In aquatic toxicity testing of engineered nanoparticles (ENPs) the process of agglomeration is very important as it may alter bioavailability and toxicity. In the present study, we aimed to identify test conditions that are favorable for maintaining stable ENP suspensions. We evaluated the influence of key environmental parameters: pH (2-12) and ionic strength using M7, Soft EPA (S EPA) medium, and Very Soft EPA (VS EPA) medium; and observed the influence of these parameters on zeta potential, zeta average, and acute immobilization of Daphnia magna for three different ENPs. Despite being sterically stabilized, test suspensions of silver (Ag) ENPs formed large agglomerates in both VS EPA and M7 media; and toxicity was found to be higher in VS EPA medium due to increased dissolution. Low-agglomerate suspensions for zinc oxide (ZnO) could be obtained at pH 7 in VS EPA medium, but the increase in dissolution caused higher toxicity than in M7 medium. Titanium dioxide (TiO2) ENPs had a point of zero charge in the range of pH 7-8. At pH 7 in VS EPA, agglomerates with smaller hydrodynamic diameters (similar to 200 nm) were present compared to the high ionic strength M7 medium where hydrodynamic diameters reached micrometer range. The stable suspensions of TiO2 ENPs caused immobilization of D. magna, 48-h EC50 value of 13.7 mg L-1 (95% CI, 2.4 mg-79.1 mg L-1); whereas no toxicity was seen in the unstable, highly agglomerated M7 medium suspensions, 48-h EC50 > 100 mg L-1. The current study provides a preliminary approach for methodology in testing and assessing stability and toxicity of ENPs in aquatic toxicity tests of regulatory relevance. (C) 2016 Elsevier Inc. All rights reserved.