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Comparison of two mathematical prediction models in assessing the toxicity of heavy metal mixtures to the feeding of the nematode *Caenorhabditis elegans*

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ABSTRACT

The combined toxicity of four heavy metals (copper, zinc, cadmium and chromium) to the nematode *Caenorhabditis elegans* was determined by using feeding as an endpoint. Six equivalent-effect concentration ratio (EECR) mixtures and six uniform design concentration ratio (UDCR) mixtures were designed to fully explore the combined toxicities of these heavy metals. Observed toxicities were compared with predictions calculated by two basic models, concentration addition (CA) and independent action (IA). All the concentration–response relationships of the mixtures can be well characterized and described by the Weibull function. CA provided a relatively better prediction for the mix-toxicity of the four heavy metals, which share a similar mode of action on the feeding of *C. elegans*, although the prediction calculated by IA was also reliable, from the viewpoint of model prediction.

1. Introduction

Human activities introduce significant quantities of pollutants into the environment. Heavy metals are of primary concern, as they persist in the environment, biomagnify through the food chain, and could cause serious health problems, including morphological abnormalities, neurophysiological disturbances, carcinogenesis, teratogenesis, and mutation (Moore and Ramamoorthy, 1984; Rainbow, 1995). Organisms are exposed to multiple mixtures of chemicals, rather than isolated compounds. Unfortunately, most of the toxicity data from laboratory tests relate to single chemicals, rather than mixtures (Foulkes, 2000; Johnson et al., 2007; Priel and Hershfinkel, 2006). Due to fluctuating numbers and concentrations of individual toxicants in mixtures, it is impractical and not feasible to test every possible mixture combination. Thus, there is an urgent need for a simple and powerful model to predict the toxicities of complex mixtures from the effects of the individual components.

Numerous models for toxicological prediction of combined effects of mixed compounds have been introduced. Most of them are based on two different fundamental concepts: Concentration addition (CA), first described by Loewe in, and Independent action (IA), described by Bliss in (Backhaus et al., 2003). Concentration addition is based on the assumption that mixture components exhibit a similar mode of action in the exposed organism that results in sublethal or lethal effects. Accordingly, components of a mixture act on the same biochemical pathway and the same molecular target, and therefore can be regarded as dilutions of one another. Independent action is the theoretical counterpart of CA, and assumes that the components in a mixture present completely different and independent modes of action. Research has shown that CA can explain a wide range of mixture effects, where the components share the same mode of action (Altenburger et al., 2000, 2003; Belden et al., 2007; Faust et al., 2001), while IA can predict the overall toxicities of chemicals representing different modes of action (Backhaus et al., 2000; Belden et al., 2007; Faust et al., 2003).

Various organisms have been employed to test the toxicities of mixtures, including algae, bacteria, invertebrates and fish. For detecting the toxicity, a number of biological endpoints are applied, including survival, growth, reproduction, and behavior (Faust et al., 2003; Gonçalves et al., 2008; Loureiro et al., 2009; Pavlaki et al., 2011; Zhang et al., 2008). *Caenorhabditis elegans*, a widespread, free-living nematode that lives mainly in the interstitial pore water of soil, has been suggested as a good model organism in ecotoxicological studies. A number of its characteristics make it not just relevant but powerful as a model for toxicology. First, it is one of the best-characterized model animals at the genetic, physiological, molecular, and developmental levels (Riddle et al., 1997). Second, its comparatively short life cycle

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(3-4 days at 20 °C), small size (1 mm), transparency, and ease and low cost of culturing, make it simple to use in toxicity assays. Third, it can tolerate wide variations in pH, salinity, and water hardness (Khanna et al., 1997).

Numerous studies have been performed to evaluate the effects of heavy metals on the nervous system of *C. elegans* by assessing behavior, reporter expression, and neuronal morphology. Exposure in heavy metals could lead to the locomotion behavior defects of *C. elegans*, which indicates possible dysfunction of the nervous system ((Du and Wang, 2009; Wang and Xing, 2008; Xing et al., 2009). M3, M4, MC, NSM, RIP, and I are neurons involved in the feeding process (Riddle et al., 1997). Any damage to this neuronal circuitry may alter feeding behavior. Exposure to heavy metals can induce *numr*-1 and *numr*-2 transcription in pharyngeal and intestinal neurons, which are metal-specific, stress-responsive genes (Leung et al., 2008; Tvermoes et al., 2010). These studies indicated that the four heavy metals copper, zinc, cadmium, and chromium share the same or similar mechanism of action, and affect the nervous system of *C. elegans*, leading to potential inhibition of feeding behavior.

Many endpoints of toxicity have been assessed in *C. elegans*, including lethality, reproduction, behavior, feeding, growth, lifespan, and production of stress proteins. When comparing the sensitivity of lethal and sublethal endpoints of *C. elegans* in aquatic tests, both reproduction and behavior were much more sensitive than lethality to low concentrations of metals (Anderson et al., 2001; Boyd and Williams, 2003; Dhawan et al., 1999, 2000; Thompson and de Pomerai, 2005). In toxicity tests of mixtures, the most commonly used endpoints are lethality and reproduction. There are only a few studies focused on quantitative models. Testing the accuracy of predictions calculated by CA and IA models usually involves lethality and reproduction tests (Gomez-Eyles et al., 2009; Martin et al., 2009; Svendsen et al., 2010). In comparison, the feeding endpoint is less time-consuming than reproduction assays and much more sensitive than lethality. To date, we have found no reports of feeding assays used to test the overall toxicity of mixtures with quantitative prediction models. Therefore, it would seem worthwhile to employ the feeding endpoint of *C. elegans* to the study of mixture toxicity in heavy metals.

Our objectives in this study were to assess the joint toxicity of four heavy metals, which share a similar mode of action, on the feeding of the nematode *C. elegans*, and to test the predictive ability of CA and IA models on the mixture. To assess the mixture of heavy metals at different component ratios and concentration levels, the equivalent-effect concentration ratio (EECR) method and the uniform design concentration ratio (UDCR) method were employed in this study.

2. Materials and methods

2.1. Test organisms

Caenorhabditis elegans wild type strain N2, was originally obtained from the *Caenorhabditis* Genetics Center (CGC) (Minnesota, USA), for use in this experiment. The worms were maintained on nematode growth medium (NGM) plates seeded with OP50 (a uracil-deficient strain of *Escherichia coli*) at 20 °C as described by Brenner (1974). A stock solution of dauerlarvae in M9 buffer was prepared, kept at 20 °C, and renewed monthly (Donkin and Williams, 1995). The dauers were used to generate age synchronous adult worms for toxicity tests. The dauers were placed on NGM plates with a lawn of OP50 for a food source and were incubated at 20 °C. After 3 days, gravid nematodes were washed off the plates into centrifuge tubes and were lysed with a bleaching mixture (0.45 mol/L NaOH, 2 percent HOCl), which killed all life stages but the eggs (Emmons et al., 1979). The eggs were collected and placed on NGM plates and incubated to obtain age-synchronous young adults. Three day old nematodes were collected and rinsed with K-medium (0.032 M KCl, 0.051 M NaCl) at least three times (Williams and Dusenbery, 1990).

2.2. Test chemicals and analysis

Four analytical grade chemicals were used in this experiment: copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, Camycal, 98%), zinc chloride ($ZnCl_2$, Sigma-Aldrich, 98%), cadmium chloride ($CdCl_2$, Sigma-Aldrich, 98%) and potassium dichromate ($K_2Cr_2O_7$, Camycal, 98%).

All metal solutions were prepared by dissolving their salts in K-medium. The concentration of all metal solutions was analysed by inductively coupled plasma mass spectrometer (ICP-MS). The results showed that measured concentrations varied generally less than 5% from the nominal concentrations. Thus, all calculations were based on nominal concentrations.

2.3. Feeding assay

Feeding assay was performed according to the method described (Anderson et al., 2001; Jones and Candido, 1999) in 12-well culture plates with some modifications. Feeding was quantified for the worms exposed in liquid medium (OP50 suspended in K-medium with or without toxicants) by detecting the change in optical density (OD) of the suspension at 24 h. The OD of all liquid medium was adjusted to 1.0 at 590 nm. Each well contained 2 mL of solution. The adult worms were added to the wells such that each well had 2000 worms. The plates were incubated at 20 °C for 24 h while being shaken gently. At the beginning and the end of this period, the worms were allowed to settle for 15 min. Supernatants were then transferred to 96-well plates and the optical density of each supernatant was read by microplate reader (E11383, Molecular Devices, USA) at 590 nm. Wells without worms were run for each toxicant and K-medium to correct for intrinsic bacterial lysis or growth, which might occur during the course of an exposure. The effects of single and mixtures of heavy metals on feeding were expressed as the inhibition value (E), which is calculated as follows

$$E(\%) = \left(1 - \frac{\Delta\text{ODheavy metal}(+\text{worm}) - \Delta\text{ODheavy metal}(-\text{worm})}{\Delta\text{ODK} - \text{medium}(+\text{worm}) - \Delta\text{ODK} - \text{medium}(-\text{worm})}\right) \times 100 \tag{1}$$

where ΔOD represents the net change of OD in 24 h.

2.4. Single and mixture design

To obtain the concentration–response curves of every single metal, twelve different test concentrations were arranged in triplicate in 12-well plates. Wells without worms were also run for each concentration and K-medium controls. The microplate test was repeated four times. To meet the requirement of the CA and IA models, which require complete concentration–response relationships of all components of the mixture, the inhibition effect of the twelve test concentrations covered the range from 5 to 90%. The initial concentrations of Cu, Zn, Cd, and Cr were 20, 100, 100, 100 mg/L, and the dilution factor was 0.618.

Two different design methods united with fixed-ratio ray design (FRRD) were employed in the mixture toxicity study. The fixed-ratio ray design is a technique that makes a mixture point change to a line. This means that the ratio of the components was maintained constant, while only the total concentration of the mixture was varied, and then a complete concentration–response relationship for every mixture was obtained. One of the design methods was EECR, which is a classical method commonly used in mixture toxicity tests (Backhaus et al., 2000; Faust et al., 2003; Olmstead and LeBlanc, 2005). The EECR mixtures were prepared in the ratio of the same effect-concentrations of all individual compounds. Six EECR mixtures were examined (EE-5, EE-10, EE-20, EE-30, EE-40, and EE-50), based on the relative toxicities of the components. It is well known that the concentration compositions in EECR mixtures are limited to a narrow space in the experimental region. Therefore, to effectively expand the space, the uniform design (UD) (Fang et al., 2000; Fang and Ma, 2001), which can effectively examine various concentration combinations of the chemicals in the mixture with less experimental effort, was introduced into the mixture toxicity design (Liu et al., 2009; Zhang et al., 2010; Zhou et al., 2010). The UD experiment is planned in terms of the uniform design table (U-table). U-tables of form $U_n(n^s)$, where the subscript n refers to the number of mixture experiment, and the superscript s to the number of heavy metals (factors) in the experiment were purposely chosen to meet the need of experiment design. In this study, $U_6(6^4)$ table with four factors (heavy metals, superscript) and six levels (concentrations) and six experiments (mixtures, subscript), was selected (Supplementary material: Tables 1 and 2). Six EECR mixtures (EE-5, EE-10, EE-20, EE-30, EE-40, and EE-50) and six UDCR ones (UD-1, UD-2, UD-3, UD-4, UD-5, and UD-6) combined with FRRD were designed in the study. Each EECR or UDCR mixture (point) with a definite concentration composition was extended by FRRD as a fixed-ratio mixture ray with twelve different concentration points. The concentration compositions and ratios of four heavy metals to the total concentration of the mixture are listed in Table 1.

2.5. Concentration–response curve fitting

To quantitatively describe various effect concentrations (ECx), the observed concentration-effect data were fitted by a non-linear Weibull function with two

Table 1
Concentration compositions and ratios p_i (%) of six equivalent-effect concentration ratio (EECR) and six uniform design concentration ratio (UDCR) mixtures.

Mixture	Cu	Zn	Cd	Cr
EE-5	EC_5 (19.42)	EC_5 (37.86)	EC_5 (7.77)	EC_5 (34.95)
EE-10	EC_{10} (16.34)	EC_{10} (37.97)	EC_{10} (9.05)	EC_{10} (36.64)
EE-20	EC_{20} (13.37)	EC_{20} (37.98)	EC_{20} (10.38)	EC_{20} (38.27)
EE-30	EC_{30} (11.72)	EC_{30} (37.85)	EC_{30} (11.27)	EC_{30} (39.16)
EE-40	EC_{40} (10.61)	EC_{40} (37.60)	EC_{40} (12.02)	EC_{40} (39.77)
EE-50	EC_{50} (9.73)	EC_{50} (37.37)	EC_{50} (12.66)	EC_{50} (40.25)
UD-1	EC_5 (2.21)	EC_{10} (9.48)	EC_{20} (5.95)	EC_{50} (82.36)
UD-2	EC_{10} (3.28)	EC_{30} (29.49)	EC_{50} (20.84)	EC_{40} (46.39)
UD-3	EC_{20} (6.16)	EC_{50} (61.51)	EC_{10} (1.82)	EC_{30} (30.51)
UD-4	EC_{30} (20.64)	EC_5 (7.82)	EC_{40} (31.66)	EC_{20} (39.88)
UD-5	EC_{40} (32.59)	EC_{20} (46.14)	EC_5 (1.87)	EC_{10} (19.39)
UD-6	EC_{50} (22.28)	EC_{40} (61.05)	EC_{30} (12.22)	EC_5 (4.44)

Table 2
Parameter values of the Weibull function with some statistics and EC_{50} values for the individual heavy metals.

Substance	α	β	R^2	RMSE	EC_{50}(mg/L)
Cu	−1.89	2.73	0.951	0.091	3.61
Zn	−2.74	2.08	0.968	0.062	13.87
Cd	−1.56	1.77	0.971	0.057	4.70
Cr	−2.68	1.97	0.981	0.044	14.94

Note: R^2, squared correlation coefficient; RMSE, root mean square errors.

parameters. Then the fitted Weibull function was used to estimate the *ECx*. The goodness of fit is characterized by the squared correlation coefficient (R^2) and the root mean square error (RMSE). The Weibull function is written as follows (Faust et al., 2001)

$$E = 1 - \exp(-\exp(\alpha + \beta \log_{10}(c))) \quad (2)$$

where E is the inhibition ratio (response) of a single compound or mixture, c represents the concentration of a single compound or mixture, and α and β are the parameters of the Weibull function.

2.6. Mixture toxicity prediction

Based on the concentration–response relationships of the single components, predictions for effect concentrations of the test mixtures (ECx_{mix}) were generated according to CA and IA models. Predictions under the assumption of CA were expressed mathematically as (Faust et al., 2001)

$$ECx\text{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECxi} \right)^{-1} \quad (3)$$

where ECx_{mix} is the total concentration of the mixture that elicits an x percent combined effect, ECx_i is the concentration of the ith component that provokes an x percent effect when tested individually, and p_i denotes the molar concentration ratio of the ith component in the mixture.

The alternative model IA is commonly defined as (Faust et al., 2001)

$$x\% = 1 - \prod_{i=1}^{n} (1 - Fi(p_i \times (ECx\text{mix}))) \quad (4)$$

where $x\%$ is the overall effect caused by the total concentration of a mixture; F_i is the concentration response functions of the ith component.

3. Results and discussion

3.1. Toxicity of single compounds

The Weibull function exhibited good fits for all the concentration–response data of the four heavy metals, with R^2 greater than 0.95, RMSE less than 0.099 and showed statistical significance ($p < 0.01$). The corresponding fitted parameters (α and β), some statistics (RMSE and R^2) and the EC_{50} values of four metals are presented in Table 2. The fitted concentration–response curves (CRCs) are shown in Fig. 1. Various *ECx* values required in the mixture toxicity tests (such as the EC_{50}, EC_{40}, EC_{30}, EC_{20}, EC_{10} and EC_5 of every single metals), can be easily computed from the fitted Weibull function.

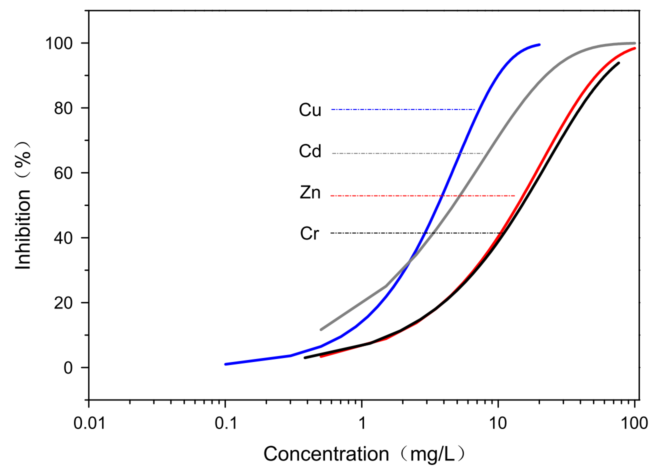

Fig. 1. Concentration–response curves (CRCs) of the four heavy metals.

Table 3
Parameter values of the Weibull function with some statistics for six equivalent-effect concentration ratio (EECR) and six uniform design concentration ratio (UDCR) mixtures and EC_{50} values observed and predicted by CA and IA.

Mixture	α	β	R^2	RMSE	EC_{50} (mg/L)	$EC_{50(CA)}$ (mg/L)	$EC_{50(IA)}$ (mg/L)
EE-5	−2.21	2.07	0.976	0.051	7.74	8.21	7.41
EE-10	−2.78	2.59	0.973	0.054	8.53	8.54	7.64
EE-20	−2.58	2.25	0.946	0.068	9.64	8.85	7.85
EE-30	−2.44	2.15	0.978	0.035	9.19	9.04	6.44
EE-40	−2.52	2.25	0.950	0.064	9.02	9.15	8.04
EE-50	−2.67	2.37	0.983	0.042	9.39	9.22	8.07
UD-1	−2.92	2.40	0.958	0.050	11.5	12.34	10.86
UD-2	−2.57	2.33	0.972	0.046	8.80	9.42	7.99
UD-3	−3.04	2.59	0.962	0.053	10.84	11.62	10.64
UD-4	−1.74	1.80	0.942	0.050	5.76	6.32	5.75
UD-5	−2.18	2.37	0.953	0.044	5.81	7.09	6.70
UD-6	−2.61	2.65	0.955	0.059	7.01	7.38	6.82

The EC_{50} values in our study are similar to, and consistent with, earlier findings showing that feeding as an endpoint is of similar sensitivity to movement, growth and reproduction, and is much more sensitive than lethality (Anderson et al., 2001; Boyd and Williams, 2003; Dhawan et al., 2000). In the case of copper, the 24 h EC_{50} values of the feeding endpoint is 3.61 mg/L and not significantly different from the reported values of 2.06–3.11 mg/L. The 24 h LC_{50} values of Cu range from 22 to 108.65 mg/L (Tatara et al., 1998; Williams and Dusenbery, 1990), which is less sensitive compared with the EC_{50} values of the feeding endpoint.

3.2. Toxicity of multi-component mixtures

The concentration–response data of six EECR mixtures and six UDCR mixtures were determined by using the same feeding assay procedure for the individual heavy metals. The observed concentration–response data were also fitted to the Weibull function. The optimal regression coefficients (α and β) and other statistics (RMSE and R^2) are listed in Table 3. To compare with the results from the classical "point to point" method, the EC_{50} values of twelve mixtures predicted by the CA and IA models were listed in Table 3. It has been shown that the CRCs of twelve mixtures exhibited statistical significance ($p < 0.01$) with the estimated R^2 value of more than 0.94, and the RMSE value of less than 0.070,

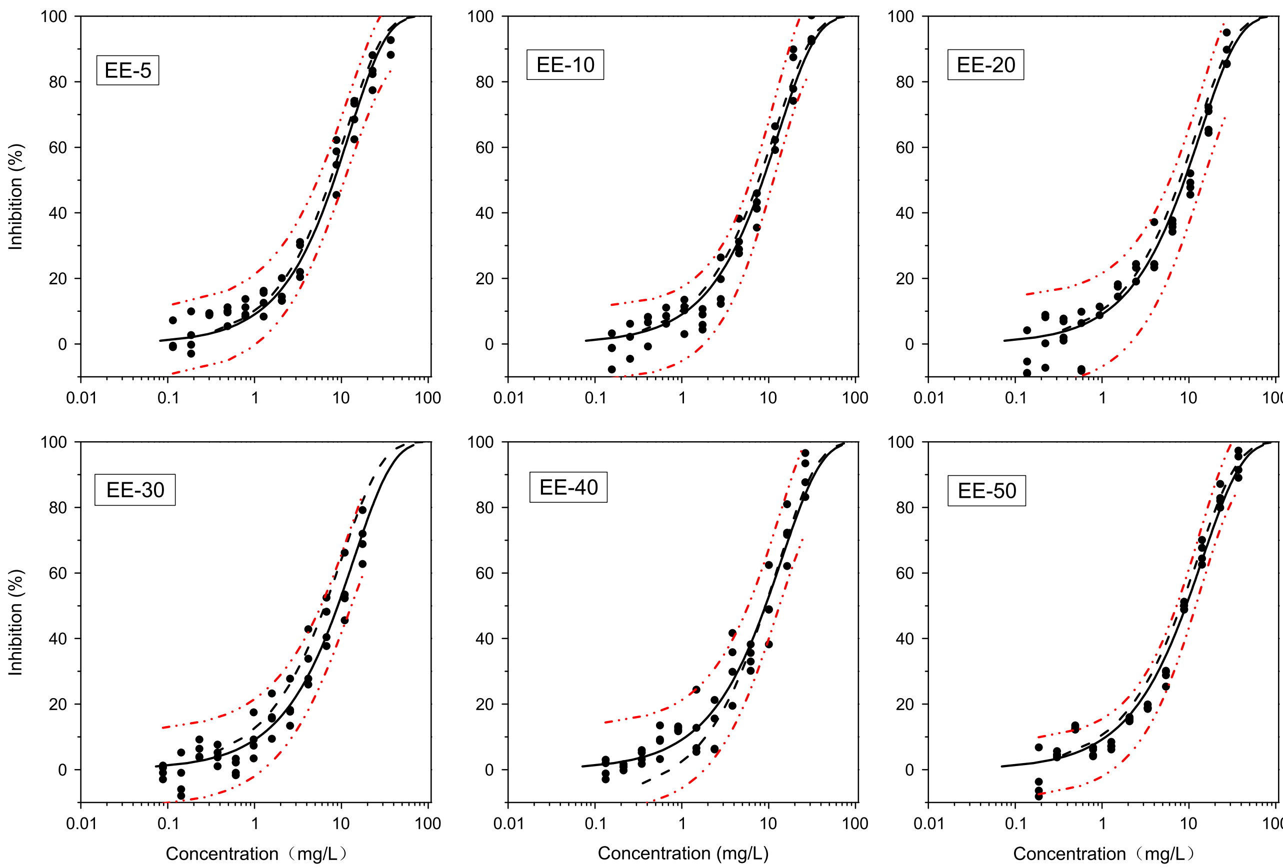

Fig. 2. Concentration–response relationships observed and predicted by CA and IA for six EECR mixtures. ●, observed; —— predicted by CA; – – –, predicted by IA; – – – –, 95% confidence intervals observed (two red short dash lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

which indicated that the reproducibility of experimental data was high and that the Weibull models were appropriate for this study.

The concentration–response data points observed and the 95% confidence intervals (CIs) of the CRCs for six EECR mixtures and six UDCR mixtures are shown in Figs. 2 and 3, respectively. To explore the toxicity interaction between various heavy metals in the mixtures, the predictive CRCs of six EECR and six UDCR mixtures were calculated by the CA and IA models and are also displayed in Figs. 2 and 3.

As shown in Figs. 2 and 3, there was a very high level of agreement between the experimental mixture toxicity data and the predictions based on the assumption of CA, irrespective of the mixture ratio and the effect level. The CRCs predicted by CA were allocated in the 95% CIs of the experimental CRCs of six EECR and six UDCR mixtures, and the deviations between the observed and that predicted *ECx* values by CA never exceeded a factor of 1.3. In classical "point to point" method, the EC_{50} of a mixture is commonly selected as the reference point to analyse the predictive accuracy of the models. On the 50% effect level, the observed and the CA-predicted mixture toxicities to *C. elegans* were virtually identical with deviation values less than 1.22 (Table 3). This result held true for the twelve heavy metal mixtures. The deviation values between observed and predicted toxicities (within 2) indicate that the model used is valid, and good enough to provide reliable and accurate predictions (Arrhenius et al., 2004; Belden et al., 2007). CA is shown to have high predictive power on the mixture toxicity of heavy metals to *C. elegans* in this study.

Since the four heavy metals shared the same or similar mechanism of action, CA was expected to have good predictive power for their mixture toxicity. However, in this experiment, IA was also shown to have power for predicting the toxicities of heavy metal mixtures, although previous research showed that IA was commonly suitable for the prediction of the mixture effects of substances with different or dissimilar modes of action (Backhaus et al., 2000; Faust et al., 2003). As shown in Figs. 2 and 3, the CRCs predicted by IA were, on the whole, allocated in the 95% CIs of the experimental CRCs of six EECR and six UDCR mixtures, and the deviations between the observed and that predicted *ECx* values by IA never exceeded a factor of 1.5. For the twelve mixtures, the deviations between the observed EC_{50} values of the mixture and those predicted by IA never exceeded a factor of 1.3 (Table 3). In Figs. 2 and 3, for all the mixtures except EE-30 and EE-40, 95% CIs of mean observed effect concentrations covered or overlapped with those predicted by IA. Although CA provided a better fit in the EE-30 and EE-40 mixtures, the deviations between observed and predicted values by IA still did not exceed a factor of 2.This indicates that IA can also provide reliable predictions for the EE-30 and EE-40 mixtures. Svendsen et al. (2010) have determined the mixture toxicity of five nerve toxicants to *C. elegans* by using the reproduction endpoint, and both CA and IA were found to be valid models in predicting the toxicity of the mixtures, although CA provided a mathematically better fit in most cases. As shown in Figs. 2 and 3, CA and IA were somewhat overestimated the toxicities of the twelve mixtures except for EE-5, UD-4 and UD-5 in the low effect region ($>$ 30%), and showed a slight antagonistic effect. This might be attributed to the relatively higher ratios of Zn and the relatively lower ratios of Cu, which may have cause this slight antagonism. In the mixture toxicity study of heavy metals to

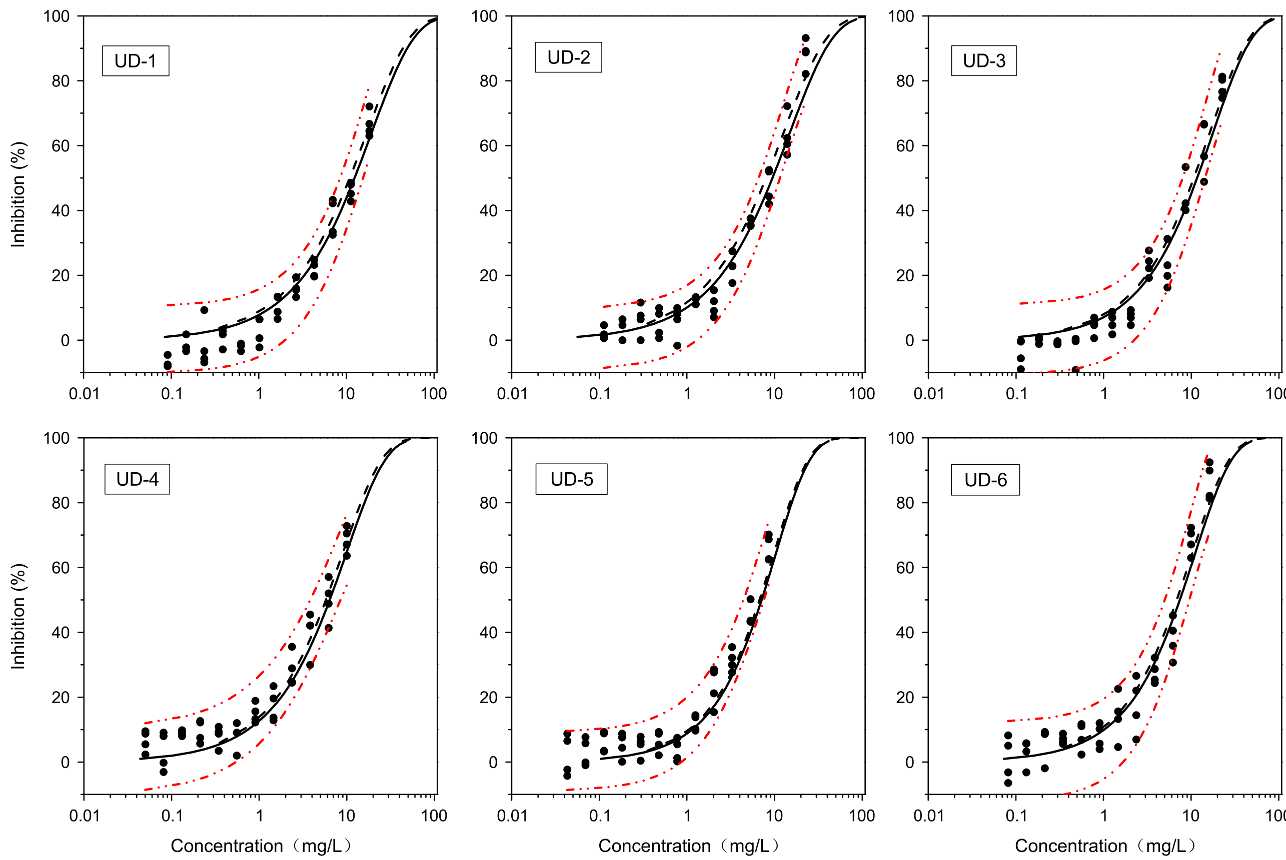

Fig. 3. Concentration–response relationships observed and predicted by CA and IA for six UDCR mixtures. ●, observed; —— predicted by CA; – – –, predicted by IA; - - - -, 95% confidence intervals observed (two red short dash lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the LC_{50} of *C. elegans,* Chu found that Cu might act synergistically with other metals, while Zn could neutralize the toxicity of many metals (Chu et al., 2005; Chu and Chow, 2002). However, whether there is an antagonistic effect of Zn to the feeding of *C. elegans* will require further investigation.

In our present study, CA and IA models made equally good predictions for the toxicities of heavy metal mixtures. Since CA and IA are based on opposing assumptions, this might be hard to explain from the mechanistic point of view, but not from the mathematical one. Some studies showed that under specific conditions, the *ECx* values predicted by CA and IA models in fact may be identical (Boedeker et al., 1993; Drescher and Boedeker, 1995). In general, the quantitative relationships between CA and IA are considered to be associated with four parameters: the number of mixture components, the concentration ratio of mixture components, the response level under consideration, and the slopes of the CRCs of individual toxicants (Drescher and Boedeker, 1995). It is easy to define the first three parameters, while no universal measurement can characterize the slope of a whole concentration–response curve. Therefore, equal prediction can be produced by CA and IA when the following conditions are met: The concentration–response relationship of every individual mixture component can be described by the two-parameter Weibull model, the curves are strictly parallel, and the slope parameter β takes a value of 2.3 (decadic logarithm) (Backhaus et al., 2004). This assumption has been confirmed for some phenylurea herbicides with similar mechanisms of action, where the prediction values obtained from CA and IA were virtually identical (Backhaus et al., 2004). This is also supported by studies of phenolic compounds and organophosphorus pesticides (Huang et al., 2011; Zhang et al., 2008). In those studies, the concentration–response relationship of individual heavy metals was well described by the Weibull function. Although the curves were not strictly parallel, the average value of β was 2.14 which is close to 2.3, indicating that the parameter β may play a very important role in explaining the negligible difference between CA and IA. This may explain the equal prediction ability of CA and IA in our study.

Commonly, CA tends to predict a higher toxicity than IA, and some authors have therefore suggested that CA may be used as a worst case approach for the predictive hazard assessment of mixtures (Berenbaum, 1985; Boedeker et al., 1993; Faust et al., 2003). However, the results from our study suggest that the toxicity predicted by IA was higher than CA. Similar results were provided by Zhu et al. (2011), who determined the mixture toxicity of heavy metals on the Chinese rare minnow (*Gobiocypris rarus*), and found that the higher toxicity predictions were given by IA, not CA. Some other experimental cases have also found that IA can predict higher effect than CA (Drescher and Boedeker, 1995; Huang et al., 2011; Junghans et al., 2003; Zhang et al., 2008;). But the nature of the factor causing the changes of α and β in the Weibull function, and how the parameters affect the relationship between CA and IA, will require further research.

There are some models have been commonly used in predicting mixture toxicity, including CA, IA, QSAR, and DEB models. CA and IA models can demonstrate excellent predictive power in case that the composition of mixtures definitely complies with the underlying ideas of similar and dissimilar action, respectively. If modes of action of components were not available, the experimental toxicity could be compared with QSAR models, which are promising techniques for the identification of congeneric compounds with a similar toxicological mode of action, based on the analysis of structural similarities, for baseline toxicity. One of the drawbacks of the CA and IA approaches for assessing effects of mixtures is that these approaches are purely descriptive, with the concentrations as the starting point, and every endpoint is considered as being unrelated to other endpoints. This problem may be solved by DEB models. The DEB approach takes the organism as a starting point instead of the concentrations, which allows us to interpret lethal or sublethal effects in a single framework, using the same parameters. In our study, the modes of action of the four heavy metals were identified, and no interactions between them, it is more appropriate and effective to use CA and IA models to predict mixture toxicity. Nevertheless, the mathematic assumptions of CA and IA models are no interactions between components of mixtures. For those mixtures, whose components may interact with each other, these two models may fail to accurately predict combined effects. A biologically-based framework, which incorporates external exposure with toxicokinetics and toxicodynamics, is proposed to support experimental studies to investigate the basis of observed interactions. This framework may support the better mechanistic understanding of interactions in mixture toxicology (Spurgeon et al., 2010).

4. Conclusions

Feeding, as a sensitive sublethal endpoint in *C. elegans*, appears to be suitable for the study of mixture toxicity of heavy metals. Our results demonstrated that the mixture toxicity of six EECR and six UDCR heavy metal mixtures can be accurately predicted by CA. Further, our results showed that deviations between observed and predicted mixture toxicity by IA were relatively small (factor < 1.3), which indicated that IA could also provide reliable predictions. All the concentration–response relationships of the mixtures built by different design methods can be well characterized and described by the Weibull function. Our study also provided an unusual case where the toxicity predicted by IA may be higher than that predicted by CA.

Acknowledgments

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Appendix A. Supporting information

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