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Mixture toxicity of zinc oxide nanoparticle and chemicals with different mode of action upon *Vibrio fischeri*

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Abstract

Background: Zinc oxide nanoparticle (*n*ZnO) and chemicals with different mode of action (MOA, i.e., narcotic and reactive) were frequently detected in the Yangtze River. Organisms are typically exposed to mixtures of *n*ZnO and other chemicals rather than individual *n*ZnO. Toxicity of *n*ZnO is caused by the dissolution of Zn^{2+}, which has been proved in the field of single toxicity. However, it is still unclear whether the released Zn^{2+} plays a critical role in the *n*ZnO toxicity of *n*ZnO–chemicals mixtures. In the present study, the binary mixture toxicity of *n*ZnO/Zn^{2+} and chemicals with different MOA was investigated in acute (15 min) and chronic (12 h) toxicity test upon *Vibrio fischeri* (*V. fischeri*). The joint effects of *n*ZnO and tested chemicals were explored. Moreover, two classic models, concentration addition (CA) and independent action (IA) were applied to predict the toxicity of mixtures.

Results: The difference of toxicity unit (TU) values between the mixtures of Zn^{2+}–chemicals with those of *n*ZnO–chemicals was not significant ($P > 0.05$), not only in acute toxicity test but also in chronic toxicity test. The antagonistic or additive effects for *n*ZnO-chemicals can be observed in most mixtures, with the TU values ranging from 0.75 to 1.77 and 0.47 to 2.45 in acute toxicity test and chronic test, respectively. We also observed that the prediction accuracy of CA and IA models was not very well in the mixtures where the difference between the toxicity ratios of the components was small (less than about 10), with the mean absolute percentage error (MAPE) values ranging from 0.14 to 0.67 for CA model and 0.17–0.51 for IA model, respectively.

Conclusion: We found that the dissolved Zn^{2+} mainly accounted for the *n*ZnO toxicity in the mixtures of *n*ZnO–chemicals, and the joint effects of these mixtures were mostly antagonism and additivity. CA and IA models were unsuitable for predicting the mixture toxicity of *n*ZnO–chemicals at their equitoxic ratios.

Keywords: Zinc oxide nanoparticle, Zn^{2+}, Mixture toxicity, *Vibrio fischeri*

Introduction

The nanoparticles (NPs) have been increasingly manufactured in industry because of the well-known characteristics such as high reactivity, electromagnetic properties and high antibacterial property [1]. Zinc oxide nanoparticle (*n*ZnO), one of the most popular manufactured metal oxide nanomaterials, has unique properties (i.e., surface area and reactive sites) due to the extremely small size and is increasingly used in a range of products, such as sunscreens, cosmetics and antibacterial ointments [2]. The wide applications have caused a rapid increase in the production of *n*ZnO, with 30,000 tons worldwide in 2010 [3]. As a result, the amount of *n*ZnO entering the environment is increasing and the occurrences of *n*ZnO, in the range 1–10 μg/L or higher, have been commonly reported in natural water and sediments

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[4, 5]. Consequently, there is a critical need to investigate the toxic effect and the potential health risk of *n*ZnO [6].

To date, the studies of toxic effects for *n*ZnO were mostly focused in the field of individual pollution, and the results demonstrated that *n*ZnO can produce toxic effect upon bacterial, crustaceans, earthworms and mammalian cells [7, 8]. Adams et al. [9] determined toxicity of *n*ZnO, nano-titanium dioxide ($n\mathrm{TiO_2}$) and nano-silicon dioxide ($n\mathrm{SiO_2}$) upon the *Escherichia coli* and found that *n*ZnO was the most toxic nanoparticle. Furthermore, some studies were also performed for the purpose of understanding the toxicity mechanism of *n*ZnO [10]. *n*ZnO can cause damage to the organ and change osmoregulatory of *Oreochromis niloticus* [11]; the phosphodiester bond of L-R-phosphatidylethanolamine in *Escherichia coli* can be broken by *n*ZnO [12]. Moreover, a variety of studies proved that the toxicity of *n*ZnO is related to the dissolution of Zn^{2+} [13]. For instance, *n*ZnO caused the cytotoxicity by means of interfering with the homeostasis of Zn^{2+} [14]; Zhang et al. [15] proved the toxic difference of various *n*ZnO particles mainly depended on their dissolution.

Organisms are typically exposed to multiple mixtures of pollutants rather than single chemicals [16]. In the process of transportation and disposal, it is conceivable that NPs are able to form nanoparticle–toxin complexes due to their high surface area and large aggregates [17]; thus, there are ongoing concerns on evaluating the environmental risk for the mixtures containing NPs. Recently, the toxic effects of *n*ZnO combined with other chemicals were investigated in few studies [18]. The joint effects of the *n*ZnO and surfactants, for example, were investigated at equitoxic mixtures in acute toxicity test, which showed that the joint effects can be explained by the interactions between the Zn^{2+} and the surfactants [19]. In the field of toxicology, chemicals are classified as narcotic or reactive compounds based on their mode of action (MOA) for a better mechanistic understanding of interactions in the mixture toxicity [20]. A variety of studies proved that mixtures of compounds exerting only one MOA (narcotic or reactive) can be assumed as additive behavior, whereas the interactions of differently acting compounds tend to yield a less or more mixture toxicity [21]. Unfortunately, the toxic effects of *n*ZnO combined with other chemicals were rarely investigated, leading to the fact that in the mixture pollution, it is still unclear whether the dissolved Zn^{2+} also mainly accounts for the *n*ZnO toxicity in the mixtures of *n*ZnO–chemicals.

In the field of mixture toxicology, the interactions of chemicals always cause the changes in the different joint effects, including synergism, antagonism and additivity [22]. For instance, the joint effects of the *n*ZnO and pollutants were investigated in acute toxicity test, and the results showed antagonism [23]; the additive effect between (*n*ZnO) with nano-copper oxide (*n*CuO) was identified in the mixture toxic effects upon *Scenedesmus obliquus* [24]; the antagonistic effect between *n*ZnO with Pb was observed in the mixture toxic effects upon *Leucaena leucocephala* seedling [25]. However, the joint effects between *n*ZnO and chemicals with different MOA have been rarely investigated, and the predictive powers of the concentration addition (CA) and independent action (IA) models have not been verified, although CA and IA models were extensively employed to predict the toxic effects of mixtures [26].

Vibrio fischeri, the marine bacterium, has been widely used as the test organism for investigating the toxicity of pollutants, including *n*ZnO [27], antibiotics [26, 28], and heavy metals [29]. In recent years, reactive compounds (i.e., antibiotics) and narcotic compounds (i.e., lignin phenols) were frequently detected in Yangtze River Basin and reported in many previous studies [30, 31]. In addition, researches have suggested that organisms are exposed to the metal NPs and metal ions in the Yangtze River [32, 33]. Therefore, the purpose of this study is to (1) explore the role of Zn^{2+} in *n*ZnO toxicity of the binary mixtures containing *n*ZnO and chemicals with different MOA, (2) evaluate the joint effects of *n*ZnO and tested chemicals, and (3) investigate the predictive powers of CA and IA models for the mixture toxicities of *n*ZnO and tested chemicals.

Materials and methods

Test materials

The freeze-dried marine bacterium, *V. fischeri*, was supplied by the Institute of Soil Science, Chinese Academy of Sciences, Nanjing PRC. *n*ZnO (30 ± 10 nm), $ZnSO_4$ (Zn^{2+}), four narcotic compounds (aniline (AL), 2-nitroaniline (NAL), *p*-toluidine (TD) and hydroquinone (HQ)) and five reactive compounds (sulfamethoxazole (SMZ), sulfapyridine (SPY), sulfadiazine (SD), tetracycline hydrochloride (TTC) and oxytetracycline hydrochloride (OTC)) were purchased from Aladdin Reagent Company (Shanghai, China, www.aladdin-e.com) and were used without further purification. The detailed information of 9 organic chemicals is listed in Additional file 1: Table S1.

Toxicity test

Single toxicity test

The single toxicity test was performed following our previous methods [28]. That is, a 3% NaCl solution was used as the diluent and the bioluminescence of *V. fischeri* was recorded over a range of chemical concentrations by a SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, California). The exposure time of

tested chemicals with *V. fischeri* for acute and chronic toxicity test was 15 min and 12 h, respectively. The inhibition of the tested chemicals toward bioluminescence was calculated as Eq. 1. Based on the decrease in light emission, the obtained concentration relationship data were fitted using dose–response model (Eq. 2) [34] and reported in unit of mg/L. The detailed information about single toxicity test is presented in Additional file 1: Fig. S1.

$$Y = \frac{I_{\text{control}} - I_s}{I_{\text{control}}} \times 100\% \tag{1}$$

where Y is the inhibition ratio or response, I_{control} and I_s are the average relative light units of *V. fischeri* exposed to the controls and test chemicals, respectively.

$$Y = A_2 + \frac{A_2 - A_1}{1 + 10^{(\log C_0 - C) \times P}} \tag{2}$$

where A_1 and A_2 are bottom inhibition and top inhibition, respectively. C is the concentration of the tested chemical, C_0 is the value of C at 50% of the inhibition ratio, and P is the parameter of slope for the concentration–response relationship curve.

Mixture toxicity test

The binary mixtures, including mixtures at equitoxic ratios and the mixtures at non-equitoxic ratios based on the single toxicity results (EC_{50}), were prepared in ratios ($1{:}10^{2.5}$, $1{:}10^{2}$, $1{:}10^{1.5}$, 1:10, $1{:}10^{0.5}$, 1:1, $10^{0.5}{:}1$, 10:1, $10^{1.5}{:}1$, $10^{2}{:}1$, $10^{2.5}{:}1$) of the individual concentration (n (*n*ZnO or Zn^{2+}):m (chemicals), mg/L). The binary mixture toxicity tests were conducted in a same method as the analysis of individual toxicity test. Mixture toxicity data were fitted and described as $EC_{i,M}$ (Eq. 3) [35]. The joint effects of the mixtures were represented as the sum of toxic units (TU) [36], as shown in Eq. 4.

$$EC_{i,M} = \frac{C_A + C_B}{\frac{C_A}{EC_{i,A}} + \frac{C_B}{EC_{i,B}}} \tag{3}$$

$$TU = \frac{C_A}{EC_{50A}} + \frac{C_B}{EC_{50B}} \tag{4}$$

where EC_{50A} and EC_{50B} are median effective inhibition concentrations of components A and B, respectively. $EC_{i,M}$ is the effective concentration of the mixtures. C_A and C_B are the concentrations of the individual chemical in mixtures at median inhibition. Simple additivity is characterized by $1.2 > TU > 0.8$, while $TU > 1.2$ represents antagonism and $TU < 0.8$ indicates synergism [37].

Toxicity prediction

Concentration addition (CA) and independent action (IA) are two classical models for mixture toxicity prediction and are widely used to predict the joint effect of mixtures [38]. CA and IA models are expressed mathematically as Eqs. (5) and (6), respectively:

$$EC_{x,m} = \left(\frac{P_A}{EC_{x,A}} + \frac{P_B}{EC_{x,B}} \right)^{-1}, \tag{5}$$

where $EC_{x,m}$ is the concentration of the mixture eliciting X% effect, P_A and P_B are the concentration ratios of A and B components in the mixture, $EC_{x,A}$ and $EC_{x,B}$ denote the concentrations of the A and B components that elicit an X% effect.

$$E(c_m) = 1 - (1 - E(c_A)) \times (1 - E(c_B)), \tag{6}$$

where $E(C_m)$ is the toxic effect of mixture, $E(C_A)$ and $E(C_B)$ are the effect from analyte A and B if applied singly at an exposure concentration of A and B, respectively.

Statistics

SPSS 25.0 software (SPSS Inc.) was used to test the significant difference of the results and $P < 0.05$ was considered to be statistically significant. The statistic quality of linear models was evaluated by determination coefficients (R^2), the formula as shown Eq. 7 [39]. The parameters of mean absolute percentage error (MAPE) (Eq. 8) and root mean square error (RMSE) (Eq. 9) were applied to measure the prediction accuracy of CA and IA models [40]. These indices were obtained by the following equations:

$$R^2 = \left(\frac{\sum_{i=1}^{n} (y_i - \bar{y})(\hat{y}_i - \bar{\hat{y}})}{\sqrt{\sum_{1=1}^{n} (y_i - \bar{y})^2 \sum_{i=1}^{n} (\hat{y}_i - \bar{\hat{y}})^2}} \right)^2, \tag{7}$$

where $\bar{\hat{y}}$ is the mean predicted responses.

$$\text{MAPE} = \frac{1}{M} \sum_{k=1}^{M} \left| \frac{\hat{p}_{ettc}(k) - p_{ettc}(k)}{p_{ettc}(k)} \right| \times 100\%, \tag{8}$$

$$\text{RMSE} = \sqrt{\frac{1}{M} \sum_{k=1}^{M} \left[\hat{p}_{ettc}(k) - p_{ettc}(k) \right]^2}, \tag{9}$$

where M is the number of sample intervals.

Results and discussions

The single toxicity of tested chemicals

To investigate the mixtures' toxicity of *n*ZnO and chemicals with different MOA, the single toxicity of

*n*ZnO, Zn^{2+} and other 9 chemicals to *V. fischeri* was determined in acute and chronic toxicity test. The data were fitted by the model of dose–response and obtained curves are presented in Fig. 1. The values of R^2 suggested a good fitting (0.977–0.999). In the case of acute toxicity (Fig. 1a), *n*ZnO and Zn^{2+} presented higher toxic effects than other tested chemicals, EC_{50} values for tested chemicals were ranging from 1.17 mg/L to 319.24 mg/L, and the order of acute toxicity was as follows: *n*ZnO > Zn^{2+} > HQ > SMZ > NAL > TD > AL > OTC > TTC > SD > SPY. As far as chronic toxicity test (Fig. 1b), results showed that OTC presented higher toxic effects than other tested chemicals, EC_{50} values for tested chemicals were ranging from 1.17E−2 mg/L to 100.64 mg/L, and the order of chronic toxicity was as follows: OTC > TTC > HQ > SMZ > NAL > SD > TD > *n*ZnO > Zn^{2+} > SPY > AL.

Mixture toxicity of nZnO and chemicals with different MOA

Acute toxicity test

Based on the results of the single acute toxicity test, the mixture toxicity of *n*ZnO/Zn^{2+} and 9 tested chemicals with different MOA was determined at their equitoxic ratios (Additional file 1: Fig. S2). As shown in Additional file 1: Fig. S2, the relationship between the luminescent inhibition ratio and the concentration of these mixtures was good, with R^2 ranging from 0.978 to 0.999 for *n*ZnO–chemicals and from 0.982 to 0.993 for Zn^{2+}–chemicals, respectively. The difference between EC_{50M}^{15min} for binary mixture containing *n*ZnO and EC_{50M}^{15min} for binary mixture of Zn^{2+}–chemicals is presented as Fig. 2a. As shown, the difference was not significant for tested chemicals ($P > 0.05$). To further verify the results, the acute mixture toxicity of *n*ZnO/Zn^{2+} combined with SMZ (a reactive compound) and AL (a narcotic compound) was subsequently determined at non-equitoxic ratios (Additional file 1: Fig. S3). Figure 2b indicates that in acute toxicity test, the difference between EC_{50M}^{15min} for *n*ZnO–SMZ and EC_{50M}^{15min} for Zn^{2+}–SMZ was still not significant ($P > 0.05$). Furthermore, the same conclusion can be obtained for *n*ZnO–AL and Zn^{2+}–AL at their non-equitoxic ratios ($P > 0.05$, Fig. 2c).

Chronic toxicity test

Based on the results of the single chronic toxicity, the mixture toxicity of *n*ZnO/Zn^{2+} and these chemicals was determined at their equitoxic ratios (Additional file 1: Fig. S4). As shown in Additional file 1: Fig. S4, the relationship between the luminescent inhibition ratio and the concentration of these mixtures was good, with R^2 ranging from 0.966 to 0.994 for *n*ZnO–chemicals and from 0.956 to 0.991 for Zn^{2+}–chemicals. In the case of equitoxic ratios for chronic toxicity test, the difference between EC_{50M}^{12h} for binary mixtures containing *n*ZnO and EC_{50M}^{12h} for binary mixtures containing Zn^{2+} is presented as Fig. 2d. It can be observed that the difference was still not significant for tested mixtures ($P > 0.05$). To further verify above results, the chronic mixture toxicity of *n*ZnO/Zn^{2+} combined with SMZ (a reactive compound) and AL (a narcotic compound) was determined at their non-equitoxic ratios (Additional file 1: Fig. S5). The results of Fig. 2e, f consistently indicated that in chronic toxicity test, the difference between EC_{50M}^{12h} for binary mixtures of *n*ZnO–SMZ/AL with EC_{50M}^{12h} for binary mixtures of Zn^{2+}–SMZ/AL at their non-equitoxic ratios was still not significant ($P > 0.05$).

Consequently, dissolved Zn^{2+} mainly accounted for the *n*ZnO toxicity in the mixtures of *n*ZnO-reactive chemicals and in the mixtures of *n*ZnO-narcotic chemicals, not only in acute toxicity test but also in chronic toxicity test.

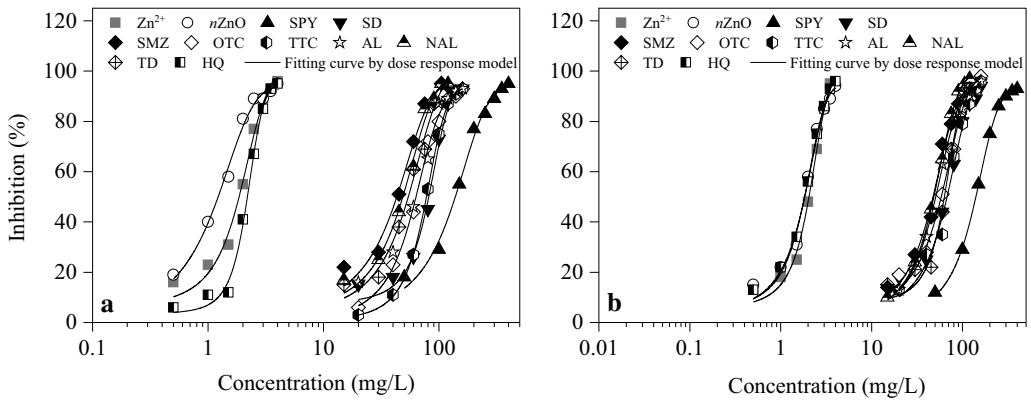

Fig. 1 The single toxic effects of chemicals upon *V. fischeri* and the fitted dose response curves. **a** The acute toxicity test results, **b** the chronic toxicity test results

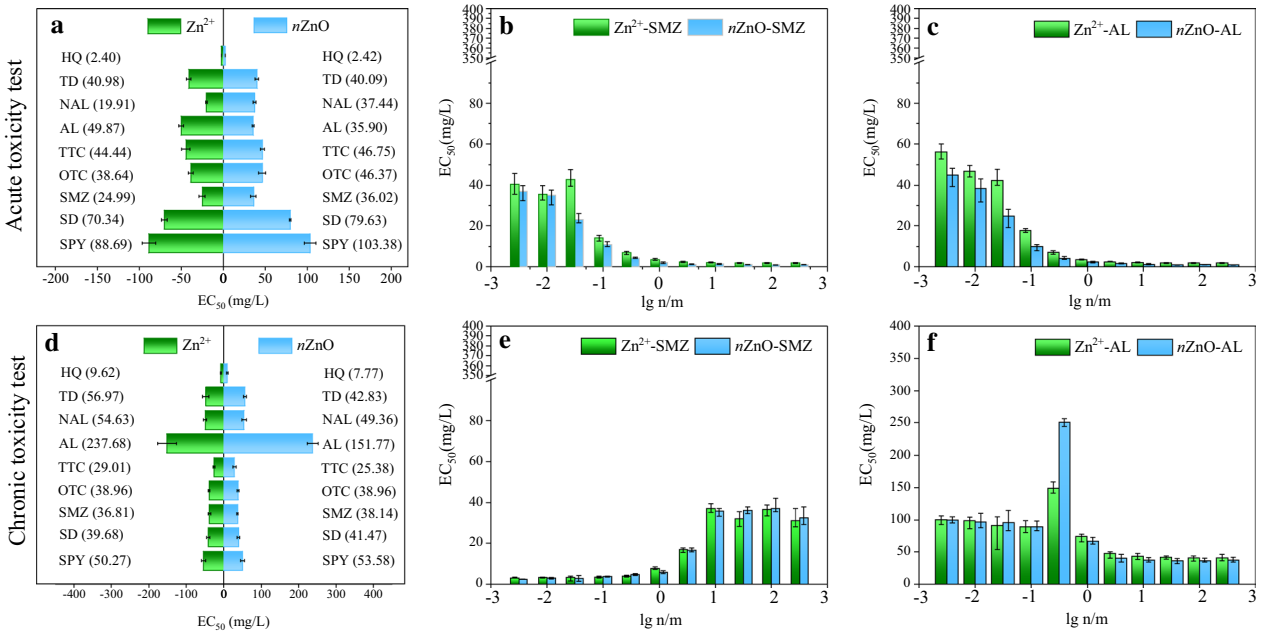

Fig. 2 Comparison of mixture toxicity between Zn^{2+}–chemicals with *n*ZnO–chemicals. **a** Zn^{2+}–chemicals and *n*ZnO–chemicals at equitoxic ratios. B in acute test, **b** Zn^{2+}–SMZ and *n*ZnO–SMZ at non-equitoxic ratios in acute test, **c** Zn^{2+}–AL and *n*ZnO–AL at non-equitoxic ratios in acute test, **d** Zn^{2+}–chemicals and *n*ZnO–chemicals at equitoxic ratios in chronic test, **e** Zn^{2+}–SMZ and *n*ZnO–SMZ at non-equitoxic ratios in chronic test, **f** Zn^{2+}–AL and *n*ZnO–AL at non-equitoxic ratios in chronic test

Joint effects of nZnO and chemicals with different MOA

Based on the mixture toxicity results, the joint effects of mixtures of *n*ZnO and tested chemicals were analyzed according to Eq. 3. In the case of acute toxicity test, it can be observed from Fig. 3a that TU values for the mixtures of *n*ZnO–chemicals at equitoxic ratios ranged from 0.75 to 1.77. Figure 3b shows that TU values for *n*ZnO–SMZ and *n*ZnO–AL at their non-equitoxic ratios were ranging from 0.93 to 1.25 and from 0.99 to 1.88, respectively. According to the study of Broderius et al. [37], those results indicated that in acute toxicity test, (1) the joint effects of *n*ZnO and chemicals with different MOA were mainly additivity or antagonism, but rarely synergism. For example, the TU value lower than 0.80 can only be obtained in the mixture of *n*ZnO–NAL and the joint effect was viewed as synergism; and (2) the joint effects for *n*ZnO–SMZ and *n*ZnO–AL were consistent additivity in the acute test at non-equitoxic ratios. In the case of the mixture of *n*ZnO–SMZ, for example, the TU values were 0.86–1.15.

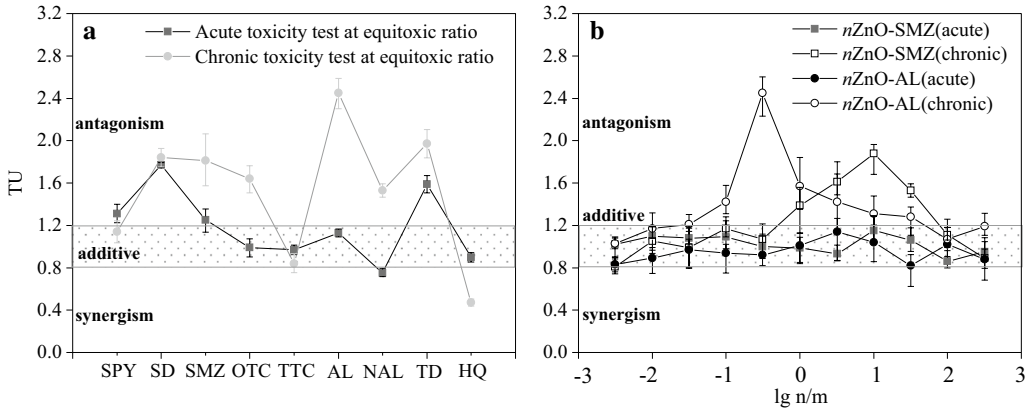

Fig. 3 Joint effects of *n*ZnO–chemicals at equitoxic ratios (**a**) and at non-equitoxic ratios (**b**)

As for chronic toxicity test, it can be observed from Fig. 3a that TU values for *n*ZnO and tested chemicals at equitoxic ratios ranged from 0.47 to 2.45, indicating the joint effects of *n*ZnO and chemicals with different MOA were additivity or antagonism or synergism. The synergism can only be obtained in one mixture (*n*ZnO–HQ), and the joint effects for other mixtures were mainly additivity or antagonism. Furthermore, Fig. 3b suggests that for the mixtures of *n*ZnO–SMZ and *n*ZnO–AL, the joint effects were additivity in the mixtures where the difference between the concentrations of the components is large (e.g., lg (n/m) = − 2.5, 2, 2.5), whereas the joint effects were antagonism in the mixtures where the difference between the concentrations of the components is small. In the case of the mixture of *n*ZnO–AL, for example, the TU values was 1.57, the corresponding lg n/m was 0.

Consequently, it can be concluded that for both acute toxicity test and chronic toxicity test, the joint effects of *n*ZnO and chemicals with different MOA were mainly additivity or antagonism. Similar results were obtained for the joint effects of *n*ZnO combined with propiconazole by Hackenberger et al. [41]. Zhang et al. [42] also found that the binary joint effects of Zn^{2+} and 11 nitro-substituted benzenes to *Photobacterium phosphoreum* were mainly antagonism.

Mixture toxicity of nZnO–chemicals predicted by CA and IA models

Based on the mixture toxicity results of *n*ZnO and chemicals, the validity and applicability of CA and IA models were further verified (Fig. 4). Results indicated that (1) the prediction accuracy of CA and IA models was satisfied in the mixtures when the difference between the concentrations of the components was large (Fig. 4b, c). When lg n/m was − 2.5 or 2.5 for all test mixtures, for example, the values of MAPE and RMSE were mostly ranging from 0.13 to 0.24, 0.00302 to 0.00319 for CA model and were from 0.17 to 0.30, 0.00307 to 0.00340 for IA model, respectively; (2) the prediction accuracy of CA and IA models was poor in the equitoxic mixtures when the joint effects were antagonism or synergism (Fig. 4a). For example, the MAPE values were 1.26 for CA model and 1.97 for IA model in the mixture of *n*ZnO–AL in chronic test; and (3) overall, the prediction accuracy of IA model was better than that of CA model, not only in the mixtures at equitoxic ratios but also in the mixtures at non-equitoxic ratios, as proved by the MAPE values of 0.105 to 2.506 and 0.108 to 2.242 for CA and IA model, respectively. It is well known that CA and IA models were used to predict the toxicity of mixture based on the theoretical assumption that chemicals in the mixture do not interact with each other, therefore both models may underestimate or overestimate the joint effects of binary mixtures [43]. CA model was used by Azevedo et al. [44] to predict the mixture toxicity of *n*ZnO and nano-silver (*n*Ag), the antagonism effect was observed and the mixture toxicity was overestimated. Wang et al. [45] reported the CA model was unsuitable to predict the mixture toxicity of Zn^{2+}–sodium dodecyl benzene sulfonate at equivalent-effect concentration ratio on *Vibrio qinghaiensis* sp. Q67.

The mixture toxicity mechanism of nZnO chemicals

By now, the mixture toxicity of engineered nanomaterials (ENMs) with chemicals is of great interest in the field of toxicology. The mechanisms for the mixture toxicity can

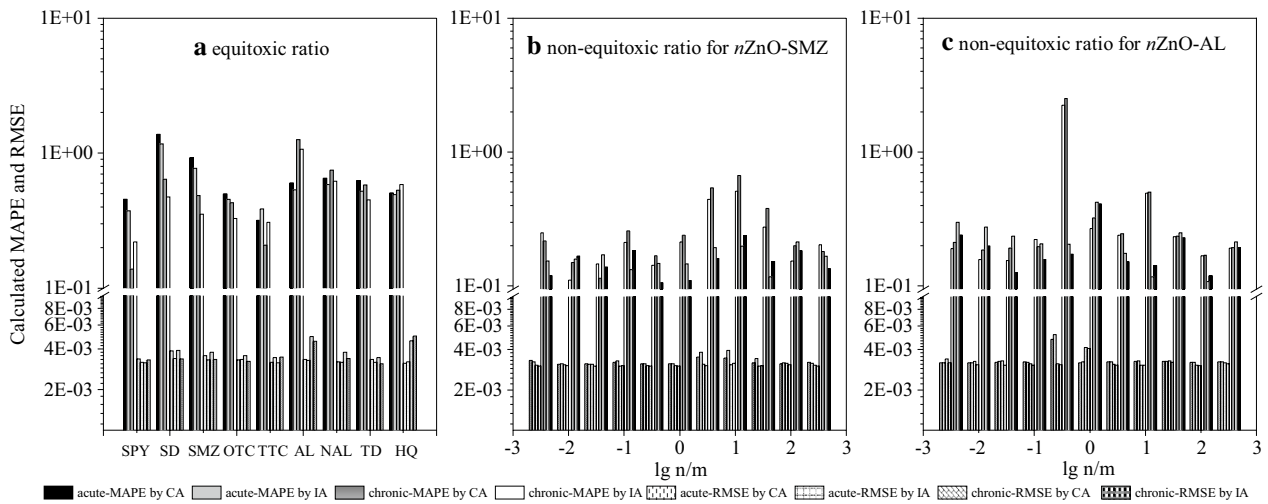

Fig. 4 The results of MAPE and RMSE values for CA and IA models from binary mixtures. **a** *n*ZnO–9 chemicals at equitoxic ratios, **b** *n*ZnO–SMZ at non-equitoxic ratios, **c** *n*ZnO–AL at non-equitoxic ratios

be mainly classified into the following types (Fig. 5): (1) ENMs effectively affect bioavailability of pollutants either positively or negatively by adsorption, complexation and degradation [46]; (2) the toxicokinetics of pollutants, including the process of uptake, biotransformation, distribution and elimination of pollutants in test organism, can be affected by ENMs by modifying the structure and function of cellular membrane, changing the metabolism pathways and altering the chemical species of pollutants [47]; and (3) ENMs influence the toxicodynamics of pollutants by interfering with the interactions of a toxicant with a biological target and its biological effects [48]. For example, ENMs may ease the entering and transport of pollutants in organisms via "Trojan horse effect" [49], because of the high surface to volume ratio [50]; Carbon nanotubes enter cells through damaging the cell membrane, which subsequently facilitated the entry of pollutants and induced a synergistic toxicity [51]; De La Torre-Roche et al. [52] indicated the dissolved Ag^{+} from silver nanoparticles (AgNPs) inhibited the activity of aquaporin and decreased the uptake of *p,p′*-DDE, and therefore, reducing the bioaccumulation of *p,p′*-DDE in test organisms.

In the case of single toxicity, studies proved that the released Zn^{2+} mainly accounted for the *n*ZnO toxicity upon *V. fischeri* and *Escherichia coli*, respectively [27, 53]. In the field of mixture toxicity, the role of released Zn^{2+} in *n*ZnO toxicity remains controversial. The work of Yi et al. [54], for example, indicated no significant difference between the toxicity of *n*ZnO–triphenyltin and Zn^{2+}–triphenyltin to *Tigriopus japonicas*. While Lakshmi Prasanna et al. [55] reported that the surface defects of *n*ZnO induced antibacterial activity via reactive oxygen species generation rather than by dissolved Zn^{2+}. The possible reason for the above difference can be concluded as following: the main toxicity mechanism of *n*ZnO may be different for the divergence of exposure condition, because the species of dissolved zinc ion could be changed by the components in the nature [56].

In the present study, the difference of the joint effects between *n*ZnO–chemicals and Zn^{2+}–chemicals was not significant ($P > 0.05$), not only in acute toxicity test but also in chronic toxicity test, suggesting that the toxicity of *n*ZnO on *V. fischeri* was due mainly to the dissolved Zn^{2+}. Thus, the joint effects of the *n*ZnO and tested chemicals can be explained by the interactions between the dissolved Zn^{2+} and these chemicals. The joint effects of tested mixtures were mainly antagonism and additivity (Fig. 3a). The possible reasons for the antagonistic effect can be explained as follows: first, metal ions interact with organic compounds which reduce the effective dose of the pollutants in organism. In case of antibiotics, interactions between metal ions and the functional groups of antibiotics are the main mechanism for decreasing the mixture toxicity [57]. The work of Kim et al. [58], for example, revealed that the complexation reactions between Cu^{2+} and the phenolic compounds (narcotic compounds) played an important role in reduction of the Cu^{2+} concentrations and therefore decreased toxicity of the binary mixtures. Second, the components of a binary mixture may compete for binding sites [59]. Hackenberger et al. [41] found that two compounds in the

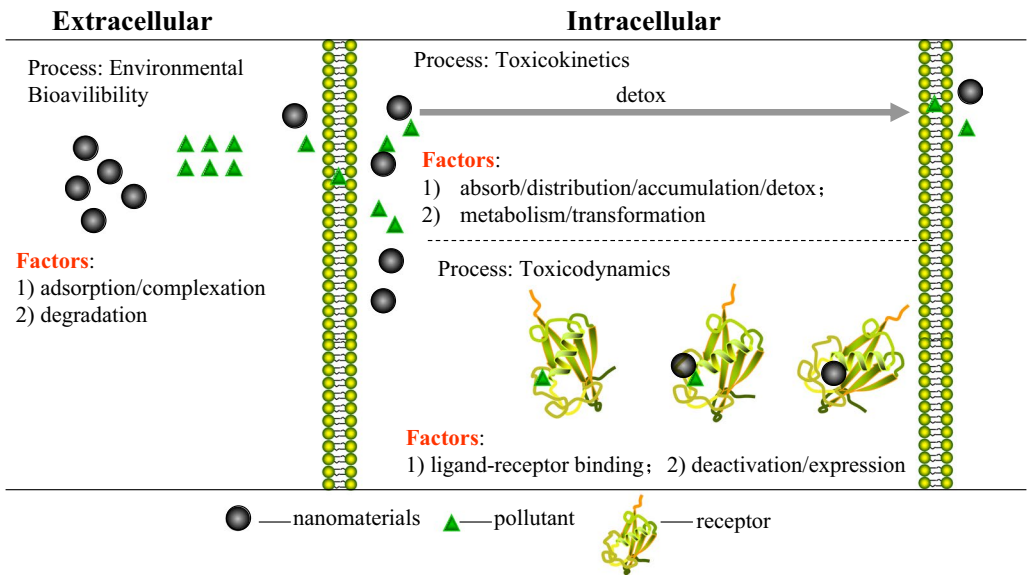

Fig. 5 The mechanisms underlying the toxicities of NMs and pollutant

treatment canceled the effect of one another in the mixtures of *n*ZnO–propiconazole and Zn^{2+}–propiconazole. The only synergism effect on *V. fischeri* occurred in the *n*ZnO–HQ binary experiment. It was well known that phenols can cause damage to cell membrane [60]. Consequently, we speculated that the HQ disrupted the cell membrane integrity of *V. fischeri*, which facilitated the entry of released Zn^{2+} and increased the mixture toxicity.

Conclusions

Our results indicated that no significant difference of the toxicity between *n*ZnO and Zn^{2+} was observed not only in single-component system but also in mixture systems of *n*ZnO/Zn^{2+} and chemicals with different MOA, suggesting that *n*ZnO toxicity was mainly caused by released Zn^{2+}. Furthermore, the joint effects of *n*ZnO and chemicals at equitoxic ratios were mainly antagonism and additivity, while the joint effects of *n*ZnO–SMZ or *n*ZnO–AL were additivity at non-equitoxic ratios. Moreover, the prediction accuracy of CA and IA models was not very well in binary mixtures at equitoxic ratios.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12302-020-00320-x.

Additional file 1: Table S1. Detail information of test chemicals. **Fig. S1.** The setting of test groups in 96-well microplate. **Fig. S2.** Comparison of dose response curves in acute toxicity test at equitoxic ratios derived from determined data and predicted data by CA and IA models. (a1–a9) *n*ZnO–chemicals, (b1–b9) Zn^{2+}–chemicals, Obs stands for observation. **Fig. S3.** Comparison of dose response curves in acute toxicity test at non-equitoxic ratios derived from determined data and predicted data by CA and IA models. (a1–a11) *n*ZnO–SMZ, (b1–b11) *n*ZnO–AL, Obs stands for observation. **Fig. S4.** Comparison of dose response curves in chronic toxicity test at equitoxic ratios derived from determined data and predicted data by CA and IA models. (a1–a9) *n*ZnO–chemicals, (b1–b9) Zn^{2+}–chemicals, Obs stands for observation. **Fig. S5.** Comparison of dose response curves in chronic toxicity test at non-equitoxic ratios derived from determined data and predicted data by CA and IA models. (a1–a11) *n*ZnO–SMZ, (b1–b11) *n*ZnO–AL, Obs stands for observation.

Abbreviations

*n*ZnO: Zinc oxide nanoparticle; *V. fischeri*: *Vibrio fischeri*; AL: Aniline; NAL: 2-Nitroaniline; TD: *p*-Toluidine; HQ: Hydroquinone; SMZ: Sulfamethoxazole; SPY: Sulfapyridine; SD: Sulfadiazine; TTC: Tetracycline hydrochloride; OTC: Oxytetracycline hydrochloride; CA: Concentration addition; IA: Independent action; TU: Toxic units; MAPE: Mean absolute percentage error; RMSE: Root mean square error; MOA: Mode of action; ENMs: Engineered nanomaterials.

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Authors' contributions

Conceptualization: XZ, LR and ML. Methodology: XZ, LW. Toxicity test: LW, FC, XX and LR. Data curation: XZ, LW and ML. Writing: XZ and LW. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article (and in Additional file: 1).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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