Dose Metric Conversion Using Silver Nanoparticles

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ABSTRACT

While use of the standard mass dose metric in nanoectoxicology may be used as a matter of convenience, additional consideration needs to be given to alternative dose metrics. In this paper we entered particle diameter, particle concentration and test organism survival from several available ecotoxicology nanosilver datasets into a new web based dose metric conversion tool and determined total particle surface area appeared to better standardize the toxicity of different sized nanosilver particles relative to total mass concentration and particle number concentration options.

Keywords: nanosilver, dose metric, surface area, particle number, toxicity

1 INTRODUCTION

While the aerosol science literature has considered alternative dose metrics to mass-concentration [1], mass is the primary dose metric applied in the nano(eco)toxicology literature [2]. A few more recent studies [3,4,5] have reported ecotoxicity in terms of both mass and surface area. Figure 1 illustrates that total measureable mass fails to account for properties related to surface area and particle number concentration, which become more important with smaller particle diameter. Particle surface area or number may be more relevant than mass and size for representing particle interactions with biological surfaces [6].

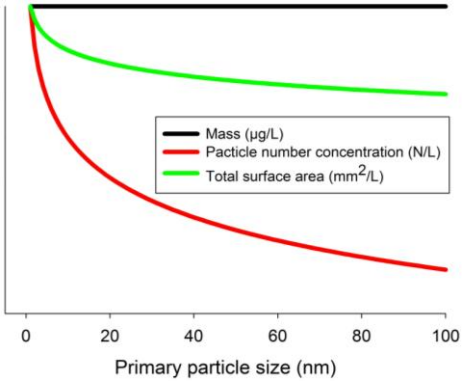


Figure 1. Conceptual illustration of the importance of smaller particle size on number concentration and total surface (at equal mass).

The objective was to use a web-based tool to determine if particle number and surface area were predictive of the aquatic toxicity of nanosilver.

2 MATERIALS AND METHODS

2.1 Particle parameters

Concentration and particle diameter data from both published [4,5] and unpublished datasets were converted to visually compare how toxicity relationships were expressed by different dose metrics. Generally, the data from these studies were generated using silver nanoparticles (10, 20, 30, 50, 60 and 100 nm) with citrate or PVP coatings that were obtained from a commerical source (NanoComposix, San Diego, CA) and characterized for primary particle diameter by transmission electron microscopy (TEM) and hydrodynamic diameter by Field Flow Fractionation (FFF; PostNova F-1000 symmetrical flow FFF, Salt Lake City, UT, USA) and dynamic light scattering (DLS; 635 nm laser; 90 Plus/BI-MAS, Brookhaven Instruments, Holtsville, NY, USA). Total particle concentration, including dissolved mass, was determined by ICP-MS (Elan DRC-II, Perkin-Elmer, Waltham, MA, USA). Dissolved concentrations were functionally determined by ultracentrifugation, as described previously [5].

2.2 Ecotoxicity data generation

Ecotoxicology data from the above studies were generated using standard 48-hour, acute lethality bioassays using water fleas (*Ceriodaphnia dubia*, *Daphnia magna*) or the fathead minnow (*Pimephales promelas*) in accordance with the U.S. Environmental Protection Agency standard methods for discharges to surface waters [7]. The number of individuals alive versus immobilized was assessed 24 and 48-h into the exposure.

2.3 Dose metric conversions

Particle number concentration and total surface area were calculated using equations we published previously [2]. These equations use measured particle diameter and concentration as inputs and are summized below.

$$\text{Particle Number Concentration (N)} = \frac{c_t}{V_{NP}} \bullet \frac{1}{\rho_b} \quad (1)$$

$$\text{Total Surface Area (TSA)} = N \bullet 4\pi R^2 \quad (2)$$

where c_t = total mass concentration, ρ_b = bulk density, R = particle radius, V_{NP} = volume of nanoparticle

Using these equations, we then developed a Dose Metric Tool that is as part of the NanoExPERT tool suite, which is available for free at http://el.erdc.usace.army.mil/nano/. The applicability of the different dose metrics were visualized within the webtool using datasets from two from previous studies [4,5] and one Engineer Research and Development Center (ERDC) unpublished dataset. Both particle coatings in the ERDC dataset were graphed together based on the particle size input. Each dose metric was compared by determining if dose response curves for different size particles overlapped and if the coefficient of variation (CV) between lethal median effect values, such as lethal median concentration (LC50) values, was reduced. For ease of demonstration, the particle concentration measured at test initiation and the particle size measured in the working stocks were entered into NanoExPERT. It should be recognized that it may be more appropriate to use weighted average values for dynamic particle dispersions in which suspended particle concentration or agglomerate size change substantially during bioassays.

3 RESULTS

3.1 Particle characterization

The measured primary particle diameter (by TEM) of the nanosilver particles were similar to nominal diameter in Kennedy et al [5] and in the unpublished ERDC data set, with a few exceptions. The measured sizes of the 10, 20, 30, 60 and 100 nm citrate nanosilver particles were 9, 19, 18, 39 and 102 nm, respectively. Thus, the 30 nm particle was smaller than expected. The measured sizes of the 10, 20, 30, 60 and 100 nm PVP nanosilver particles were 32, 21, 31, 70 and 122 nm, respectively. Thus, the measured size of the 10 nm particle was considerably larger than expected. This conclusion for the 10 nm particle was confirmed by measurement of hydrodynamic diameter (27 - 47 nm). The hydrodynamic diameter was generally slightly larger than primary particle size, as expected, and larger for PVP relative to citrate coated particles.

3.2 Toxicity data used as inputs

In the unpublished ERDC dataset, Total silver as measured by ICP-MS indicated that concentrations were relatively stable in the water column during the first 24-hours of the exposures, when the majority of toxicity was observed (data not presented). Based on mass concentration derived LC50 values, toxicity was strongly negatively correlated with the measured diameter of the citrate and PVP nanosilver particles in both the to *C. dubia* ($R \geq 0.96$) and the *P. promelas* ($R \geq 0.88$) exposures.

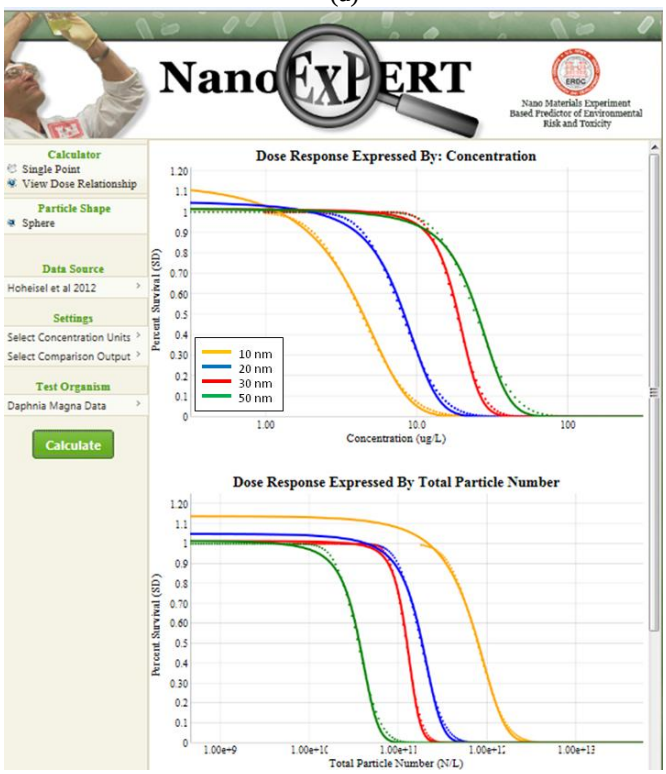
3.3 Dose metric conversions

Dose response curves from Hoheisel et al [4], Kennedy et al [5] and the in-house data set were visualized in NanoExPERT using the measured particle diameter and concentration at test initiation. When expressed as total mass concentration, dose response curves for the different sized particles in both the Hoheisel et al [4] (Figure 2a), unpublished ERDC (Figure 2b) and Kennedy et al [5] data sets clearly did not overlap, with smaller sized particles generally plotted to the left of larger particles indicating greater toxicity. When expressed as particle number concentration, dose response curves for the smaller and larger nanosilver particles reversed relative to the concentration plots for both the Hoheisel et al [4] (Figure 2a) and the ERDC (Figure 2b) data sets. This indicated particle number concentration over-predicted the toxicity of the larger particles. However, for both the Hoheisel et al [4] (Figure 3a), ERDC (Figure 3b) and Kennedy et al [5] (Figure 4) data sets, total surface area resulted in the most similar dose response curves, with the different particle sizes nearly overlapping. In addition, the range in CVs between the graphically determined median effects values of the different nanosilver sizes for the surface area dose metric (30 – 74%) were generally less than the CV between median effect values for the different particle sizes for total mass (54 – 91%). While not perfect, the lower CVs between median effect values generated from the surface area dose metric indicates that surface area reduced the variability in toxicity expression between different sized particles relative to mass and thus more consistently described toxicity across the various particle diameters evaluated. In the ERDC dataset, when total surface area was calculated from hydrodynamic diameter, the coefficient of variation between median effect values further decreased (25 – 66%). The applicability of comparing median effect values using the CV approach is more clearly illustrated by entering data from Kennedy et al [5] into the user entered data function of NanoExPERT (Figure 4); the smaller CV and overlapping dose response curves for the surface area dose metric clearly indicated an improvement over mass dosimetry for nanosilver. It was also previosly found that the surface area dose metric had greater efficacy than mass for different shaped CuO nanoparticles [8].

4 CONCLUSION

Total particle surface area converted by NanoExPERT from measured particle diameter and mass better described the toxicity of silver and CuO nanoparticles as a dose metric than mass or particle number concentration.

(a)



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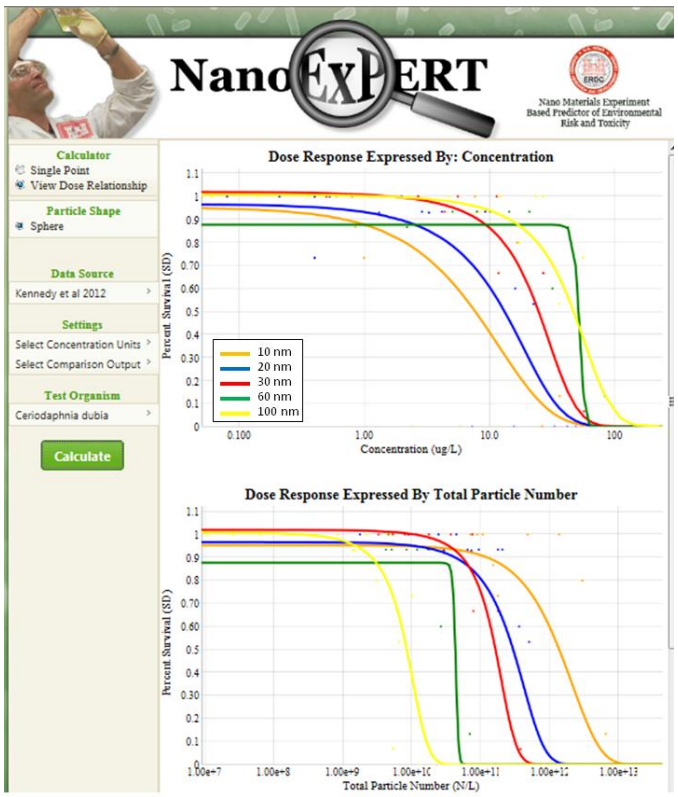
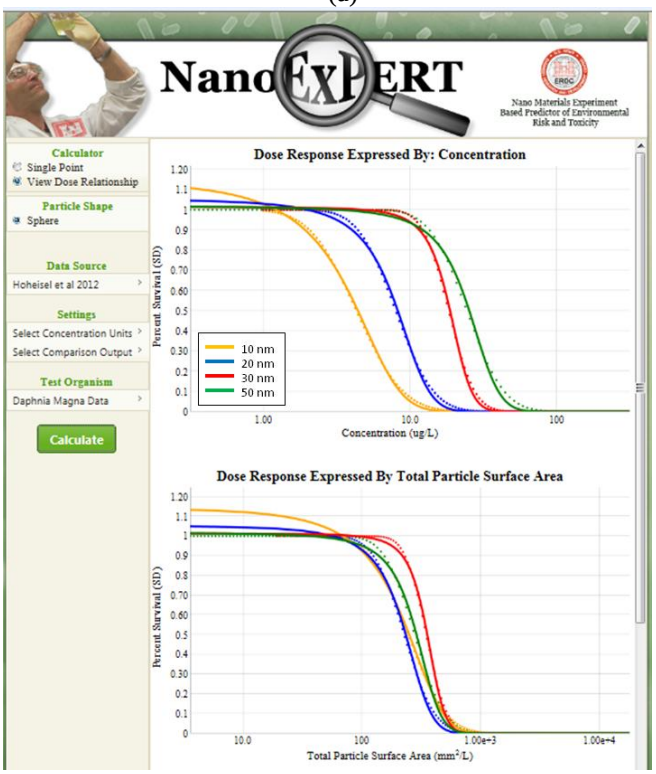


Figure 2. Comparison of total mass and particle number concentration dose metrics using data from (a) Hoheisel et al [4] and (b) ERDC data sets.

(a)



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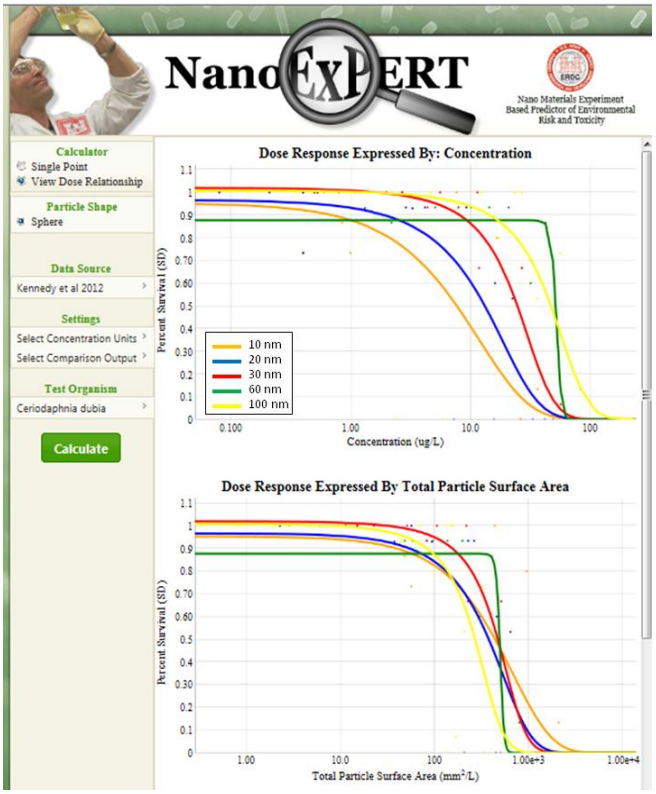

Figure 3. Comparison of total mass and total particle surface area dose metrics using data from (a) Hoheisel et al [4] and (b) the ERDC data sets.

In the case of silver and CuO nanoparticles, the surface area dose metric may have better described the observed toxicity because total exposed surface area provides a larger relative area for reactivity, oxidation and ion release. Previous studies [4,5,6,8] have suggested ion release to be related to particle size, surface area and the toxicity of certain metal nanoparticles including silver and CuO.

In a typical exposure study of soluble metal nanoparticles, quantities of silver or Cu ions outnumber the particles by several orders of magnitude. This likely relates to the total exposed surface area of the suspended particles. However, particle number may provide a relevant dose metric for particles that are less prone to dissolve, such as nano-gold [9]. In absence of a clear relationships between individual dose-metrics and toxicity, it may be most expedient to convert particle size and mass concentration to a range of dose metrics and visualize the toxicity expression using comparative data visualization tools such as NanoExPERT to determine the best fit. It must be caveated that such conversions are only as good as the available particle characterization information. Further, uncertainty is increased by large particle size distributions and heterodispersity.

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Figure 4. User entered data option in NanoExPERT illustrating the improved toxicity metric by total surface area calculated from entered size and mass. The table provides coefficients of variation between median effect values generated from the two dose metrics. Data entered are from Kennedy et al [5].