The acute toxicity of monodispersed 6 nm and <100 nm poly-dispersed copper oxide nanoparticles toward *Daphnia magna* was assessed using 48 h immobilization tests. CuSO₄ was used as a reference. Four different exposure conditions were tested, to study whether the toxicity of the nanoparticle suspensions changed in a way similar to what is known for dissolved Cu: first in ISO standard test conditions (pH 7.8), second with slight acidity (pH 6.5), third in the presence of citric acid, and fourth in the presence of humic acid. For all four exposure conditions, the toxicity of Cu employed in the three forms followed the same sequence, i.e., CuSO₄ > monodispersed 6 nm CuO ≫ poly-dispersed CuO. The toxicity of all Cu forms decreased from pH 6.5, ≫ pH 7.8, > pH 7.8 + citric acid, to ≫ pH 7.8 + humic acid. This pattern is in agreement with concentrations of Cu²⁺ calculated using the equilibrium model MINTEQ. These findings show that the acute toxicity of copper oxide nanoparticles is governed by test water composition and the chemical species Cu²⁺.