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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 2.69–2.73 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).

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 and risks 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; Kahru and Dubourguier, 2010). Some studies showed that fullerenes, 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).

Nanometals 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, hatching 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 altering 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

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(*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 antimicrobial 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 (Cioffi et al., 2005). These diverse applications of NMs such as antimicrobial 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. Uptake 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 C_{60} fullerenes on large-mouth bass (*Micropterus Salmoides*) (Oberdöster, 2004), daphnid (*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 (TiO_2) NPs or $nTiO_2$ 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. Griffitt 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 TiO_2 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 $nTiO_2$ 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 (LC_{10}) 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 nanoecotoxicity 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 (Griffitt 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 Griffitt 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 Griffitt 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:

$$M(C) = \frac{M_{\max}}{1+(\mathrm{LC50}/C)^n}; \tag{1}$$

where M_{max} is the maximum measured response, $M(C)$ is the mortality rate for a specific Cu/Ag NPs exposure 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 a low concentration, $n>1$ represents a sublinear (sigmoidal) response indicating a positive cooperatively, and $n<1$ represents a supralinear response.

To protect the health of top predators and other organisms living in aquatic environments, conservative LC_{10} values representing 10% mortality when exposed to waterborne nanometals 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

$$P(M|C) = \Phi\left(\frac{M_{\max}}{1+(\mathrm{LC10}/C)^n}\right), \tag{2}$$

where $\Phi(\bullet)$ 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

$$F(C) = \begin{cases} 1-\exp\left[-\left(\dfrac{C-\gamma}{\alpha}\right)^{\beta}\right], & C>\gamma, \\ 0, & C\le\gamma \end{cases} \tag{3}$$

where $F(C)$ represents the LC_{10} CDF data corresponding to the specific Cu/Ag NPs concentrations, α is the scale parameter, β is the shape parameter, and γ is the fitted threshold (mg L^{-1}). The Weibull shape parameter, α, is known as the Weibull slope. This is because, in the CDF plot, the value of α is equal to the slope of the line. In addition, the Weibull scale parameter, β, 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 (γ) 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

$$RQ = \frac{PEC}{\gamma}. \tag{4}$$

Here PEC and γ 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

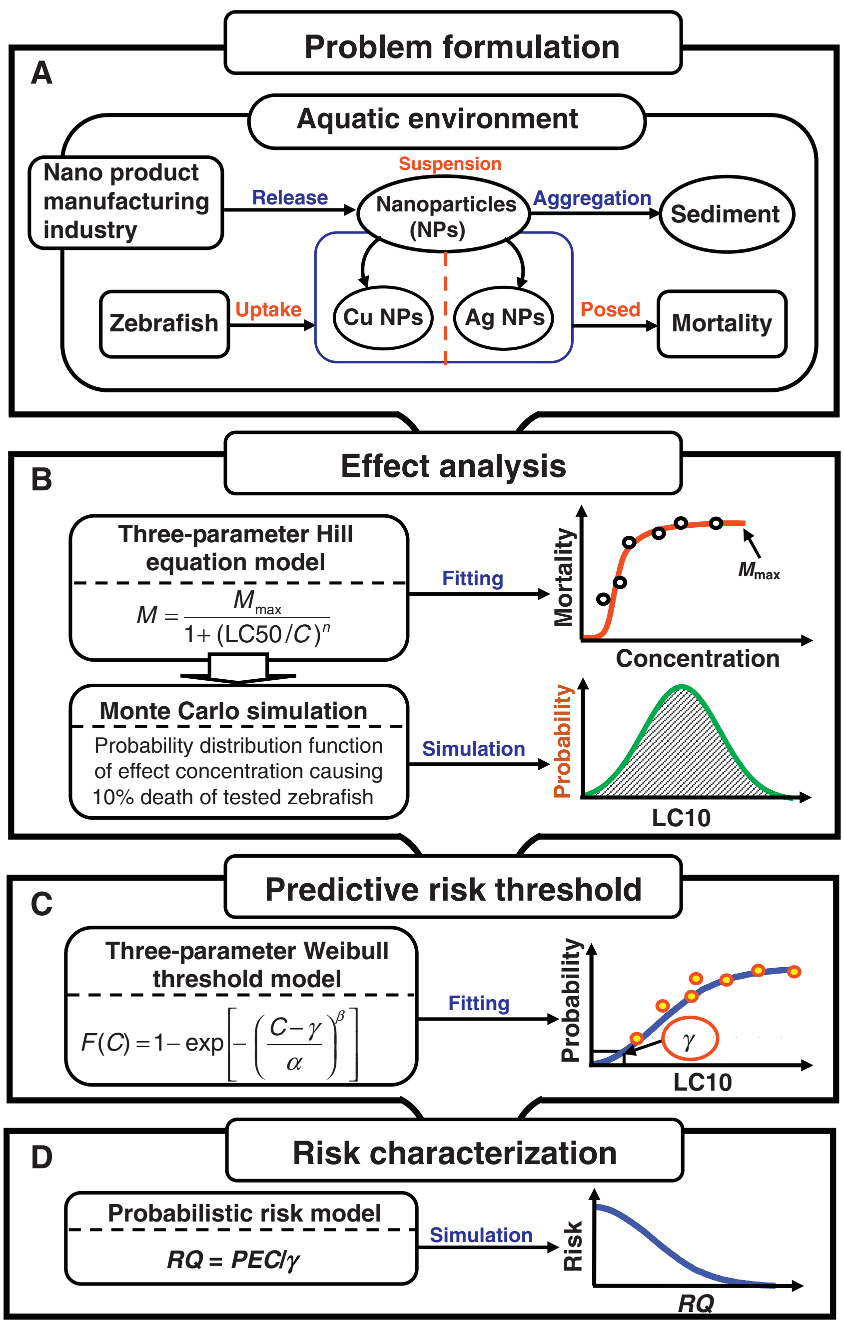

Fig. 1. Framework and computational algorithms used in this study.

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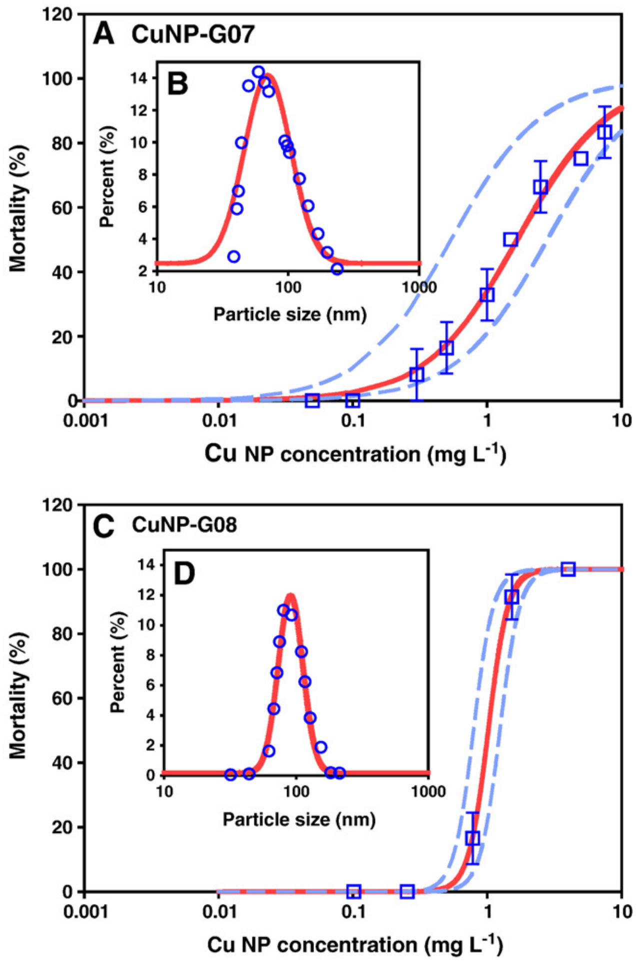

Fig. 2. (A, C) Reconstructed concentration–mortality profiles and (B, D) corresponding size distributions of Cu nanoparticle (NP) suspensions for the Cu NPs-G07 and Cu NPs-G08 scenarios. Open circles are the size distribution of Cu NP suspension data, whereas open squares are the concentration–mortality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

Table 1
Summary of size-dependent Hill model fitted parameters of concentration–mortality profiles for zebrafish (*Danio rerio*) exposed to Cu and Ag nanoparticles (NPs) in different model scenarios.

Scenario[a]	Particle size distribution	Fitted Hill model parameters
CuNP-G07	LN(69.7 nm, 1.51)[b] (Original: 80 nm)	$n=1.28$; $r^2=0.96$ $LC_{50}=1.68$ (0.54–2.81) mg L^{-1}[c] $LC_{10}=0.3$ (0.1–0.5) mg L^{-1}
CuNP-G08	LN(90.68 nm, 1.23) (Original: 15–45 nm)	$n=5.96$; $r^2=0.99$ $LC_{50}=1.02$ (0.79–1.24) mg L^{-1} $LC_{10}=0.7$ (0.55–0.86) mg L^{-1}
AgNP-A08	LN(10.85 nm, 1.42) (Original: 5–20 nm)	$n=1.54$; $r^2=0.97$ $LC_{50}=26.2$ (13.79–38.59) mg L^{-1} $LC_{10}=6.26$ (3.29–9.23) mg L^{-1}
AgNP-G08	LN(47.93 nm, 2.01) (Original: 20–30 nm)	$n=4.88$; $r^2=0.95$ $LC_{50}=7.23$ (5.12–9.34) mg L^{-1} $LC_{10}=4.61$ (3.27–5.95) mg L^{-1}

[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.

reconstructed concentration–mortality profile (Fig. 2a) and corresponding size distribution of the Cu NPs suspension (Fig. 2b) were well fitted ($r^2=0.96$). The fitted LC_{50} 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 LC_{10} 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. 2c) and the corresponding size distribution of Cu NPs suspensions (Fig. 2d) were also found for the CuNP-G08 scenario ($r^2=0.99$). The fitted LC_{50} and n were estimated to be 1.02 (95% CI: 0.79–1.24) mg L^{-1} and 5.96, respectively. The fitted LC_{10} was 0.7 (95% CI: 0.55–0.86) mg L^{-1}. The best-fitted particle size distribution was LN(90.68 nm, 1.23). Table 1 summarizes the fitted values for the CuNP-G07 and CuNP-G08 scenarios. Compared to the original particle sizes (Table 1), the results indicate that the aggregation occurred in Cu NPs suspensions.

For the AgNP-A08 scenario, the fitted LC_{50} and n values were estimated to be 26.2 (95% CI: 13.79–38.59) mg L^{-1} and 1.54, respectively, with $r^2=0.97$. The fitted LC_{10} was 6.26 (95% CI: 3.29–9.23) mg L^{-1} (Fig. 3a). The function of LN(10.85 nm, 1.42) best described the particle size distribution (Fig. 3b). Results showed that the final Ag NPs suspensions had similar patterns of the particle size range compared to the original Ag NPs which ranged 5–20 nm (Table 1).

For the AgNP-G08 scenario, the fitted LC_{50}, LC_{10}, and n values were estimated to be 7.23 (95% CI: 5.12–9.34) mg L^{-1}, 4.61 (3.27–5.95) mg L^{-1}, and 1.54, respectively, with $r^2=0.97$ (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

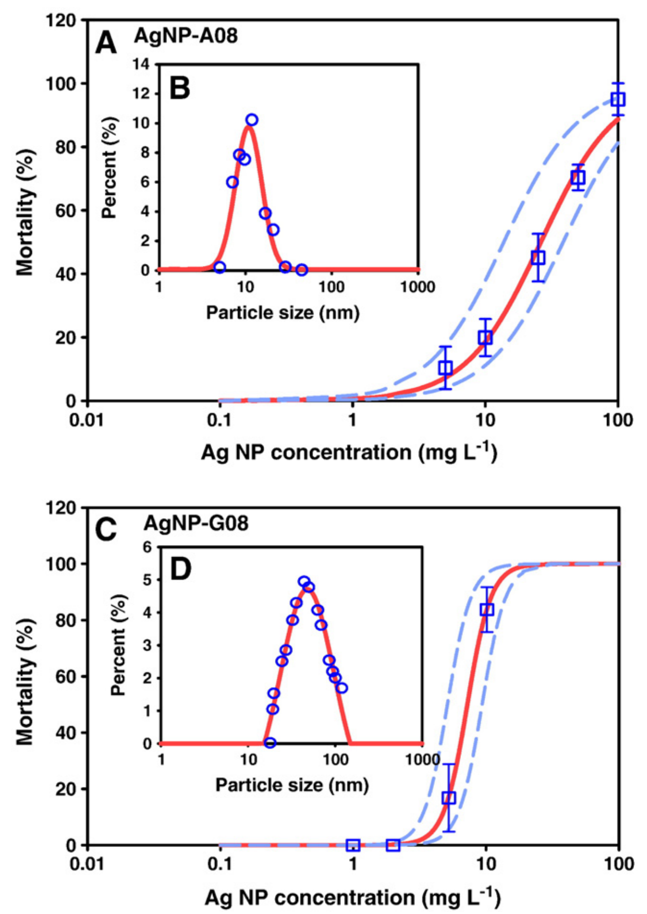

Fig. 3. (A, C) Reconstructed concentration–mortality profiles and (B, D) corresponding size distributions of Ag nanoparticle (NP) suspensions for the Ag NPs-A08 and Ag NPs-G08 scenarios. Open circles are the size distribution of Ag NP suspension data, whereas open squares are the concentration–mortality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

Table 2
Per 10th-percentile data points (mg L^{-1}) 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.

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

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, γ, 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, γ, 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, 2008). The partition coefficients (k_{sp}) for Cu/Ag NPs were adopted from Griffitt et al. (2008). Based on the laboratory-based study, k_{sp} values were estimated to be 0.213 and 0.084 for Cu and Ag nanometals, respectively. Therefore, to determine the PEC of Cu/Ag NPs, the original monitoring dataset (with the minimum, median, and maximum values for soluble Cu/Ag) were converted into the most likely environmentally relevant concentrations of Cu/Ag NPs with median and 95% CI values via k_{sp}.

For the soluble Cu concentration, the median values monitored from major Taiwanese rivers located in five regions of Taipei, Hsinchu, Taichung, Tainan, and Kaohsiung, ranged 0.002–0.01 mg L^{-1}. The median Cu NP PEC value with the 95% CI was estimated to be 0.06 (95% CI: 0.01–0.92) mg L^{-1} for all of Taiwan (Table 3). On the other hand, the Ag NP PEC value was estimated to be 0.04 (95% CI: 0.01–0.11) mg L^{-1} based on measured Ag soluble concentrations ranging 0.001–0.011 mg L^{-1} (Table 3).

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 organism exposed to emerging NPs through an analysis of selected available data.

In most review studies, the particle characteristics in aquatic environment were also examined (Baun et al., 2008; Shaw and Handy, 2011), because these NPs released by nano-product manufacturing industries into aquatic environments might form suspensions, aggregations, 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

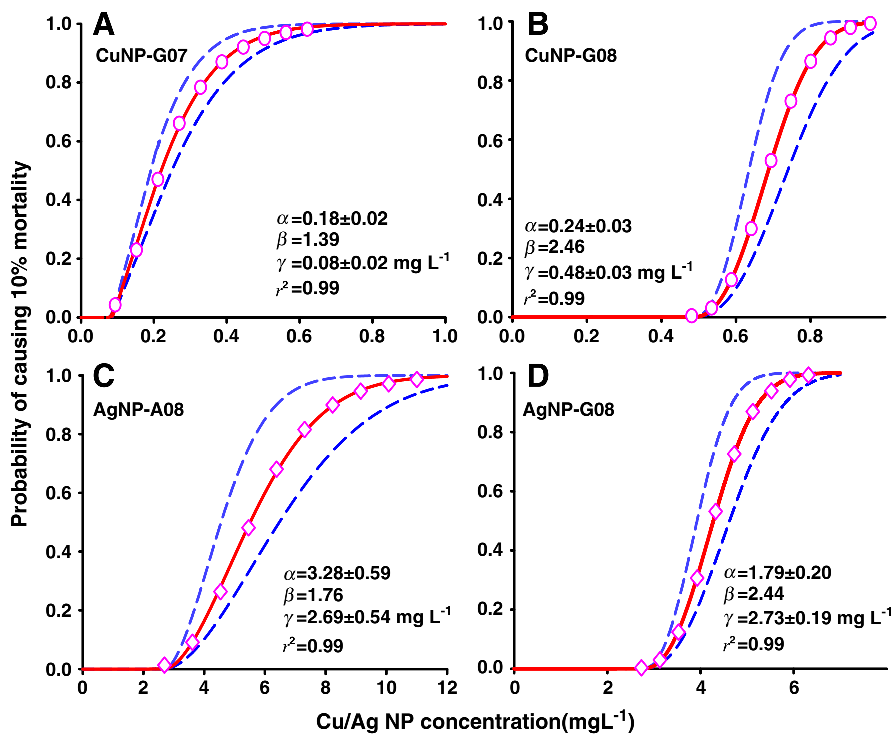

Fig. 4. The best fit of the Weibull threshold model to the data adopted from the 10% lethal concentration (LC_{10})-derived cumulative distribution functions for zebrafish exposed to (A, B) Cu nanoparticles (NPs) and (C, D) Ag NPs for four study scenarios. LC_{10} denotes the lethal concentration yielding 10% death of zebrafish exposed to waterborne Cu/Ag NPs. Open circles are the extracted Cu NP data, whereas open diamonds are the extracted data for Ag NPs. Solid and dashed lines are fitted models and their 95% confidence intervals, respectively.

these two selected scenarios. Otherwise, for Ag NPs, the fitted LC_{50} and LC_{10} 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 made 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 $nTiO_2$), 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; Kahru and Dubourguier, 2010; Savolainen et al., 2010; Shaw and Handy, 2011).

Table 3
Measured soluble and predicted environmental concentrations (PECs) of nano-Cu and nano-Ag in major Taiwanese rivers.

Selected major river region	Measured soluble concentration[a] (mg L^{-1})			PEC (mg L^{-1})	k_{sp}[b]
	Min	Median	Max	Median (95% confidence interval)	
Nano-Cu					0.213
Taipei (73)[c]	0.001	0.01	0.262	0.08 (0.01–0.80)[d]	
Hsinchu (49)	0.002	0.004	0.160	0.08 (0.01–0.52)	
Taichung (26)	0.001	0.002	0.014	0.02 (0.01–0.06)	
Tainan (75)	0.001	0.006	2.190	0.22 (0.01–5.28)	
Kaohsiung (35)	0.001	0.006	0.036	0.03 (0.01–0.12)	
Taiwan				**0.06 (0.01–0.92)**[e]	
Nano-Ag					0.084
Taiwan (31)	0.001	0.002	0.011	**0.04 (0.01–0.11)**	

Values with bold entries represent the pooled data in Taiwan.
[a] Adopted from Taiwan Environmental Protection Administration (TEPA, 2008).
[b] k_{sn}, partition coefficient that is the ratio of soluble to nanoparticulate Cu/Ag in water (Griffitt et al., 2008).
[c] Available sample sizes.
[d] PEC of nano-Cu/Ag calculated by the soluble concentration divided by k_{sp} factor with 10,000 iterative Monte Carlo simulations to generate the median and 95% confidence interval.
[e] From the PEC of nano-Cu calculated with data from the Taipei, Hsinchu, Taichung, Tainan, and Kaohsiung regions.

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

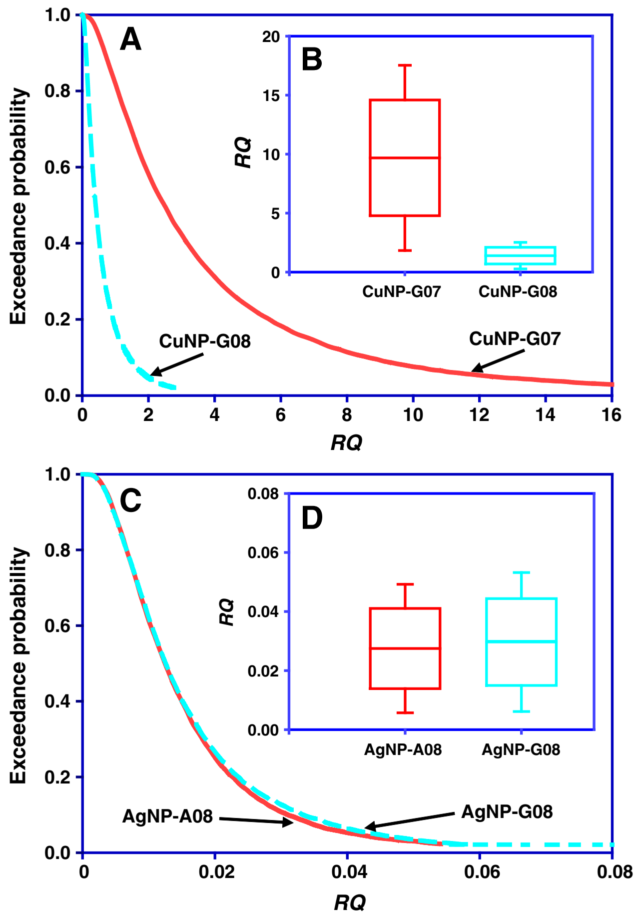

Fig. 5. Estimated exceedance probabilistic curves and box-and-whisker plots of risk quotients for zebrafish exposed to (A, B) Cu nanoparticles (NPs) and (C, D) Ag NPs in major Taiwanese rivers based on different model scenarios.

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 (Purcell 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 fish and drinking water from aquatic systems (Howard, 2004). Thus, fish and other living organisms are relevant test organisms for studies investigating the environmental effects of manufactured NMs. The zebrafish is commonly used as a model organism to test/monitor pollution levels of emerging materials, such as Cu/Ag NPs, nC_{60} and so on, emitted into aquatic environments (Griffitt et al., 2007, 2008; Asharani et al., 2008). In the present study, we provide a quantitative method to assess the risk to aquatic organisms exposed to emerging NPs. Our results support arguments mentioned in much of the qualitative literature.

The SSD approach (Posthuma et al., 2002) was also widely used in ecological risk assessment that was based on the lower confidence interval of the hazardous concentration for 5% of species. This study, however, had lack of information regarding the SSD of aquatic organisms, and therefore we might consider and adapt the idea to estimate ecological risks in our future studies. Recent study (Verschoor et al., 2011) used biotic ligand model to extrapolate the site-specific no observed effect concentrations to construct the SSD based on site-specific no observed effect concentrations. The spatial and temporal variations of water type-specific would affect the SSD and further risk characterization. Hence, we recommended that future research should focus on a more geographical and temporal variations of bioavailability through site-specific SSD evaluation of aquatic species with toxic NMs.

On the other hand, zebrafish may not be a nature species in Taiwan ecosystems. However, zebrafish is a very good model fish to evaluate vertebrate on chemical toxicities or other issues in Taiwan (http://www.zebrafish-nthu-nhri.org/tzcf/). Substantial information had gathered from toxicities of NPs to zebrafish. Therefore, we used the toxicity data from zebrafish exposed to NPs to estimate the possible ecological risk by linking the NPs predicting data in Taiwan rivers.

Griffitt et al. (2008) indicated that metal NPs, with the exception of Cu in zebrafish fry, were less toxic than the soluble forms of metals based on the mass of metal added with each exposure. Griffitt et al. (2007) suggested that existing regulations based on soluble metals may be adequate to protect aquatic life from metal NPs. Griffitt et al. (2007) also concluded that Cu NPs are acutely lethal to zebrafish at much lower concentrations than those illustrated by experiments with TiO_2 particles in *Daphnia* or with CNTs, such as fullerenes in fathead minnows. In our analysis, we found that different NPs can cause different adverse effects. Therefore, we speculated that different model organisms exposed to different NPs might produce more-complex results. These knowledge gaps should be studied and overcome through analyzing more experimental data.

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 (Griffitt et al., 2007, 2008, 2009; 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 LC_{10} CDF data points estimated from toxicity data as the acute mortality risks.

Recently, Zhu et al. (2010) investigated the food chain transfer of $nTiO_2$ 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 $nTiO_2$ 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 mercenariamay* are one of the shellfish for human consumption and have the potential risk entry into the human body by food chain (Ferry et al., 2009).

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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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