Optimization of high-throughput nanomaterial developmental toxicity testing in zebrafish embryos

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Nanomaterial (NM) developmental toxicities are largely unknown. With an extensive variety of NMs available, high-throughput screening methods may be of value for initial characterization of potential hazard. We optimized a zebrafish embryo test as an *in vivo* high-throughput assay for NM developmental toxicity by assessing 5 embryo culture solutions for their effects on NM aggregation as well as embryo development. We compared the aggregation of NM in 5 zebrafish embryo rearing solutions [10% Hank's, Danieau, 60 mg/L artificial seawater (ASW), full strength (1X) and 10% EPA moderately hard reconstituted water (MHRW)] by dynamic light scattering (DLS) analysis. Silver nanoparticles (nano-Ag) coated with citrate-, polyvinylpyrrolidone (PVP)-, or gum arabic (GA) were suspended in these 5 solutions as well as deionized water (ddH_2O) for 24 h. All nano-Ag aggregate sizes in both MHRWs were similar to that in ddH_2O. GA-coated nano-Ag aggregates were largest in Hank's, followed by Danieau, and distantly by ASW. PVP-coated 10 nm nano-Ag and citrate-coated nano-Ag aggregate sizes were slightly larger in Hank's than in ddH_2O. PVP-coated 25 nm nano-Ag sizes were similar in all 5 solutions and ddH_2O. Overall, the aggregates were largest in Hank's, followed by Danieau, ASW, and smallest in MHRWs and ddH_2O. This is consistent with the notion that aggregation is generally more severe in ion-rich solutions. Zebrafish embryos were cultured from days 0-6 post-fertilization in the same 5 solutions without NM, and their health was assessed using death, non-hatching and malformations as endpoints. Preliminary data showed the healthiest embryos were cultured in 1X MHRW and Hank's. 1X MHRW appears to be optimal for NM testing in zebrafish embryos, because it is both ideal for embryo development and least likely to promote NM aggregation. *This abstract does not necessarily reflect Agency policy.*