

QD acute toxicity assessment in zebrafish

i) Acute embryo toxicity testing

Three replicates of 12 zebrafish embryos were exposed at 4-6 hours post-fertilization to four different surface coated QDs, two amphiphilic polymers poly(acrylic acid)-octylamine copolymer (PAA) and poly(maleic anhydride-alt-1-octadecene) (PMAO) with and without polyethylene glycol (PEG) conjugation, prepared in standard zebrafish E3 embryo media. The mortality was 8% among the hatched larvae in the control group after 120 hr. Although mortality increased with exposure to the PAA-coated QDs, the difference was not statistically significant. No LC_{50} was reached up to the maximum concentration of 200 μM Cd equivalent PAA-coated QDs tested and the average viability was $83 \pm 10\%$ at 120 hpf. Almost all mortality occurred during the first 48 hpf, for both the control and exposure sets. Since the PAA-coated QDs exhibited the least toxicity after 120 hr of exposure (Figure S1), we chose to use these in our trophic transfer study.

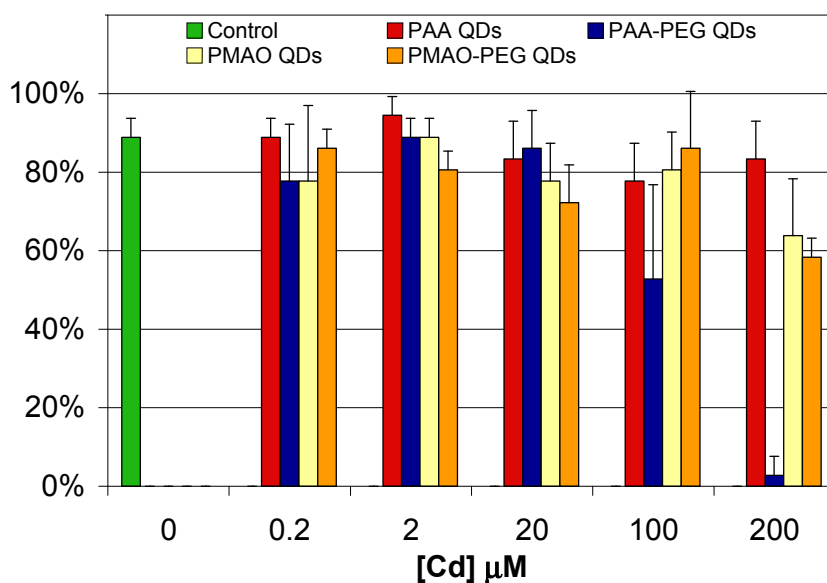


Figure S1. Viability of zebrafish larvae exposed to four different amphiphilic polymer coated QDs after 120 hr exposure duration. Error bars represent standard deviation of the mean from three replicate experiments ($n = 12$).

The incidence of developmental malformations was much higher for the exposure sets above 20 μM as show in Figure S2.

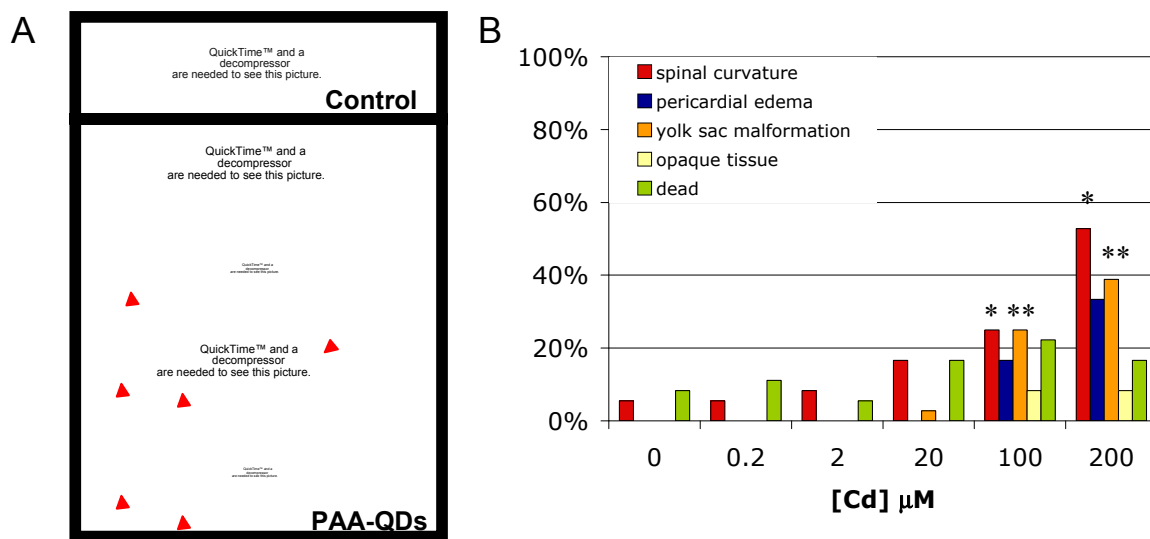


Figure S2. Effects of exposure to PAA-QDs at 200 μM Cd equivalents on zebrafish larvae. A) Representative images of deformities observed in exposed larvae. Red arrows indicate pericardial edema, yolk sac malformation, and spinal curvature. B) Incidence of deformations in zebrafish larvae exposed to graded concentrations of PAA-coated QDs. Results across combined replicates (3 replicates of $n = 12$) are shown. Asterisks indicates incidence above unexposed control ($p < 0.05$).

ii) Acute adult toxicity testing

Three replicates of 3 adult zebrafish were exposed for 72 hr to PAA-coated QDs prepared in system water (reverse osmosis water amended with Instant Ocean salt, pH 7-8 and conductivity 400–700 μS). The fish were held in 1.8 L tanks containing 1 L of synthetic freshwater with QD concentrations up to 0.5 ppm cadmium equivalents, with daily water changes. No abnormal behavior or mortality was observed.