strong binding of EB to nucleic acids, which results in their increased stability and viscosity. The effect on DNA is one of intercalation (demonstrated in model systems by X-ray crystallography [Tsai et al., 1975]) in a ratio of 1 molecule of EB per 4 to 5 nucleotides, followed by secondary ionic binding until a maximal 1:1 ratio is achieved (Waring, 1965). Binding to RNA is only ionic. EB is very selective in inhibiting synthesis of extranuclear (mitochondrial) DNA probably through inhibition of DNA polymerases.
- Carcinogenic effects: There is no report on any carcinogenicity of EB. On the contrary, EB acts as an antitumor agent against some transplantable tumors in rats (Kramer and Grunberg, 1973).
- Mutagenic and teratogenic effects: EB is a strong mutagen in the Ames test but only after activation by a rat liver metabolizing system. There is no evidence for teratogenicity.

Emergency Treatment

1. Skin and eye exposure: For skin exposure, remove contaminated clothing and wash skin with soap and water. For eye exposure, irrigate immediately with copious quantities of running water for at least 15 minutes.
2. Ingestion: Drink plenty of milk or water. Induce vomiting.
3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary.
4. Refer to physician.

References

- Hudson, B. and R. Jacobs. 1975. The ultraviolet transitions of the ethidium cation. *Biopolymers* 14:1309-1312.
- Kandaswamy, T.S. and J.F. Henderson. 1963. The metabolism of ethidium bromide in normal and neoplastic tissues. *Cancer Res* 23:250-253.
- Kramer, M.J. and E. Grunberg. 1973. Effect of ethidium bromide against transplantable tumors in mice and rats. *Chemotherapy* 19:254-258.
- LePecq, J-B., P. Yot, and C. Paoletti. 1964. Interaction of ethidium bromide with nucleic acids. *CR Acad Sci[D] Paris* 259:1786-1789.
- Lunn, G. and E.B. Sansone. 1987. Ethidium bromide: Destruction and decontamination of solutions. *Anal Biochem* 162:453-458.
- MacGregor, J.T. and T.W. Clarkson. 1971. Metabolism and biliary excretion of phenanthridinium salts. I. Nature of the biliary metabolites. *Biochem Pharmacol* 20:2833-2846.
- Pauluhn, J., A. Nanjok, and H.W. Zimmermann. 1980. On the quantitative fluorimetric determination of intracellular DNA with ethidium bromide and the constancy of the quantum yield of the ethidium-DNA complex in the biological environment. *Z Naturforsch* 35C:585-598.
- Shabanowitz, J., P. Brynes, A. Maelicke, D.V. Bowen, and F.H. Field. 1975. Chemical ionization mass spectrometry of quaternary amines. *Biochem Mass Spectrom* 2:164-167.
- Thomas, G. and B. Roques. 1972. Proton magnetic resonance studies of ethidium bromide and its sodium borohydride-reduced derivative. *Fed Eur Biochem Soc Lett* 20:169-175.
- Tsai, C-C., S.C. Jain, and H.M. Sobell. 1975. X-ray crystallographic visualization of drug-nucleic acid intercalative binding: Structure of an ethidium-dinucleoside phosphate crystalline complex, ethidium-5-iodouridyl(3',5') adenosine. *Proc Natl Acad Sci USA* 72:628-632.
- Waring, M.J. 1965. Complex formation between ethidium bromide and nucleic acids. *J Mol Biol* 13:269-282.
- Waring, M. 1975. Ethidium and propidium. Pages 141-165 in Corcoran, J.W., and F.E. Hahn (eds.) *Antibiotics*, vol. 3. Springer, New York, NY.

atkins, T.I. 1952. Trypanocides of the phenanthridine series.
The effect of changing the quaternary grouping in dimidium
bromide. J Chem Soc 74:3059-3064.

immermann, I. and H.W. Zimmermann. 1976. pKa values of ethidium
bromide and 7-amino-9-phenyl-10-ethylphenanthridinium bromide.
Z Naturforsch 31C:656-660.