Thimerosal, also known as thimersal, Merthiolate, or sodiumethylmercurithiosalicylate, is an organic mercurial compound that is used in a variety of commercial as well as biomedical applications. As a preservative, it is used in a number of vaccines and pharmaceutical products. Its active ingredient is ethylmercury. Both inorganic and organic mercurials are known to interfere with glutamate homeostasis. Brain glutamate is removed mainly by astrocytes from the extracellular fluid via high-affinity astroglial Na\(^{+}\)-dependent excitatory amino acid transporters, glutamate/aspartate transporter (GLAST) and glutamate transporter-1 (GLT-1). The effects of thimerosal on glutamate homeostasis have yet to be determined. As a first step in this process, we examined the effects of thimerosal on the transport of \(^{3}\)H-D-aspartate, a nonmetabolizable glutamate analog, in Chinese hamster ovary (CHO) cells transfected with two glutamate transporter subtypes, GLAST (EAAT1) and GLT-1 (EAAT2). Additionally, studies were undertaken to determine the effects of thimerosal on mRNA and protein levels of these transporters. The results indicate that thimerosal treatment caused significant but selective changes in both glutamate transporter mRNA and protein expression in CHO cells. Thimerosal-mediated inhibition of glutamate transport in the CHO-K1 cell line DdB was more pronounced in the GLT-1-transfected cells compared with the GLAST-transfected cells. These studies suggest that thimerosal accumulation in the central nervous system might contribute to dysregulation of glutamate homeostasis.