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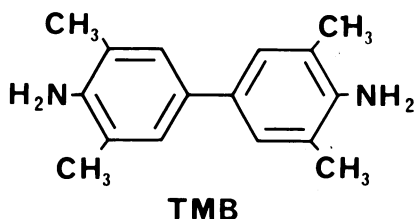


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Tetramethylbenzidine

A reported noncarcinogenic analog of benzidine



For many years benzidine has been used as a sensitive and specific reagent for the detection of blood.¹ However, its extreme carcinogenicity has curtailed its use in recent years. In fact, in 1974 the Occupational Safety and Health Administration banned its manufacture and use in the United States.²

One of the early hypotheses for the carcinogenicity of aromatic amines involved *ortho* hydroxylation.³ Thus, it seemed that 3,3',5,5'-tetramethylbenzidine (TMB) (in which *ortho* hydroxylation is impossible) might be an effective and safe substitute for benzidine.⁴ Indeed, it was found that subcutaneous injection of TMB into rats "produced no tumors specifically attributable to it, in doses greater than those in which benzidine or *o*-tolidine cause a high yield of neoplasms."⁴ The *Salmonella* microsome test (Ames test)⁵ showed TMB to be nonmutagenic,^{6,7} suggesting that it is noncarcinogenic.

Garner *et al.*⁸ have evaluated the use of TMB as a presumptive test for blood in forensic work. In various concentrations of glacial acetic acid, TMB reacted with blood in the presence of H₂O₂ to form a colored product. Comparative studies with benzidine showed TMB to be equally sensitive in blood detection.

Standefler and Vanderjagt⁹ found that methods employing TMB for plasma hemoglobin assay compared well with those using benzidine in accuracy, precision and sensitivity. Iron porphyrins, which can exhibit a peroxidase-like action, were detected at very low levels on paper chromatograms with TMB as visualizing agent.¹⁰ The peroxidase activity of the heme protein cytochrome P-450 was determined by sodium

dodecyl sulfate-polyacrylamide gel electrophoresis with TMB staining.¹¹ In this method TMB-H₂O₂ stain is superior to benzidine-H₂O₂. The blue-stained bands are distinct and the color is stable.¹¹ The activity was also detected in immunoprecipitates in Ouchterlony double-diffusion plates.¹¹

In the area of neurohistochemistry, TMB proved more sensitive than 3,3'-diaminobenzidine in demonstrating retrograde and anterograde axonal transport of horseradish peroxidase (HRP) in rat brain by light microscopy.¹² TMB also gave results comparable to those obtained with benzidine dihydrochloride in the demonstration of retrograde transport of HRP.¹³

We also offer TMB dihydrochloride which, in contrast to the water-insoluble free base, dissolves slowly in water or in citrate buffer.¹⁴ TMB dihydrochloride appears to be suitable for the quantitative determination of hemoglobin in solutions, and for the identification and localization of myeloperoxidase in cells.¹⁴

TMB and TMB dihydrochloride are rapidly gaining acceptance as reliable and safe substitutes for benzidine and its carcinogenic derivatives.

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