Submission for TIC by Steve Diamond, 05/31/2012

Title: Life-stage dependent response in zebrafish (*Danio rerio*) to phototoxicity of TiO_2 nanoparticles

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Abstract:
The Zebrafish, and especially its embryo stage, has been increasingly used as a model to evaluate toxicity of manufactured nanomaterials. However, many studies have indicated that the chorion may protect developing embroys from the toxic effects of nanomaterials, suggesting that post-hatch life-stages may be more susceptible to nanomaterial toxicity and should continue to be tested to fully discern the potential hazard and risk of nanomaterials. The current study aimed to identify the "window of susceptibility" (the life stage that is most sensitive to toxicity) of zebrafish to phototoxicity of TiO_2 nanoparticles. To this end, 96-h toxicity tests were conducted to zebrafish at different life-stage (embryo [< 2-h post fertilization, "yolk-sac" larvae [< 48-h post hatch], "free-swimming" larvae [21-d post hatch], and juvenile [50-d post hatch]) by exposing test organisms to TiO_2 nanoparticles under simulated solar radiation (4 h within every 24 h during the 96-h exposure period). Toxicity endpoints included hatching rate, % malformation, and % mortality for embryonic test, and mortality only for all other tests. For zebrafish embryo, TiO_2 concentration at 1-20 mg/l caused no mortality, had no impact on 72-h hatching rate, but induced malformation in fish embryo/larvae by up to 20%. TiO_2 at higher concentration (up to 200 mg/l) caused mortality to fish embryo/larvae with a 96-h LC50 of 34.4 mg/l (95% CI: 24.5, 48.4), but had no impact on hatching rate, suggesting that mortality mainly occurred after hatching. The "yolk-sac" larvae had a 96-h LC50 of 20.3 mg/l (95% CI: 18.9-21.8) and the "free-swimming" larvae had a 96-h LC50 of 134.6 mg/l (95% CI: 103.7, 174.7). For juvenile fish, TiO_2 concentration up to 200 mg/l caused mortality less than 25% after 96-h exposure. The finding that newly hatched zebrafish larvae (i.e., < 48-h post hatch) are more sensitive than fish embryo in response to TiO_2 phototoxicity suggest that zebrafish embryo test may not always be appropriate to evaluate toxicity of nanomaterials and relying on embryo toxicity data alone may substantially underestimate the potential risk of nanomaterials. These results will be discussed relative to risk assessment for nano-TiO2.

Impact Statement: Nanotechnology is an emerging field that will produce hundreds of new and novel substances that have the potential for biological activity not typical of their bulk forms. One concern with many of these materials is their nano scale facilitates the conversion of photon energy to electron energy, leading to production of reactive oxygen species. The work presented here indicates that nano-scale TiO2 exhibits at least two orders of magnitude greater toxic potency in simulated sunlight. The work will be of significant value to regulators of nanomaterials.