The purpose of this in vitro study was to investigate the affinity of mepiquat chloride for subtypes of muscarinic acetylcholine receptors (M₁, M₃) in membranes of animal origin and to compare these findings with those obtained with standard muscarinic reference compounds.

The membranes, prepared in the testing facility and stored at -196°C until needed, were those of bovine cerebral cortex (M₁, M₂), rat heart (M₃) and rat submaxillary gland (M₃). The subtype-specific substances with high affinity for muscarinic acetylcholine receptors (reference compounds) were pirenzepine (M₁), methoctramine (M₃), 4-diphenylacetoxy-N-methylpiperidine methiodide [4-DAMP] (M₃) and atropine (a high affinity compound without subtype specificity). The radioligand was [N-Methyl-³H]N-methylscopolamine (³H-NMS). The rats (males of Sprague-Dawley strain, weighing about 200 g) were obtained from the local supplier.
(Charles River) and the bovine brains were obtained from the local slaughterhouse. The affinity of Mepiquat chloride for the receptors was studied by incubating membranes with $^3$H-NMS and Mepiquat chloride ($10^{-5}$, $10^{-4}$ or $10^{-3}$ moles/liter) or with $^3$H-NMS and reference compounds ($10^{-10}$-$10^{-3}$ moles/liter) for 1 hour at 37°C. After incubation, the membranes were washed, the radioactivity measured and the $K_i$ values (inhibition constants of Mepiquat chloride and reference compounds) calculated.

Compared with the reference compounds, Mepiquat chloride had very low and unselective affinity for the muscarinic receptors ($^3$H-NMS binding sites). The inhibition constants ($K_i$) of Mepiquat chloride were 88, 160 and 200 micromoles/liter for the receptors ($M_1$), ($M_2$) and ($M_3$), respectively. The $K_i$ values for the reference compounds were in the nanomolar range. According to this submission, "there is only a small risk of side effects with muscarinic receptors with high concentrations of mepiquat chloride in animals and man ", meaning, apparently, that Mepiquat chloride is not a serious health hazard to humans and animals.

**Classification of Study:** Acceptable as a Nonguideline, special (neuropharmacological) in vitro study.