

How to test nanomaterials in the aquatic environment?, observations on the current regulatory framework



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Introduction

To ensure regulatory compliance with relevant legislative regimes it is necessary to understand the toxicity, fate, and environmental effects of nanomaterials prior to release into the environment. As such there is a requirement to be able to assess the risk of nanomaterials in the aquatic environment; therefore appropriate testing regimes are required without which it is impossible to do so. The range and variety of nanomaterials available is likely to require a specific risk assessment for each new compound due to the current inability to predict fate or effects from standard testing approaches.

Currently accepted standard regulatory testing procedures have been questioned about their reliability when adopted for the testing of nanomaterials in the aquatic environment. Cefas reports on the findings of a recent fish exposure study to differentiate the impacts of cadmium sulphide, in the form of bulk material and quantum dots upon sticklebacks.

Various biological endpoints were measured in sticklebacks exposed in a flow through test system. This poster focuses on the practicalities of testing nanomaterials in such test systems including the uncertainty created in the dosing regime and how this and other parameters may influence chemical availability.

Consideration is given to the appropriateness of such test systems, including words of caution about experimental design, the influence of photo exposure and the requirement to understand the behaviour of the compound in the aqueous phase. Few workers report the downsides of their exposure studies, generating a false impression of the testing requirements for nanomaterials. By discussing these findings in an open forum, the opportunity for debate is afforded. This work is important for all those involved in the regulation of chemicals in the environment and serves to provide a word of caution about adopting without consideration or modification standard testing protocols.

This study highlights some of the problems associated with testing nanomaterials; much of the biological/ toxicological information from this study is not reported.

Methods

Fish (stickleback) were exposed to cadmium nanomaterials in a flow through saline test system. The experimental tanks were divided in sections: such that in each tank 5 male and 13 female sticklebacks were exposed to one of three concentrations of cadmium sulphide nanoparticles coated in methyl polyethylene glycol (nCdS) at the nominal concentrations of 5, 50 or 500 $\mu\text{g L}^{-1}$ or either bulk cadmium sulphide (CdS) or methyl polyethylene glycol (MPG) at 500 $\mu\text{g L}^{-1}$. An additional control treatment received freshwater alone. Each treatment and control was duplicated.

Each tank received brackish water (5ppt) was maintained at $18\pm 2^\circ\text{C}$ under a photoperiod of 16L:8D. Stock solutions were prepared for the required concentrations for each treatment and delivered to the tanks by means of peristaltic pumps at a flow rate of 50ul min^{-1} . With the exception of the CdS stock solution, which required sonication and constant agitation, all the stock solutions were left static. The stock solutions were replaced weekly. Water samples were collected from each compartment of each tank on days 6, 13, 18 (replicate 1) & 19 (replicate 2) and pooled to provide 50ml of tank water per replicate for Cd analysis via ICP-MS.

Results and Discussion

In both tanks receiving the highest treatment (500 $\mu\text{g L}^{-1}$ nCdS), the average cadmium concentrations were in excess of twice the nominal concentration. Similarly the concentration of Cd in replicate 1 of the 5 $\mu\text{g L}^{-1}$ nCdS treatment was six times higher than nominal, although the average concentration in replicate 2 was 4.22 $\mu\text{g L}^{-1}$.

Conversely the Cd concentrations in the 50 $\mu\text{g L}^{-1}$ nCdS replicates were 29.80 and 38.26 $\mu\text{g L}^{-1}$, respectively, slightly less than the nominal values (Fig 1).

It has not been possible to identify the reason why the nominal and measured values differed so greatly, nor why there were such differences between replicates. Figure 1 shows that the flow from the pump treating the tanks was in most cases consistent with the required flow of 50ul min^{-1} and therefore fluctuations in the dosing of the tanks is unlikely to be responsible for differences in the measured Cd concentrations.

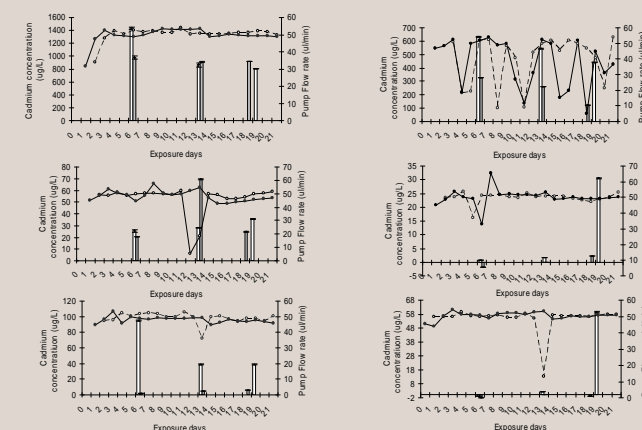
The insolubility of the bulk CdS resulted in its accumulation in both the tank and the tubing from the pump to the tank. The latter resulted in frequent blocking of the tubing supplying the material to the tank and this is reflected in the erratic predicted flow rate from the pump (Fig 1).

There is no consistent explanation for the discrepancies between the nominal and the measured Cd concentration. It has been suggested that the distribution differences may have arisen as a consequence of the size and density of the nanoparticles, compounded by effects from the polymer coating (MPG) on the outside of the nanoparticles resulting in heterogeneous diffusion limited distributions.

The expected effect would be similar to that observed in some polymer systems (Paul Christian, pers. comm.) resulting in accumulating levels of Cd in the tanks. While it is possible that the mixing in the tanks was not sufficient to ensure homogeneity, the hypothesis is not supported by the results from the other treatments, particularly that of 50 $\mu\text{g L}^{-1}$ nCdS.

However, should polymer interaction and density differences represent contributing factors to the variability in the measured Cd levels.

Figure 1: Dosing rates ($\mu\text{l min}^{-1}$) and cadmium concentration ($\mu\text{g/L}$) for each replicate (Replicate 1; open circles with broken line, or open bars, Replicate 2; solid circles with continuous line, or solid bars): (A) 500 $\mu\text{g nCdS L}^{-1}$, (B) 50 $\mu\text{g nCdS L}^{-1}$, (C) 5 $\mu\text{g nCdS L}^{-1}$, (D) 500 $\mu\text{g CdS L}^{-1}$, (E) 500 $\mu\text{g MPG L}^{-1}$, (F) control



Summary

Biological changes were observed in the fish following exposure but these are not reported in detail. However, male sticklebacks following exposure to nCdS at concentrations of 1000 $\mu\text{g L}^{-1}$ showed retarded nest building. This inhibition occurred in males exposed to nCdS concentrations considerably higher than those experienced by individuals exposed to the bulk form of CdS. It is therefore not possible to draw direct comparisons between the effects of CdS compared to CdS nanoparticles. Dose dependant effects were not apparent in this study, primarily due to the discrepancies between nominal and measured concentrations. Some of the issues surrounding the discrepancies observed between bulk and nanomaterials in solution evolve from their fundamental chemical and physical properties, including enhanced photo reactivity, their ability to drop out of solution through aggregation and clumping and other chemical peculiarities. Many of these problems are similar to those encountered through routine toxicological testing of difficult substances in the early days of the emergence of ecotoxicology.

Studies with nanomaterials should at least consider the technical aspects of the dosing route and the need to obtain better parity between the nominal and the absolute concentrations.