Chemosphere 156 (2016) 8–13

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Prediction of joint algal toxicity of nano-CeO_2/nano-TiO_2 and florfenicol: Independent action surpasses concentration addition

Zhuang Wang [a, *], Se Wang [a], Willie J.G.M. Peijnenburg [b, c]

[a] Collaborative Innovation Center of Atmospheric Environment and Equipment Technology (AEET), Jiangsu Key Laboratory of Atmospheric Environment Monitoring and Pollution Control (AEMPC), School of Environmental Science and Engineering, Nanjing University of Information Science and Technology, Nanjing 210044, PR China

[b] Centre for Safety of Substances and Products, National Institute of Public Health and the Environment (RIVM), Bilthoven 3720 BA, The Netherlands

[c] Institute of Environmental Sciences (CML), Leiden University, Leiden 2300 RA, The Netherlands

HIGHLIGHTS

- Ecotoxicological risks associated with $nCeO_2$/$nTiO_2$ were enhanced by concomitant FLO.
- Joint toxicity of $nCeO_2$ and FLO was significantly higher than that of $nTiO_2$ and FLO.
- The IA and CA models underestimate the joint toxicity of $nCeO_2$/$nTiO_2$ and FLO.
- Predictions based on IA performs better than CA for the joint toxicity.

ARTICLE INFO

Article history:
Available online 6 May 2016

Handling Editor: Tamara Galloway

Keywords:
Engineered nanoparticles
Antibiotics
Joint toxicity
Independent action
Concentration addition

ABSTRACT

Co-exposure of aquatic organisms to engineered nanoparticles (ENPs) and antibiotics is likely to take place in the environment. However, the impacts of co-exposure on aquatic organisms are virtually unknown and understanding the joint toxicity of ENPs and antibiotics is a topic of importance. The independent action (IA) model and the concentration addition (CA) model are two of the most common approaches to mixture toxicity assessment. In this study, the joint toxicity of two ENPs ($nCeO_2$ and $nTiO_2$) and one antibiotic (florfenicol, FLO) to *Chlorella pyrenoidosa* was determined to compare the applicability of the IA and the CA model. Concentration-response analyses were performed for single toxicants and for binary mixtures containing FLO and one of the ENPs at two suspended particle concentrations. The effect concentrations and the observed effects of the binary mixtures were compared to the predictions of the joint toxicity. The observed toxicity associated with the $nCeO_2$ or $nTiO_2$ exposure was enhanced by the concomitant FLO exposure. The joint toxicity of $nCeO_2$ and FLO was significantly higher than that of $nTiO_2$ and FLO. Predictions based on the IA and CA models tend to underestimate the overall toxicity (in terms of median effect concentration) of the binary mixtures, but IA performs better than CA, irrespective of the effect level under consideration and the types of mixtures studied. This result underpins the need to consider the effects of mixtures of ENPs and organic chemicals on aquatic organisms, and the practicability of the IA and CA methods in toxicity assessment of ENPs.

1. Introduction

Due to their remarkable characteristics, engineered nanoparticles (ENPs) have been the focus of various industrial and biomedical applications (Nel et al., 2006). A growing number of products containing ENPs emerge in commercial markets and are present in our daily life (Mueller and Nowack, 2008). Of these various nanotechnology products, those composed of metal oxide ENPs such as nano-titanium dioxide ($nTiO_2$) and nano-cerium dioxide ($nCeO_2$) are attractive for a large variety of applications including catalysis, sensors, electronic materials, and environmental remediation (Pan et al., 2009; Van Hoecke et al., 2009). With the increased applications of ENPs, the concerns about their potential human health effects and the environmental hazards also increased (Baker et al., 2014; Farkas et al., 2015; Galloway et al.,

* Corresponding author.
E-mail address: zhuang.wang@nuist.edu.cn (Z. Wang).

http://dx.doi.org/10.1016/j.chemosphere.2016.04.072
0045-6535/

2010; Lowry et al., 2012; Velzeboer et al., 2008; Wang et al., 2015a, 2014, 2012).

Once released into the aquatic environment, ENPs may interact with concomitant organic pollutants inevitably and voluntarily (Baun et al., 2008; Lee et al., 2015; Navarro et al., 2008). Antibiotics are emerging pollutants that are known to be of particular concern as they are frequently detected in surface water (Kümmerer, 2009; Wang et al., 2015b) and it is reasonable to assume that combined exposure to mixtures of ENPs and antibiotics is inevitable in the environment. However, the effects of combined exposure of aquatic organisms by ENPs and concomitant antibiotics are relatively unknown. Therefore, understanding the joint toxicity of ENPs and antibiotics is a topic of importance for the risk assessment and pollution control of both ENPs and antibiotics.

To date, several approaches for the assessment of combined effects of chemical pollutants have been explored (Wang et al., 2009), for which two basic concepts are generally applicable, namely concentration addition (CA) and independent action (IA). CA assumes that all mixture components exert their effects through a similar mode of toxic action (MOA) (Altenburger et al., 2000). In contrast, IA has been employed in computing the toxicity of mixtures when the individual components share dissimilar MOAs (Backhaus et al., 2004). The two methodologies have also been applied to predict the joint toxicity of pollutants in the field of environmental toxicology (Altenburger et al., 2012; González-Pleiter et al., 2013). Based on the current theory, to seek a reliable method with the capability of assessing toxicity of ENPs and organic pollutants is necessary and indispensable. However, the predictive powers of the CA and IA methods have not been verified for mixtures composed of ENPs and organic pollutants.

It is the purpose of the present study to verify whether the IA and CA models are suitable for predicting toxicity of mixtures comprising metal oxide ENPs ($nCeO_2$ or $nTiO_2$) and one antibiotic (florfenicol, FLO). FLO is frequently detected in the aquatic environment (Lin et al., 2008; Sørensen and Elbæk, 2004). Important issues addressed in this study include: (1) application of the IA and the CA model for estimating the overall toxicity of the mixtures; (2) assessment of the predictive power of the IA and the CA model. Furthermore, the application and the predictive power of the IA and CA models were assessed and validated by purposely designed algal toxicity experiments.

2. Material and methods

2.1. Test chemicals

The two nano-sized metal oxides, $nCeO_2$ and $nTiO_2$ were purchased from Sigma-Aldrich. Their primary particle diameters as provided by the manufacturer were 1.07 and 3.90–4.26 nm for $nCeO_2$ and $nTiO_2$, respectively. The nanoparticles were obtained as 10% (w/w) dispersions in deionized water. The $nTiO_2$ used was mostly in its anatase phase, with a ratio of rutile: anatase of 30: 70 provided by the manufacturer. FLO was purchased from Aladdin Industrial Corporation (Shanghai, China) with 98% purity. The algae growth and test media (pH 7.8 ± 0.2) were prepared according to OECD guideline (OECD 201, 2002).

2.2. Preparation and characterization of nanoparticle suspensions

$nCeO_2$ and $nTiO_2$ stocks were prepared by adding the original nanoparticle suspensions into the algae culture media and stirring for 24 h in the dark at 25 °C. Then the stocks were sonicated for 30 min in a temperature controlled sonication bath (150 W, 40 Hz, 25 °C) prior to addition to the experimental tanks. Toxicity test suspensions were prepared by drop-wise addition of the stock suspensions to the algae culture media and stirring for 24 h in the dark at 25 °C. The pH values of the test suspensions were adjusted to 7.8 ± 0.2 using a 0.1 M NaOH or HCl solution. The intensity averaged hydrodynamic diameters and zeta (ζ) potential of the nanoparticles were measured respectively by the dynamic light scattering technique and phase analysis light scattering technique using a ZetaSizer (Nano ZS90, Malvern Instruments Ltd., Worcestershire, UK). The concentrations of $nCeO_2$ applied in the hydrodynamic diameter and ζ-potential measurements were 5.81 and 581 μM, and the concentrations of $nTiO_2$ were 12.52 and 501 μM, which represent a toxicologically relevant concentration and the highest exposure concentration in the toxicity testing respectively.

The FLO stock solution was prepared in ultrapure water. A suspension of the ENPs and the FLO complex was prepared by adding the $nCeO_2$/$nTiO_2$ stocks into the FLO solutions, afterwards stirring for 24 h in the dark at 25 °C. The pH values of the suspensions of the complexes were adjusted to 7.8 ± 0.2 using a 0.1 M NaOH or HCl solution. The actual FLO concentrations in the toxicity tests were determined using an Agilent 1100 HPLC with a Diode array detector. In order to obtain the FLO concentrations during the exposure time, the nanoparticle suspensions first were centrifuged at 15,000 rpm for 30 min (using a D3024 high speed micro-centrifuge; Scilogex, Rocky Hill, CT, USA), then each sample was passed through a 0.22-μm Durapore membrane filter, and then the FLO concentration in the supernatant was analyzed using the HPLC.

2.3. Biotest

The freshwater green algae *Chlorella pyrenoidosa* obtained from the Institute of Hydrobiology of Chinese Academy of Sciences (Wuhan, China) was used in 96 h growth inhibition experiments, conducted in accordance with the OECD guideline (OECD 201, 2002). The test solutions were inoculated with 10^4 algal cells/mL. Control experiments were included to ensure that the observed effects were associated with the exposure to the test chemicals. During the exposure, all flasks were incubated at a temperature of 25 °C under climatic chambers and were shaken manually three times a day. Three replicates were included for each treatment. The algal cell density was determined after 96 h to allow the specific growth rate to be calculated. Growth inhibition (%) was calculated by dividing the specific growth rate for a treatment by the mean specific growth rate for the controls. Algae were exposed to increasing initial exposure concentrations of FLO (from 2.79 to 279 μM), $nCeO_2$ (from 0.58 to 581 μM), and $nTiO_2$ (from 1.25 to 501 μM).

2.4. Joint toxicity testing

Four binary mixtures were designed: the first and second mixtures were composed of FLO and $nCeO_2$ with two different exposure concentrations (29 and 58 μM, respectively), the third and fourth mixtures consisted of FLO and $nTiO_2$ with two different exposure concentrations (29 and 58 μM, respectively). Thus, the four mixtures were named [FLO + $nCeO_2$ (29)], [FLO + $nCeO_2$ (58)], [FLO + $nTiO_2$ (29)], and [FLO + $nTiO_2$ (58)], respectively.

2.5. Determination of concentration-response curve

The concentration-response relationships of the single toxicants and their mixtures were analyzed using biometrical approaches. Since no single statistic model was capable of depicting all concentration-response curves, a best-fit model was selected to describe the experimental data, as also done in previous studies (Scholze et al., 2001; Wang et al., 2009).

For each model, the functional effect (E) was confined to the