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Albumin, the responsible protein of the  ${\rm Cu}^{2+}$ -dependent hydrolysis of O-hexyl O-2,5-dichlorophenyl phosphoramidate (HDCP) by chicken serum "antagonistic stereoselectivity"

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#### ACCEPTED MANUSCRIPT

Albumin, the responsible protein of the Cu<sup>2+</sup>-dependent hydrolysis of O-hexyl O-2,5-dichlorophenyl phosphoramidate (HDCP) by chicken serum "antagonistic stereoselectivity"

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#### **Abstract**

O-hexyl O-2,5-dichlorophenyl phosphoramidate (HDCP) is a chiral analogous compound of the methamidophos insecticide that induces delayed neuropathy, and the R-(+)-HDCP enantiomer is an inhibitor of neuropathy target esterase (NTE). This enantiomer is not hydrolized by Ca<sup>2+</sup>-dependent phosphotriesterases in mammal tissues. Our group had reported R-(+)-HDCP hydrolysis in chicken serum enhanced by 30-250 μM copper in *ex vivo* assays, which we call "antagonistic stereoselectivity". We checked the hypothesis of the role of cupper binding proteins. Two hundred micrograms of human serum ceruloplasmine or horse kidney methallotionein in 1 mL containing 400 μM HDCP for 60 min showed no significant Cu<sup>2+</sup>-dependent hydrolysis. However under the same conditions, 10 μL of chicken serum or 10 μL of buffer containing 216 μg of chicken serum albumin (CSA) (amount of albumin content in this serum volume) with 100 μM Cu<sup>2+</sup> showed the same stereoselectivity and similar levels to the Cu<sup>2+</sup>-dependent R-(+)-HDCP hydrolysis. About 75% of R-(+)-HDCP were hydrolyzed after 120 min in the presence of 100 μM Cu<sup>2+</sup> (inhibited by 5 mM EDTA).

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