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Modeling and prediction for the acute toxicity of pesticide mixtures to the freshwater luminescent bacterium *Vibrio qinghaiensis* sp.-Q67

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Abstract

In China, water pollution by pesticide mixtures has constituted a serious environmental problem due to potential toxicity and bioaccumulation. But few pesticide combinations have exactly similar and dissimilar mechanisms of action. For this purpose, in tests with the freshwater luminescent bacterium (*Vibrio qinghaiensis* sp.-Q67), ten pesticides, including three herbicides and seven insecticides, were selected as test substances. Concentration response analysis was performed for ten individual substances, and for mixtures containing all ten substances in twelve different concentration ratios (based on UDCR and EECR methods). The observed mixture toxicity was compared with predictions by the two models, concentration addition (CA) and independent action (IA). The toxicity of the tested mixtures showed a good agreement with those predicted by the concept of CA except four UDCR mixtures: UD10-2, UD10-4, UD10-8 and UD10-10. To examine the influence of imidacloprid in the four UDCR mixtures (UD10-2, UD10-4, UD10-8, UD10-10), it was removed from the ten-pesticide mixtures and the remaining nine chemicals were combined at the same relative proportions based on the UDCR method (UD9-2, UD9-4, UD9-8, UD9-10). There was not significant departure from CA for the scattered points with the nine remaining pesticides omitting imidacloprid. Thus, imidacloprid may significantly influence the other pesticides due to its properties.

Key words: pesticides; *Vibrio qinghaiensis* sp.

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Introduction

China is the world's biggest country on pesticide production and application (Yang and Song, 2007). Water pollution by pesticide mixtures has constituted a serious environmental problem due to potential toxicity and bioaccumulation (Li et al., 2008; Tan et al., 2003). Given that pesticide contaminants in water environment exist frequently as mixtures and the behavior of chemicals in a mixture may not correspond to that predicted from the single substance toxicity, the detection and characterization of pesticide interactions in complex mixtures is critical for regulatory decisions. In aquatic toxicology, two different prediction models, concentration addition (CA) and independent action (IA) are often used for that purpose. The most widely used mathematical equation for CA is expressed as Eq. (1):

$$\sum_{i=1}^{n} \frac{c_i}{\mathrm{EC}_{xi}} = 1 \quad (1)$$

where, n is the number of components in the mixture, EC_{xi} is the concentration of the i-th component that provokes $x\%$ effect when applied individually and c_i is the concentration of the i-th component in the mixture.

Indeed, a lot of studies have demonstrated the validity of CA as a means of predicting the toxicity of multi-component mixtures of similarly acting chemicals in various assays with fish, daphnia, plant, algae, and bacteria (Altenburger et al., 2000; Barata et al., 2006; Brian et al., 2005; Munkegaard et al., 2008). The target contaminants mainly contained pesticides (Backhaus et al., 2004b; Faust et al., 2001; Junghans et al., 2003a, 2003b) and other groups of toxicants with a common mode of action, such as, uncouplers of oxidative phosphorylation (Altenburger et al., 2000), quinolones (Backhaus et al., 2000b), estrogenic chemicals (Brian et al., 2005; Silva et al., 2002).

In contrast to CA, the concept of IA is based on the assumption of a dissimilar mechanism of action of all mixture components. The most widely used mathematical equation for IA is expressed as Eq. (2):

$$E(c_{\mathrm{mix}}) = 1 - \prod_{i}^{n} (1 - E(\mathrm{c}_i) \quad (2)$$

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where, $E(c_{mix})$ is the total effect of the mixture and $E(c_i)$ is the effect of *i*-th component.

Although Bliss introduced IA into pharmacological literature as early as 1939 (Bliss, 1939), few ecological studies have investigated the concept. While precise predictions were obtained by IA for mixtures of strictly dissimilarly acting substances (Backhaus et al., 2000a, 2004a; Faust et al., 2003; Walter et al., 2002), for several multi-component mixtures of similarly acting substances, this concept was shown to underestimate the joint effects (Altenburger et al., 2000; Backhaus et al., 2000b; Faust et al., 2001; Junghans, 2004; Silva et al., 2002).

From the present studies, three possibilities for combined effects of pesticide mixtures on Q67 may occur: (1) compliance with CA; (2) some kinds of intermediate effect, approximately predictable by CA or IA, and, as long as interaction between the mixture components cannot be excluded; (3) inappropriateness of both concepts. Now there is no clear understanding of which concept is optimum for pesticide mixtures due to a dearth of related studies.

The aim of this study was to clarify the variability of toxicity of pesticide mixtures in whole concentration space and give a more reliable estimate of the toxicity of pesticide mixtures. We studied a comparative evaluation of the predictive values of both prediction models, CA and IA. Experimental mixture toxicity analyses were performed with a set of 10 selected pesticides (with unspecific mode of action to Q67), including one triazine herbicide (metamitron), one phenoxy herbicide (2,4-D), one amide herbicide (metolachlor), one carbamate insecticide (carbofuran), two chloronicotinyl insecticides (imidacloprid and acetamiprid), and four OP insecticides (dylox (TM), methyl parathion, monocrothophos, and phosphamidon). The inhibition of bacteria bioluminescence of the freshwater luminescent bacterium *Vibrio qinghaiensis* sp.-Q67 was used as toxicity parameter. Adverse effects of toxicants can all be expected to be detectable at such an integrating level of response. Mixture toxicity predictions will be calculated according to previous research works (Faust et al., 2001).

1 Materials and methods

1.1 Test substances

Ten test pesticides and their physicochemical properties are given in Table 1. All substances were obtained from ChemService (USA) in the highest available purity (all substances > 97%). Pesticide stock solutions were prepared in dimethylsulfoxide (DMSO, analytical grade, Shanghai, China) and stored at 4°C. The substance concentration in each aqueous stock solution was always lower than the water solubility of an individual chemical. All treatments solutions used for dissolving chemicals in experiment contained the same concentration of DMSO (1%, *V/V*).

1.2 Toxicity test

Toxicity tests of both individual pesticide and pesticide mixtures were performed on the SpectraMax M5 Multi-Detection Microplate Reader with a 96-well microplate (Molecular Devices Inc., USA). The procedure in detail was as follows: in 12 wells of the first row in a microplate, added 100 μL 1% (*V/V*) DMSO as 12 controls and in 12 wells of the second row, added, respectively, 12 different toxicant volumes derived by a dilution factor such as 0.618 and supplied 1% DMSO up to a total volume of 100 μL. In the same way as the second row, prepared various test solutions in 12 wells of the third, fourth, or fifth row. And then 100 μL bacterial suspension was added into each test well to make the final test volume be 200 μL. Then two duplicated microplates were done again (Ge et al., 2006; Liu et al., 2007).

Table 1 Physico-chemical properties, concentration-response models with some statistics, and two effect concentrations of ten pesticides

No.	Compound	CAS RN	MW	Purity (%)	logK_{ow}	Model	α	β	RMSE	*R*	EC_5 (mol/L)	EC_{50} (mol/L)
P1	Metamitron	41394-05-2	202.2	99.5	1.44	Logit	6.98	2.78	0.0206	0.995	2.26×10^{-4}	3.09×10^{-3}
						Weibull	5.38	2.29	0.0190	0.995		
P2	Metolachlor	51218-45-2	283.8	98.5	3.24	Logit	10.37	2.68	0.0188	0.998	6.37×10^{-6}	1.52×10^{-4}
						Weibull	6.85	1.89	0.0190	0.999		
P3	2,4-D	94-75-7	221.0	98	2.62	Logit	11.77	3.86	0.0177	0.997	1.30×10^{-4}	9.16×10^{-4}
						Weibull	8.96	3.07	0.0127	0.998		
P4	Carbofuran	1563-66-2	221.3	99	2.30	Logit	9.44	2.83	0.0202	0.997	2.86×10^{-5}	4.98×10^{-4}
						Weibull	6.57	2.1	0.0099	0.999		
P5	Imidacloprid	138261-41-3	255.7	99.5	0.56	Logit	7.93	2.26	0.0167	0.998	1.54×10^{-5}	3.10×10^{-4}
						Weibull	5.54	1.7	0.0232	0.996		
P6	Dylox (TM)	52-68-6	257.4	99	–0.28	Logit	4.86	2.49	0.0149	0.997	6.11×10^{-4}	1.12×10^{-2}
						Weibull	3.65	2.06	0.0122	0.998		
P7	Acetamiprid	135410-20-7	222.7	99.5	2.55	Logit	7.44	2.09	0.0100	0.999	1.07×10^{-5}	2.76×10^{-4}
						Weibull	4.89	1.5	0.0246	0.997		
P8	Methyl parathion	298-00-0	263.2	99.5	2.75	Logit	13.85	3.13	0.0146	0.998	4.31×10^{-6}	3.76×10^{-5}
						Weibull	9.96	2.35	0.0225	0.997		
P9	Monocrothophos	6923-22-4	223.2	99.5	–1.31	Logit	4.28	2.66	0.0166	0.995	1.92×10^{-3}	2.46×10^{-2}
						Weibull	3.29	2.26	0.0177	0.994		
P10	Phosphamidon	13171-21-6	299.7	97.6	0.38	Logit	7.66	2.73	0.0135	0.999	1.30×10^{-4}	1.56×10^{-3}
						Weibull	5.04	1.96	0.0316	0.995		

CAS RN: chemical abstracts services registry number; MW: relative molecular weight; RMSE: root mean square error; EC_5: concentrations for 5% inhibition of Q67 bioluminescence; EC_{50}: concentrations for 50% inhibition of Q67 bioluminescence.

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The relative light unit (RLU) measurements of Q67 in various wells in the test microplate were then determined using the Microplate Reader after 15 min exposure to the toxicants at (22 ± 1)°C. The toxicity of each pesticide or mixture to Q67 is expressed as an inhibition ratio (E or x) as Eq. (3):

$$E = x = \frac{I_0 - I}{I_0} \times 100\% \quad (3)$$

where, I_0 was an average of the RLU of Q67 exposed to the controls (12 parallels) and I was an average of the RLU to the test toxicant or mixture (3 parallels) in one microplate.

The bacteria (Q67) were purchased from East China Normal University as freeze-dried particles. They were stored at –24°C and rehydrated with 0.2 mL of 0.8% NaCl prior to testing. The standard methods for culture medium preparation and Q67 incubation have been introduced in previous articles (Liu et al., 2006; Ma et al., 1999; Zhang et al., 2008).

1.3 Experimental design for pesticide mixtures

Uniform design concentration ratio (UDCR) is an effective experimental design method introduced by Fang (2001). In contrast to the factorial design, such as the equivalent-effect concentration ratios (EECR), the experimental effort of the UDCR only linearly increases with the number of the components or the concentration levels of the components in the mixtures. Two EECR mixtures (EE10-05 and EE10-50) and ten UDCR ones (UD10-1, UD10-2, UD10-3, UD10-4, UD10-5, UD10-6, UD10-7, UD10-8, UD10-9, and UD10-10) including all ten pesticides were designed in this study. The concentration ratios (%) of various pesticides in the mixtures are listed in Table 2.

1.4 Data analysis

In this study, two different regression models (Scholze et al., 2001), Logit (Eq. (4)) and Weibull (Eq. (5)), were selected and the mathematical formulation are expressed as follows.

$$E = 1/(1 + \exp(-\alpha - \beta \log c)) \quad (4)$$

$$E = 1 - \exp(-\exp(\alpha + \beta \log c)) \quad (5)$$

where, c is the concentration; and α, β are the model parameters to be estimated.

Each individual set of data were fitted respectively by these two models and the best fit model was chosen using the correlation coefficient (R) and the root mean square error (RMSE) as a criterion where with the higher R or lower RMSE, the fitting will be better.

Table 2 Concentration ratios (%) of ten pesticides, concentration-response models with some statistics, and two effect concentrations of 16 mixtures

Mixtures	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
EE10-5	7.31	0.21	4.21	0.93	0.50	19.81	0.35	0.14	62.31	4.23
EE10-50	7.25	0.36	2.15	1.17	0.73	26.31	0.65	0.09	57.65	3.66
UD10-1	0.75	0.02	0.73	0.21	0.25	10.41	0.36	0.07	82.00	5.21
UD10-2	1.47	0.10	2.54	1.61	2.02	3.97	0.16	0.09	77.77	10.27
UD10-3	3.05	0.25	5.97	0.19	0.21	34.67	1.79	0.03	48.83	5.02
UD10-4	7.40	1.07	2.04	2.34	4.91	9.64	0.94	0.60	58.86	12.19
UD10-5	10.27	1.57	2.29	5.28	0.34	54.82	0.11	0.21	20.09	5.03
UD10-6	3.48	0.02	1.94	0.10	0.45	4.75	0.96	0.03	86.57	1.70
UD10-7	6.13	0.06	3.51	0.57	0.06	42.80	0.23	0.02	45.70	0.94
UD10-8	13.85	0.33	1.12	4.43	1.13	11.78	0.09	0.33	64.82	2.13
UD10-9	16.64	0.36	2.07	0.34	0.08	59.40	0.57	0.07	19.76	0.70
UD10-10	33.24	1.61	5.89	2.62	0.80	33.42	0.26	0.08	20.69	1.39
UD9-2	1.50	0.10	2.59	1.64	0.00	4.05	0.16	0.09	79.37	10.48
UD9-4	7.78	1.12	2.15	2.46	0.00	10.14	0.99	0.63	61.90	12.82
UD9-8	14.01	0.33	1.13	4.48	0.00	11.91	0.09	0.33	65.55	2.15
UD9-10	33.50	1.63	5.94	2.64	0.00	33.69	0.26	0.08	20.85	1.40

Mixtures	Model	α	β	RMSE	R	$EC_{5,mix}$ (mol/L)	$EC_{50,mix}$ (mol/L)
EE10-5	Logit	6.83	2.95	0.01223	0.9987	4.86×10^{-4}	4.84×10^{-3}
EE10-50	Logit	6.52	2.78	0.01404	0.9984	3.94×10^{-4}	4.52×10^{-3}
UD10-1	Logit	5.40	2.52	0.01655	0.9975	4.88×10^{-4}	7.20×10^{-3}
UD10-2	Logit	5.99	2.66	0.00833	0.9991	4.38×10^{-4}	5.60×10^{-3}
UD10-3	Logit	6.38	2.66	0.01562	0.9977	3.12×10^{-4}	3.99×10^{-3}
UD10-4	Logit	6.71	2.56	0.01805	0.9970	1.69×10^{-4}	2.39×10^{-3}
UD10-5	Weibull	5.10	2.12	0.01475	0.9983	1.56×10^{-4}	2.64×10^{-3}
UD10-6	Logit	5.41	2.56	0.01239	0.9983	5.45×10^{-4}	7.70×10^{-3}
UD10-7	Logit	5.64	2.67	0.01720	0.9961	6.09×10^{-4}	7.72×10^{-3}
UD10-8	Logit	6.69	2.79	0.01921	0.9958	3.52×10^{-4}	4.00×10^{-3}
UD10-9	Logit	6.65	2.86	0.01377	0.9983	4.42×10^{-4}	4.73×10^{-3}
UD10-10	Logit	8.04	3.17	0.01383	0.9985	3.43×10^{-4}	2.91×10^{-3}
UD9-2	Logit	6.01	2.72	0.01357	0.9979	5.10×10^{-4}	6.17×10^{-3}
UD9-4	Logit	7.16	2.78	0.00992	0.9991	2.32×10^{-4}	2.66×10^{-3}
UD9-8	Weibull	4.87	2.16	0.01780	0.9965	2.35×10^{-4}	3.76×10^{-3}
UD9-10	Weibull	5.37	2.28	0.01532	0.9973	2.20×10^{-4}	3.05×10^{-3}

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2 Results

2.1 Toxicity of individual pesticides to Q67

The toxicity parameters and some statistics, the root mean square error (RMSE) and relationship coefficient (R) of the best-fit models (Logit or Weibull) for all selected individual substances are summarized in Table 1, and the corresponding concentration-response functions are visualized regarding curve shape and position in Fig. 1.

From Table 1, the R between the responses observed and those from the best-fit functions were higher than 0.99 and RMSE lower than 0.019, which indicated a good statistical significance. The best concentration response model was the Logit function for five pesticides, such as imidacloprid (P5), acetamiprid (P7), methyl parathion (P8), monocrotophos (P9), and phosphamidon (P10) and the Weibull function for others. Various EC_x such as EC_{50} and EC_5 of an individual chemical can be easily computed from the fitted parameters (α and β) which are shown in Table 1. The EC_{50} values span approximately 2.8 orders of magnitude, ranging from 3.76×10^{-5} mol/L for methyl parathion (P8) to 2.46×10^{-2} mol/L for monocrotophos (P9). According to the EC_{50} values (Faust et al., 2001; Zhang et al., 2008), the toxicity sequence was: P9 < P6 < P1 < P10 < P3 < P4 < P5 < P7 < P2 < P8. For EC_5, a similar span of toxicity was observed with the range of the toxicity concentrations from 4.31×10^{-6} mol/L for methyl parathion (P8) to 1.92×10^{-3} mol/L for monocrotophos (P9).

Many researchers ever chose the effect level of $x = 1\%$ as a lower limit (Altenburger et al., 2000; Faust et al., 2001, 2003), but in this study we selected the response level of $x = 5\%$ as a low limit. Because down to lower than this level, the experimental data sets generally had poor reproducibility and the observed toxicity values were unreliable.

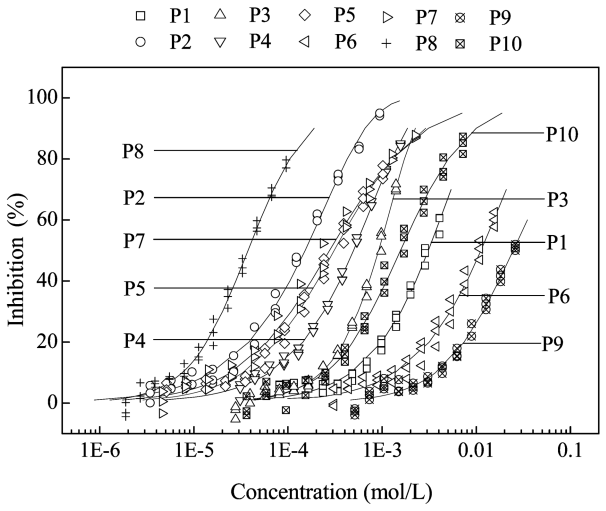

Fig. 1 Plot of inhibition vs. concentration of ten individual pesticides where the curves of five pesticides of P1, P2, P3, P4, and P6 are fitted to Weibull function and the other curves of five pesticides of P5, P7, P8, P9, and P10 are fitted to Logit function.

2.2 Toxicity of pesticide mixtures to Q67

2.2.1 Toxicity of pesticide mixtures based on EECR

The best-fit model (Logit) parameters as well as some statistics (RMSE and R) are listed in Table 2 and plots of effect (inhibition) vs. concentration of the EECR mixtures are shown in Fig. 2.

The concentration-response relationships observed were found to be in good agreement with those predicted by the concept of CA. In the case of EE10-5 mixture, the observed EC_{50} is 4.84×10^{-3} mol/L, which was only a difference of 15% with the predicted value 4.21×10^{-3} mol/L. The excellent predictive power of CA became even more prominent for the EE10-50 mixture. The EC_{50} predicted by CA was 4.27×10^{-3} mol/L and the observed EC_{50} was 4.52×10^{-3} mol/L, a difference was less than 6%. Only in the lower effect regions of the EE10-50 and the EE10-5 mixtures there were small differences between observations and predictions for CA (at maximum, a factor of 1.6). In contrast, the concept of IA overestimated the mixture toxicity at every response level. At the level of 50% effect, the predictions by IA deviated from the observed mixture toxicity at the most by a factor 1.6 for both concentration ratios tested.

2.2.2 Toxicity of pesticide mixtures based on UDCR

From Table 2, all UDCR mixtures were effectively characterized by Logit function except UD10-5 mixture (Weibull function). The correlation coefficients (R) were higher than 0.995 and the values of RMSE were lower than 0.020, which indicated that the reproducibility of experimental data was very high and the selected regression models were appropriate for this study. The corresponding $EC_{50,\,mix}$ values of all UDCR mixtures ranged from 2.39 $\times 10^{-3}$ mol/L for UD10-4 mixture to 7.72×10^{-3} mol/L for UD10-7 mixture. In comparison with the individual pesticides, the $EC_{50,mix}$ values for tested UDCR mixtures did not exceed the least toxic component (monocrotophos) of 2.46×10^{-2} mol/L and were higher than the most active component (methyl parathion) of 3.76×10^{-5} mol/L (Table 1). Similarly, the $EC_{5,mix}$ values were lower than the least toxic component (monocrotophos) of 1.92×10^{-3} mol/L and were higher than the most toxic component (methyl parathion) of 4.31×10^{-6} mol/L.

The bioluminescence response inhibited by the UDCR mixtures are shown in Fig. 2, together with the curves predicted by CA (solid lines) and IA (dotted lines). For the sake of simplicity, the best-fit concentration-response curves for the mixtures have been omitted from the figures. In general, the mixture toxicity predicted by CA was lower than IA did. This was not specific for the mixture ratio analyzed and the effect level under observation. In comparison with IA, observed concentration-response relationships were found to be in very good agreement with those predicted by the concept of CA except four UDCR mixtures, UD10-2, UD10-4, UD10-8 and UD10-10. For the four UDCR mixtures, the extent of the difference between the predictions was dependent on the mixture ratio and the effect level. In the lower effect regions

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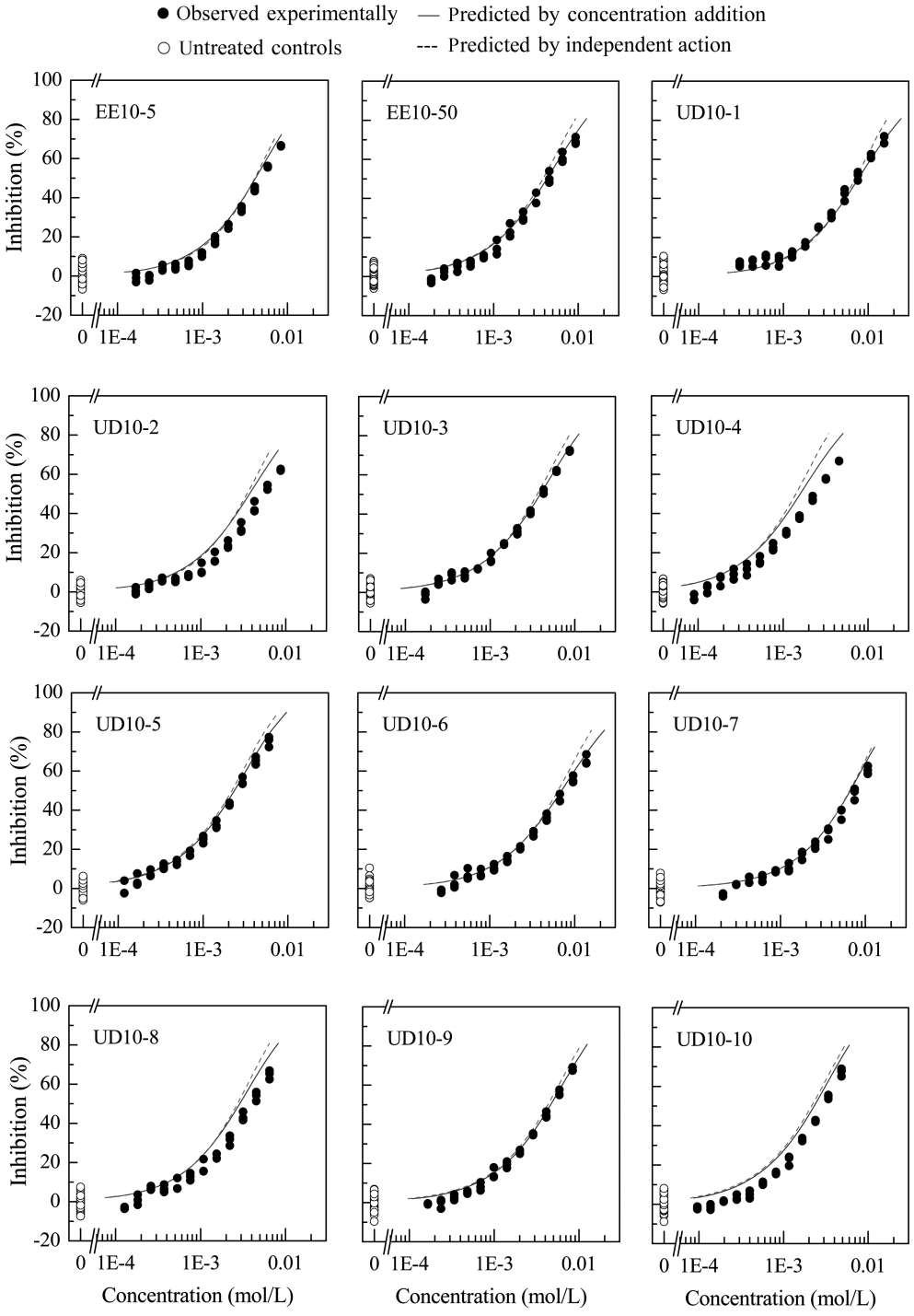

Fig. 2 Plot of inhibition vs. total concentration for two EECR and ten UDCR mixtures including ten pesticides.

(< 10%) of the four UDCR mixtures, there were small differences between observations and predictions for CA. But in the higher effect region (> 50%), the deviations were relatively large.

3 Discussion

CA model may be used as a slightly conservative, but broadly applicable model with a relatively small likelihood of underestimating effects due to interactions, and has even been proposed as the general solution for mixture toxicity analysis (Berenbaum, 1985). With this perspective, the existence of a common molecular target sites is not a guarantee for CA. Different unspecific sites or dissimilar toxicokinetic characteristics of chemicals may result in an altered mode of combined action, such as some environmentally relevant pesticide mixtures (Broderius and Kahl, 1985; Faust et al., 1994; Junghans et al., 2006). Some researchers discussed the reason for this additivity (Backhaus et al., 2000a). They thought that these compounds based on the environmental relevance were usually rather lipophilic; hence, the mixture components share at least partly a common model of action (baseline toxicity, narcotic mode of action), so that the observed toxicity may

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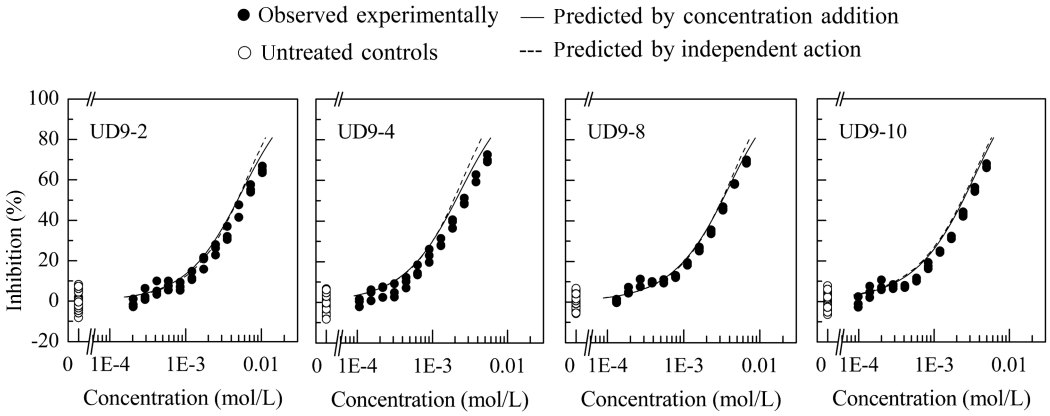

Fig. 3 Plot of inhibition vs. total concentration for four UDCR mixtures including nine pesticides without imidacloprid.

be systematically shifted toward the predictions made by CA. This theory is consistent with the result of this study.

Junghans et al. (2003b) discussed the quantitative relationship between predictions according to CA and IA. They thought that the different steepness of different mixture system was the main factor for the reverse quantitative relationships between CA and IA. The steeper the concentration-response curves were, the higher prediction values of IA would be. Using the ratio between EC_5 and EC_{50} as a measure for the steepness of a concentration-response curve, we obtained an average value of 0.073 for all pesticides presented in this study, which showed that the concentration-response relationships of the tested pesticides were rather flat compared with other studies. The same result was obtained for six OPs with the same test organism and the average value was 0.038 (Zhang et al., 2008).

As shown in Fig. 2, four UDCR mixtures, UD10-2, UD10-4, UD10-8 and UD10-10, deviated from the predicted concentration-response curves of CA model. The degree of deviation between the predictions is dependent on the mixture ratio and the effect level. In the four UDCR mixtures, the deviations between observations and CA predictions were small in the lower effect region (< 10%), but large in the higher effect region (> 50%). The concentration ratios of individual chemicals were analyzed in the four UDCR mixtures (Table 2) and we found that the concentration ratios of imidacloprid in the four UDCR mixtures were relatively higher than that in other pesticide mixtures. To examine the influence of imidacloprid in the four UDCR mixtures (UD10-2, UD10-4, UD10-8, UD10-10), it was removed from the ten-pesticide mixture and the remaining nine chemicals (UD9-2, UD9-4, UD9-8, UD9-10) were combined at the same relative proportions based on the UDCR method and the concentration ratios of various mixtures are listed in Table 2. Previous researchers have used this method to study the influence of one chemical in whole mixture (Gennings et al., 2004; Moser et al., 2005). As a result, there was no significant departure from CA for the scattered points with the nine remaining pesticides omitting imidacloprid (Fig. 3). Thus, we concluded that imidacloprid significantly influenced the other pesticides and was not dose-responsive alone.

To predict the potential mixture toxicity of pesticides detected in water bodies, several studies used the toxic unit (TU) approach (Anderson et al., 2003; Battaglin and Fairchild, 2002; George et al., 2003; Hunt et al., 2003; Steen et al., 1999; Thomas et al., 2001) which was proposed by Sprague (1970). Figure 4 shows the TU values of imidacloprid in ten UDCR mixtures. It has been found that the TU values of imidacloprid in four UDCR mixtures (UD10-2, UD10-4, UD10-8, UD10-10) were significantly higher than others, which were consistent with the extent of the deviation between observed experimental data set and concentration-response curve of CA model. The higher the TU values, the larger the deviation from concentration-response curve of CA model (Figs. 2 and 4).

Loewe and Muischnek (1926), as well as Bliss (1939), have also discussed mixtures in which the observed combination effect for a fixed endpoint cannot be predicted by CA or IA. Plackett and Hewlett (1952) concluded that, irrespective of the similarity of action, compliance with one of the two concepts can only be expected if there are no interaction between the mixture components (Plackett and Hewlett, 1952). They defined the term "interaction" on a physiological basis by stating that the mixture components can be ascribed the potential to interact by quantitatively altering the action of another component in the mixture. In

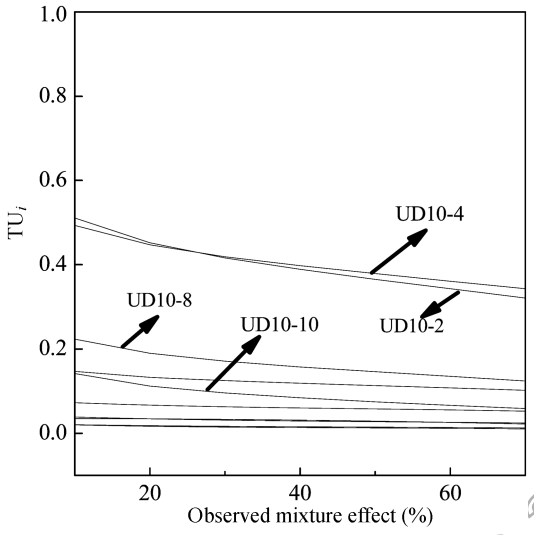

Fig. 4 Toxic unit (TU_i) of imidacloprid in ten UDCR mixtures.

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these mixtures, the deviation of the observed combination effects from the predictions by CA or IA would be strongly dependent on the ratio of the mixture components. Thus, we concluded that imidacloprid could interact with other pesticides in this study, especially when the concentration ratio of imidacloprid was far beyond the threshold value.

4 Conclusions

The study showed that overall toxicity of the multiple-component mixtures of ten pesticides were in very good agreement with those predicted by the concept of CA except four UDCR mixtures, UD10-2, UD10-4, UD10-8 and UD10-10. Imidacloprid may significantly influence the other pesticides because of the properties of itself, especially when the concentration ratio of imidacloprid was far beyond the threshold value. The concentration-response relationships of the tested pesticides were rather flat compared with other studies (an average value of 0.073), which resulted in a challenge of the precautionary principle for CA. Nevertheless, the accuracy of CA even for the prediction of the toxicity of a multiple mixture composed of chemicals with slightly different modes of action have been demonstrated by many studies (Broderius and Kahl, 1985; Faust et al., 1994; Munkegaard et al., 2008; Olmstead and LeBlanc, 2005). Moreover, the observed toxicities of pesticide mixtures did not exceed the toxicities predicted by CA and were rather accurately predictable by this concept. Although the mechanisms that control these interactions cannot be revealed from these experiments, CA may be a reasonable assumption for the hazard assessment of mixtures of chemicals with unspecific mechanisms of action. This becomes even more evident if the mechanisms of interactions at the quantitative level are understood and confidence can be gained to provide realistic risk assessments for chemical mixtures with unspecific mode of action.

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