Assessing the potential risks to zebrafish posed by environmentally relevant copper and silver nanoparticles

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ABSTRACT

The manufacture of large quantities of engineered nanomaterials (NMs) may lead to unintended contamination of aquatic ecosystems. Biologically based monitoring techniques need to be developed to detect these emerging NMs. The purpose of this study was to develop a risk-based probability model to predict the potential hazards of nanoecotoxicity toward aquatic organisms posed by waterborne copper and silver nanoparticles (Cu/Ag NPs). Published experimental evidence based on Cu/Ag NP-zebrafish (Danio rerio) systems was adopted as the study data. A Hill model was used to reconstruct a concentration–mortality response profile. A cumulative Weibull predictive model was employed to estimate exposure thresholds. The derived probabilistic model can predict the potential risk of environmentally relevant Cu/Ag NPs for major Taiwanese rivers with predicted environmental concentrations of 0.06 (95% confidence interval (CI): 0.01–0.92) mg L\(^{-1}\) for Cu NPs and 0.04 (0.01–0.11) mg L\(^{-1}\) for Ag NPs. The results indicated that estimated thresholds were 0.10–0.48 mg L\(^{-1}\) (95% CI) for Cu NPs and 2.69–7.33 mg L\(^{-1}\) for Ag NPs. The probabilities of a risk quotient (RQ) of > 1 ranged 17%–81% for zebrafish exposed to Cu NPs. This study found that Ag NP exposure scenarios posed no significant risks to zebrafish (RQ < 0.1).

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1. Introduction

The increasing use of engineered nanomaterials (NMs) in numerous industrial applications and consumer products necessitates performing risk assessments for human health and the environment (Mueller and Nowack, 2008; Schrand et al., 2010). NMs are a diverse class of small-scale (<100-nm) substances formed by molecular-level engineering to achieve unique mechanical, optical, electrical, and magnetic properties. Most toxicity studies focused on human health, and the environmental aspects are largely unexplored. Assessing the potential entry into and hazards of engineered NMs to ecosystems in order to ensure their safe use and handling are topics of great interest to environmental toxicologists, chemists, and social scientists (Auffan et al., 2009; Kabru and Dubourguier, 2010). Some studies showed that fullerences, carbon nanotubes, and various metal and metal oxide nanoparticles (NPs) can affect the physiology of different aquatic organisms such as fish (Griffitt et al., 2007, 2008; Asharani et al., 2008), algae (Baun et al., 2008), and water fleas (Lovern et al., 2007).

Nanomaterials of copper (Cu) and silver (Ag) are used in many consumer applications, mostly because of their well-demonstrated and safe use as antimicrobial agents (Lok et al., 2007). Cu/Ag NPs in the sub-50-nm range exhibit increased efficiency in inhibiting a wide range of bacteria and fungi. Although Cu/Ag NPs are already widely found in multiple products, a concrete assessment of their environmental implications is lacking. Yoon et al. (2007) indicated that Cu/Ag NPs can inhibit the activities of bacteria, including Escherichia coli and Bacillus subtilis. Antimicrobial characteristics and acute toxicities to aquatic organisms of Cu/Ag NPs were reported (Griffitt et al., 2007; Asharani et al., 2008; Schrand et al., 2010).

Several studies (Griffitt et al., 2007; Asharani et al., 2008) indicated that Cu NPs caused slightly less mortality to zebrafish (Danio rerio) than did soluble Cu, yet Ag NPs showed an adverse response (toward zebrafish embryos) compared to Ag\(^{+}\) ions. A dose-dependent relation with zebrafish mortality was also found (Griffitt et al., 2007; Asharani et al., 2008). In fact, a mortality effect and also non-mortality effects (e.g., oxidative stress, gill injury, heart rate, hatch rate, edema, and malformations) were found and addressed (Griffitt et al., 2007; Asharani et al., 2008). Based on toxicity tests of Ag NPs, chronic exposure to Ag NPs can cause thickening of epithelia gill tissue and alter gene expression profiles to adult and juvenile sheepshead minnows (Cyprinodon variegatus) (Griffitt et al., 2012). Laban et al. (2010) indicated that Ag NPs had more influence on fish in early life stage and can cause mortality for fathead minnow (Pimephales promelas) embryos. However, Bilberg et al. (2010) found that Ag NPs can reduce the diffusion conductance of gill for Eurasian perch
(Perca fluviatilis) which then leads to internal hypoxia during low water oxygen tensions.

Ag NPs are extensively used in detergents and wound dressings due to their antibacterial properties (Yoon et al., 2007). Cu NPs are also widely used as bactericides for air and liquid filtration, as coatings on integrated circuits and batteries, and to increase the thermal and electrical conductivity of coatings and sealants (Gioffredi et al., 2005). These diverse applications of NMs such as antibacterial coatings, in fuel cells, for water electrolysis, and as biomedical imaging contrast agents will very likely result in release to aquatic environments (Mueller and Nowack, 2008; Schrand et al., 2010). Possibly, NPs associating with naturally occurring colloids and particles should be considered together to determine how this could affect the bioavailability to aquatic animals and uptake into cells and organisms. Uptakes by endocytotic routes is identified as a probable major mechanism of entry into cells; leading potentially to various types of cell toxicity and injury (Moore, 2006).

Given the likelihood of exposure to metals which are toxic to aquatic species resulting from the release of NMs into aquatic systems, many studies evaluated the potential toxic effects of NMs toward aquatic species. These include the effects of C60 fullerenes on large-mouth bass (Micropterus Salmoides) (Oberdörster, 2004), daphnids (Daphnia magna) (Lovern and Klaper, 2006), and fathead minnow (Pimephales promelas) (Oberdörster et al., 2006). The effects of carbon nanotubes (CNTs) (Smith et al., 2007) and titanium dioxide (TiO2) NPs or nTiO2 on rainbow trout (Oncorhynchus mykiss) (Federici et al., 2007) and on D. magna (Hund-Rinke and Simon, 2006) were also studied. Those studies identified significant toxicities for NPs. Griffith et al. (2008) used zebrafish, daphnia, and algal (Pseudokirchneriella subcapitata) species as the test organisms in a mortality study with 48 h of exposure to Ag, Cu, aluminum, nickel, cobalt, and TiO2 NPs, and indicated that Cu/Ag NPs were strongly toxic to all aquatic organisms.

Mueller and Nowack (2008) carried out a quantitative risk assessment of engineered NPs in the environment. They showed that the predicted environmental concentrations of carbon nanotubes and Ag NPs in the environment posed little risk, whereas the effects of nTiO2 might be alarming in water bodies. Morgan (2005) developed a preliminary framework for performing risk assessment and management of the ecological and human-health risks of exposure to NPs.

Meanwhile, the major factors affecting the risks of environmental impacts, such as particle-related characteristics, surface chemistry, the presence of NPs, the uptake capacity, transport, fate, and toxic effects, were considered. Baun et al. (2008) also presented an integrated framework for human health and ecological risk assessments of ecotoxicity of engineered nanoparticles based on risk assessment protocols proposed by the USEPA (1992).

The objectives of this study were fourfold: (1) to obtain dose-response profiles based on published laboratory zebrafish exposure experiments by applying the Hill model, (2) to reanalyze acceptable levels of Cu/Ag NPs by employing the Weibull cumulative threshold model based on data of lethal concentrations yielding 10% mortality (LC10) to zebrafish, (3) to estimate the risk quotient (RQ) associated with uncertainties for possible exposure scenarios to Cu/Ag NPs in Taiwanese rivers using a probabilistic risk assessment, and (4) to integrate a risk-based framework to show that zebrafish can be a suitable bioindicator for monitoring the nanotoxicity of Cu/Ag NPs in aquatic ecosystems.

2. Materials and methods

2.1. Study data

Study data related to relationships between concentrations of Cu/Ag NPs and the mortality of zebrafish were obtained from recently published studies (Griffith et al., 2007, 2008; Asharani et al., 2008), which provided suitable datasets to reconstruct and validate the present model. Two datasets designated respectively as CuNP-G07 and CuNP-G08, adopted from Griffith et al. (2007, 2008), were used for the Cu NP-zebrafish system. For the Ag NP-zebrafish system, study data were adopted from Asharani et al. (2008) and Griffith et al. (2008) and are referred to as AgNP-A08 and AgNP-G08, respectively.

2.2. Effect analysis

Concentration–mortality profiles were reconstructed by fitting the Hill model to the published data of mortality bioassays for zebrafish exposed to Cu/Ag NPs:

\[ M(C) = \frac{M_{\text{max}}}{1 + (LC50/C)^{\beta}} \]

where \( M_{\text{max}} \) is the maximum measured response, \( M(C) \) is the mortality rate for a specific Cu/Ag NPs concentration, \( C \) (mg L\(^{-1}\)), \( LC_{50} \) is the 50% lethal concentration of Cu/Ag NPs (mg L\(^{-1}\)), and \( n \) is the fitted Hill coefficient. \( n = 1 \) represents a linear response at low concentration, \( n > 1 \) represents a sublinear (sigmoidal) response indicating a positive cooperativity, and \( n < 1 \) represents a supralinear response.

To protect the top of predators and other organisms living in aquatic environments, conservative LC\(_{10}\) values representing 10% mortality when exposed to waterborne nanomaterials were considered. The LC\(_{10}\) data adopted from the model fitted by Eq. (1) were treated probabilistically. Then, the LC\(_{10}\) cumulative distribution function (CDF) was obtained and can be expressed as

\[ P(M(C) = \Phi(F)\left(\frac{M_{\text{max}}}{1 + (LC10/C)^{\beta}}\right) \]

where \( \Phi(*) \) is the cumulative standard normal distribution.

2.3. Predictive risk threshold

A three-parameter Weibull threshold model was employed to best fit the LC\(_{10}\) CDF toxicity data to estimate the threshold concentration that can be used as a guideline to protect zebrafish from mortality when exposed to waterborne Cu/Ag NP suspensions. The toxicity data can be obtained from the estimated LC\(_{10}\) CDF (Eq. (2)). The Weibull threshold model can be written as

\[ F(C) = \left\{ \begin{array}{ll} 1 - \exp \left( - \left( \frac{C - \gamma}{\alpha} \right)^{\beta} \right), & C > \gamma, \\
0, & C \leq \gamma \end{array} \right. \]

where \( F(C) \) represents the LC\(_{10}\) CDF data corresponding to the specific Cu/Ag NPs concentrations, \( \alpha \) is the scale parameter, \( \beta \) is the shape parameter, and \( \gamma \) is the fitted threshold (mg L\(^{-1}\)). The Weibull shape parameter, \( \alpha \), is known as the Weibull slope. This is because, in the CDF plot, the value of \( \alpha \) is equal to the slope of the line. In addition, the Weibull scale parameter, \( \beta \), has the effect on the distribution as a change of the abscissa scale. If the shape parameter is constant, the CDF has the effect of stretching out with the increase of shape parameter. Per 10th-percentile of the LC\(_{10}\) CDF data points were chosen for fitting by the Weibull threshold model.

2.4. Risk characterization

The mortality risk for zebrafish at a specific Cu/Ag NPs exposure concentration can be calculated by using a joint probability function combining the predicted threshold value (\( \gamma \)) estimated by Eq. (3) as a predicted no-effect concentration (PNEC) and the PEC to define the possible risk with the various exposure...
scenarios. Therefore, a potential hazard risk can be assessed by the risk quotient \( RQ \) which is defined as

\[
RQ = \frac{PEC}{\gamma}.
\]  

(4)

Here \( PEC \) and \( \gamma \) are treated probabilistically. \( RQ > 1 \) implies that there is a potential hazard risk for the selected aquatic environment posed by waterborne Cu/Ag NPs, whereas \( RQ < 1 \) indicates that no significant risk is posed by environmentally relevant waterborne nanometals.

2.5. Uncertainty and sensitivity analysis

TableCurve 2D (vers. 5.01, AISN Software, Mapleton, OR, USA) was used to perform all model fittings. A Monte Carlo (MC) analysis was performed to evaluate the uncertainties in the concentration–mortality relationships and predicted risks. Parameterization and sensitivity analysis of variables in this study were performed using 10,000 MC simulations. The sensitivity of each variable as related to one another was assessed by calculating the rank correlation coefficients between each input and output during the simulations and then estimating each input contribution to the output variance with normalization to 100%. The MC simulation and sensitivity analysis were implemented using Crystal Ball software (vers. 2000.2, Decisioneering, Denver, CO, USA). Fig. 1 illustrates the overall framework of this study.

3. Results

3.1. Concentration–mortality assessment

Fig. 2 shows the particle size-specific concentration–mortality relationships in the Cu NP-zebrafish system. In scenario CuNP-G07, the

Fig. 1. Framework and computational algorithms used in this study.

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Based on Fig. 2a, the LC10 was also estimated to be 0.3 (95% CI: 0.1–0.5) mg L⁻¹, with a geometric standard deviation (gsd) of 1.51, denoted as LN(69.7 nm, 1.51) (Fig. 3d). The function of LN(10.85 nm, 1.42) best described the particle size distribution of the Cu NPs suspension (Fig. 2b) and the corresponding size distribution of the Cu NPs suspensions for the Cu NPs-G07 and Cu NPs-G08 scenarios. Compared to the original particle sizes (Table 1), the LC50 values were estimated to be 7.23 (95% CI: 5.12–9.34) mg L⁻¹ and 1.54, respectively, with r² = 0.97 (Fig. 3c). The final particle size distribution of Ag NPs best fit LN(47.93 nm, 2.01) (Fig. 3d). The results indicated that the aggregation occurred in Ag NPs scenarios.

For the AgNP-G08 scenario, the fitted LC50, LC10, and n values were estimated to be 2.81 (95% CI: 2.79–2.83) mg L⁻¹, 5.95 (3.27–5.95) mg L⁻¹, and 1.54, respectively. The LC10 was also estimated to be 0.3 (95% CI: 0.1–0.5) mg L⁻¹, with a geometric standard deviation (gsd) of 1.28.

The best fit of the reconstructed concentration–mortality profile (Fig. 2c) and the corresponding size distribution of Cu NPs suspensions (Fig. 2d) were well fitted (r² = 0.99). The fitted LC90 value was estimated to be 1.68 (95% CI: 0.54–2.81) mg L⁻¹ with a fitted Hill coefficient (n) of 1.28. Based on Fig. 2a, the LC10 was also estimated to be 0.3 (95% CI: 0.1–0.5) mg L⁻¹. The size distribution of the Cu NPs suspensions was optimally fitted as a lognormal (LN) function with a geometric mean (gm) of 69.7 nm and a geometric standard deviation (gsd) of 1.51, denoted as LN(69.7 nm, 1.51).

The best fit of the reconstructed concentration–mortality profile (Fig. 2a) and corresponding size distribution of the Cu NPs suspension (Fig. 2b) were well fitted (r² = 0.96). The fitted LC90 value was estimated to be 1.68 (95% CI: 0.54–2.81) mg L⁻¹ with a fitted Hill coefficient (n) of 1.28. Based on Fig. 2a, the LC10 was also estimated to be 0.3 (95% CI: 0.1–0.5) mg L⁻¹. The size distribution of the Cu NPs suspensions was optimally fitted as a lognormal (LN) function with a geometric mean (gm) of 69.7 nm and a geometric standard deviation (gsd) of 1.51, denoted as LN(69.7 nm, 1.51).

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Figure 2. (A, C) Reconstructed concentration–mortality profiles and (B, D) corresponding size distributions of Cu nanoparticle (NP) suspensions for the Cu NPs-G07 and Cu NPs-G08 scenarios. Open circles are the size distribution of Cu NP suspension data, whereas open squares are the concentration–mortality data in the model scenarios. Solid and dashed lines are the fitted models and their 95% confidence intervals, respectively.

Figure 3. (A, C) Reconstructed concentration–mortality profiles and (B, D) corresponding size distributions of Ag nanoparticle (NP) suspensions for the Ag NPs-A08 and Ag NPs-G08 scenarios. Open circles are the size distribution of Ag NP suspension data, whereas open squares are the concentration–mortality data in the model scenarios. Solid and dashed lines are the fitted models and their 95% confidence intervals, respectively.

Table 1

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Particle size distribution</th>
<th>Fitted Hill model parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuNP-G07</td>
<td>LN(69.7 nm, 1.51)</td>
<td>LC90 = 1.28; r² = 0.96</td>
</tr>
<tr>
<td></td>
<td>(Original: 80 nm)</td>
<td>LC50 = 1.68 (0.54–2.81) mg L⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC10 = 0.3 (0.1–0.5) mg L⁻¹</td>
</tr>
<tr>
<td>CuNP-G08</td>
<td>LN(90.68 nm, 1.23)</td>
<td>n = 5.96; r² = 0.99</td>
</tr>
<tr>
<td></td>
<td>(Original: 15–45 nm)</td>
<td>LC50 = 1.02 (0.79–1.24) mg L⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC10 = 0.7 (0.55–0.86) mg L⁻¹</td>
</tr>
<tr>
<td>AgNP-A08</td>
<td>LN(10.85 nm, 1.42)</td>
<td>n = 1.54; r² = 0.97</td>
</tr>
<tr>
<td></td>
<td>(Original: 5–20 nm)</td>
<td>LC50 = 26.2 (13.79–38.59) mg L⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC10 = 0.62 (3.20–9.23) mg L⁻¹</td>
</tr>
<tr>
<td>AgNP-G08</td>
<td>LN(47.93 nm, 2.01)</td>
<td>n = 4.88; r² = 0.95</td>
</tr>
<tr>
<td></td>
<td>(Original: 20–30 nm)</td>
<td>LC50 = 7.23 (5.12–9.34) mg L⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC10 = 4.61 (3.27–5.95) mg L⁻¹</td>
</tr>
</tbody>
</table>

a Scenarios CuNP-G07 and CuNP-G08 are data from Griffitt et al. (2007) and Griffitt et al. (2008), respectively, whereas AgNP-A08 and AgNP-G08 are data from Asharani et al. (2008) and Griffitt et al. (2008), respectively.
b LN(gm, gsd) denotes lognormal distribution with a geometric mean (gm) and a geometric standard deviation (gsd). c 95% confidence interval.
suspensions compared to the original Ag NPs that ranged 20–30 nm (Table 1).

3.2. Threshold estimation

To determine thresholds under different exposure scenarios, LC\(_{10}\) values were appropriately log-transformed. The data points representing the relationships between the probability of causing 10% mortality and Cu/Ag NPs concentrations could then be extracted from the LC\(_{10}\) CDFs for four exposure scenarios (Table 2). The median value and the ranges of the 10th and 100th percentiles were estimated to be 0.33 and 0.09–0.62 mg L\(^{-1}\) for the CuNP-G07 scenario, whereas those for CuNP-G08 were estimated to be 0.69 and 0.48–0.96 mg L\(^{-1}\), respectively. For the AgNP-A08 and AgNP-G08 scenarios, medium values of LC\(_{10}\) were estimated to be 6.38 and 4.33 mg L\(^{-1}\) with their corresponding ranges of 2.69–11.0 and 2.73–6.32 mg L\(^{-1}\), respectively.

The Weibull threshold models were best fitted to the LC\(_{10}\) data for four exposure scenarios \((r^2 = 0.99)\) (Fig. 4). The fitted thresholds, \(\gamma\), were estimated to be 0.08 ± 0.02 (mean ± se), 0.48 ± 0.03, 2.69 ± 0.54, and 2.73 ± 0.19 mg L\(^{-1}\) for the respective CuNP-G07, CuNP-G08, AgNP-A08, and AgNP-G08 scenarios. Results indicated that the fitted thresholds, \(\gamma\), that caused 10% mortality of zebrafish for Cu NPs were 6-fold different (Fig. 4a, b), whereas no significant difference in Ag NPs exposure scenarios was found (Fig. 4c, d).

3.3. PEC determination

Measurement data of environmentally relevant waterborne Cu/Ag NPs concentrations are limited and scarce. To overcome this predicament, a partition coefficient \((k_{sp})\) between soluble and nanoparticulate materials can be used to estimate nanoparticulate concentrations of waterborne Cu/Ag NPs. Here the estimated nanoparticulate Cu/Ag NPs in water was calculated by dividing the measured soluble Cu/Ag concentrations by \(k_{sp}\), then performing 10,000 iterative MC simulations to generate median and 95% CI values (Table 3).

Recently determined soluble Cu and Ag concentrations in major Taiwanese rivers were adopted based on Taiwan EPA data (TEPA, 2011), because these NPs released by nano-product manufacturing industries into aquatic environments might form suspensions, aggregates, or sediments. For Cu NPs, the final suspended particle size in water showed an increasing trend with a smaller original size \((2.69–11.0)\) representing the relationships between the probability of causing 10% mortality and Cu/Ag nanoparticle (NP) concentrations extracted from LC\(_{10}\) cumulative distribution functions for different model scenarios.

### Table 2

<table>
<thead>
<tr>
<th>Percentile</th>
<th>CuNP-G07</th>
<th>CuNP-G08</th>
<th>AgNP-A08</th>
<th>AgNP-G08</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.09</td>
<td>0.48</td>
<td>2.69</td>
<td>2.73</td>
</tr>
<tr>
<td>20</td>
<td>0.15</td>
<td>0.53</td>
<td>3.61</td>
<td>3.13</td>
</tr>
<tr>
<td>30</td>
<td>0.21</td>
<td>0.59</td>
<td>4.54</td>
<td>3.53</td>
</tr>
<tr>
<td>40</td>
<td>0.27</td>
<td>0.64</td>
<td>5.46</td>
<td>3.93</td>
</tr>
<tr>
<td>50</td>
<td>0.33</td>
<td>0.69</td>
<td>6.38</td>
<td>4.33</td>
</tr>
<tr>
<td>60</td>
<td>0.39</td>
<td>0.75</td>
<td>7.31</td>
<td>4.72</td>
</tr>
<tr>
<td>70</td>
<td>0.45</td>
<td>0.80</td>
<td>8.23</td>
<td>5.12</td>
</tr>
<tr>
<td>80</td>
<td>0.50</td>
<td>0.85</td>
<td>9.16</td>
<td>5.52</td>
</tr>
<tr>
<td>90</td>
<td>0.56</td>
<td>0.91</td>
<td>10.08</td>
<td>5.92</td>
</tr>
<tr>
<td>100</td>
<td>0.62</td>
<td>0.96</td>
<td>11.00</td>
<td>6.32</td>
</tr>
</tbody>
</table>

3.4. Risk characterization

The estimated exceedance risk curves and box-and-whisker plots of RQs for zebrafish exposed to Cu/Ag NPs based on different exposure scenarios in major Taiwanese rivers were obtained by the risk model of RQ = PEC/\(\gamma\) (Fig. 5). The results showed that waterborne Cu NPs may pose a potential risk to zebrafish with an RQ > 1 (Fig. 5a, b). Approximately 81% and 17% probabilities can cause an estimated RQ > 1 under the respective CuNP-G07 and CuNP-G08 scenarios. Thus, the modeling suggests that aquatic organisms may face potential risks if exposed to environmental Cu NPs in major Taiwanese rivers.

No significant risk was posed for environmental exposures in major Taiwanese rivers for the two Ag NP exposure scenarios (Fig. 5c, d). The estimated median RQ values with 95% CI were estimated to be 0.027 (0.003–0.051) and 0.029 (0.003–0.055), respectively. The estimated RQ values were both much less than 1 for the AgNP-A08 and AgNP-G08 scenarios, implying that environmental exposure to Ag NPs is unlikely to pose a potential mortality risk to zebrafish in this study (Fig. 5d).

4. Discussion

4.1. Aquatic organisms exposed to NPs/NMs

Recently, most nanotoxicology experiments on adult and embryo life stages of zebrafish also demonstrated that Cu/Ag NPs can cause acute toxicity to zebrafish (Griffitt et al., 2007; Asharani et al., 2008). Furthermore, toxicological research reports proved that Ag NPs were more lethal to cell-based in vitro systems than were other metal NPs screened (Schrand et al., 2010). In our study, we provided an integrated risk-based framework to evaluate and protect the safety of aquatic organisms exposed to emerging NPs through an analysis of selected available data.

In most review studies, the particle characteristics in aquatic environments were also examined (Baum et al., 2008; Shaw and Handy, 2011), because these NPs released by nano-product manufacturing industries into aquatic environments might form suspensions, aggregates, or sediments. For Cu NPs, the final suspended particle size in water showed an increasing trend with a smaller original size (Table 1). Characteristic LC\(_{50}\) values showed that larger-sized suspended/aggregated NPs could cause more-severe mortality. The modeled LC\(_{10}\) values, however, showed an inverse trend under...
these two selected scenarios. Otherwise, for Ag NPs, the fitted LC50 and LC10 values also showed that larger-sized suspensions/aggregations could cause more-severe mortality. This phenomenon of larger-sized suspensions/aggregations in water causing more-severe effects differs from airborne NPs (Chio et al., 2007; Liao et al., 2009), yet the knowledge gap of the likely reason still needs to be overcome.

It is difficult, if not impossible, to obtain metal NPs data in Taiwan rivers. We used a parsimonious approach by analogizing the partition coefficient (k_{sp}) of soluble to nanoparticle copper and silver (Cu/Ag NPs) from Griffitt et al. (2008) to that of Taiwan situation in this study. Since there had no related data which can correspond to partition coefficient for Cu/Ag NPs, we roughly estimated the possible values from the ratio of soluble metal ion and NPs concentration in natural aquatic system. The predicted coefficients are based on a simple assumption and back calculating method. However, we should make more efforts on this part in future studies. But the simple assumption might be inappropriate or problematic if taking into account the metal solubility, initial solid particle concentration, and several influence factors (Klaine et al., 2008). To date, we did not have available data including above mentioned factors to assess the risk posed by emerging NMs into such complex ecosystems. Therefore, we conducted and treated the partition coefficients from limited data in laboratory experiments (Griffitt et al., 2008). We agreed that the estimates of the partition coefficients of Cu/Ag NPs systems should be validated or revisited. That is because the partition coefficients play important roles in performing consequent risk assessments.

In addition, our study only adopted the zebrafish with Cu/Ag NPs data from previously studies (Griffitt et al., 2007, 2008; Asharani et al., 2008). Therefore, we just focused on a higher trophic level, such as invertebrate zebrafish, to find the important information via our proposed model for implicating human health risk assessment and emission standard regulations. On the contrary, other lower trophic aquatic organisms (such as daphnia), NPs (such as nTiO2), and evaluated approaches (such as species sensitive distribution, SSD) were discussed latter (Posthuma et al., 2002; Holbrook et al., 2008; Auffan et al., 2009; Barrena et al., 2009; Kahrhu and Dubourg, 2010; Savolainen et al., 2010; Shaw and Handy, 2011).

4.2. Modeled threshold levels and water quality criteria

Currently, the water quality criterion (WQC) levels for total Cu and Ag in the workplace and environment are regulated in some developed countries (Purcell and Peters, 1999; USEPA, 2003; Srinivasan et al., 2010; Shaw and Handy, 2011). Therefore, we just focused on a higher trophic level, such as invertebrate zebrafish, to find the important information via our proposed model for implicating human health risk assessment and emission standard regulations. On the contrary, other lower trophic aquatic organisms (such as daphnia), NPs (such as nTiO2), and evaluated approaches (such as species sensitive distribution, SSD) were discussed later (Posthuma et al., 2002; Holbrook et al., 2008; Auffan et al., 2009; Barrena et al., 2009; Kahrhu and Dubourg, 2010; Savolainen et al., 2010; Shaw and Handy, 2011).

4.2. Modeled threshold levels and water quality criteria

Currently, the water quality criterion (WQC) levels for total Cu and Ag in the workplace and environment are regulated in some developed countries (Purcell and Peters, 1999; USEPA, 2003; Srinivasan et al., 2010; Shaw and Handy, 2011). Therefore, we just focused on a higher trophic level, such as invertebrate zebrafish, to find the important information via our proposed model for implicating human health risk assessment and emission standard regulations. On the contrary, other lower trophic aquatic organisms (such as daphnia), NPs (such as nTiO2), and evaluated approaches (such as species sensitive distribution, SSD) were discussed later (Posthuma et al., 2002; Holbrook et al., 2008; Auffan et al., 2009; Barrena et al., 2009; Kahrhu and Dubourg, 2010; Savolainen et al., 2010; Shaw and Handy, 2011).
and Swain, 2007). Fitted threshold levels for Cu NPs were estimated to be 0.08–0.48 mg L$^{-1}$. The USEPA (2003) and Srinivasan and Swain (2007) reported that the present acute and chronic levels of dissolved Cu were 3.1 and 1.9 μg L$^{-1}$, respectively. Results indicated that our evaluated threshold values were about 2 orders of magnitude higher than the present WQC.

For Ag in the workplace, the WQC level ranged 0.01–0.1 mg m$^{-3}$, however they were 0–0.1 and 0.0001–1 mg L$^{-1}$ for Ag in drinking and surface water, respectively. Now, the highest emission level of Ag ranging 0.1–3.0 mg L$^{-1}$ is regulated for sewage treatment discharge (Parcell and Peters, 1999). Our estimated threshold level for Ag NPs was nearly 2.7 mg L$^{-1}$ based on a laboratory evaluation. This quantity is unacceptable for surface waters but is acceptable for sewage treatment.

4.3. Implications for environmental monitoring and risk assessment

Industrial nanoscale products that enter the aquatic environment form suspended particles that are taken up by benthic-dwelling invertebrates through different exposure routes. The routes of NPs uptake into aquatic biota include direct ingestion and entry across gills, olfactory organs, and the body wall. Fish and shellfish, filter-feeding invertebrates, are present in marine and freshwater bodies. Human are exposed through feeding invertebrates, are present in marine and freshwater bodies.

**Fig. 5.** Estimated exceedance probabilistic curves and box-and-whisker plots of risk quotients for zebrafish exposed to (A, B) Cu nanoparticles (NPs) and (C, D) Ag NPs in major Taiwanese rivers based on different model scenarios.

In the present study, we provide a quantitative method to assess the risk to aquatic organisms exposed to emerging NPs. Our models did not take into account the sub-lethal effects of Cu/Ag NPs on zebrafish. Yet a lot of researches carried out Cu/Ag NPs exposure bioassays to assess mortality and sub-lethal effects for aquatic organisms. The endpoints of sub-lethal effect have been investigated for zebrafish posed Cu/Ag NPs such as decreasing heart rate, hatching rate, abnormal development, gill filament and inhibition of branchial Na$^+$/K$^+$-ATPase activity (Griffith et al., 2007, 2008; Asharani et al., 2008). We can also use the above mentioned dose–response data to construct the relationship models between NPs and sub-lethal effect based on our proposed models. The NPs-exposed sub-lethal risk assessment for zebrafish could be obtained by our proposed framework. In the study, we used Weibull model to fit the LC50 CDF data points estimated from toxicity data as the acute mortality risks.

Recently, Zhu et al. (2010) investigated the food chain transfer of nTiO2 from a low trophic level organism (daphnia, *Daphnia magna*) and to a high trophic level organism (zebrafish, *Danio rerio*). The results showed that no biomagnifications of nTiO2 were found from prey zooplankton to predator zebrafish. However, Holbrook et al. (2008), Lewinski et al. (2011), and Werlin et al. (2011) also indicated that the dietary uptake may be a major route of NMs exposure for trophic transfer level in several prey–predator systems. Lewinski et al. (2011) pointed that zebrafish retained 1% of the steady-state CdSe/ZnS NPs doses yet from zooplankton, even though the low absorbed rate. In brief, NPs can transfer from zooplankton through food chain to other high trophic level aquatic organisms. In other lab experimental study, the gold NPs can pass from the water column to the marine food web. However, filter-feeders such as Mercenaria mercenaria may be one of the shellfish for human consumption and have the potential risk entry into the human body by food chain (Ferry et al., 2009).
Fish are renowned for their ability to bioconcentrate trace contaminants in the environment. Human consumption of fish suggests a direct impact on human health by the potential release of NMs into the environment. The zebrafish has attracted much interest as a remarkable animal model for organogenesis and human disease because it has transparent embryos, rapid embryo development, and organs and tissues that are functionally equivalent to those of mammals (Garrity et al., 2002). In the present study, a series of model assessments were applied. The most important elements, however, are useful experimental data from zebrafish-Cu/Ag NPs systems.

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