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Effect of ionic liquid on the toxicity of pesticide to *Vibrio-qinghaiensis* sp.-*Q67*

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ABSTRACT

Ionic liquids (ILs) are novel green chemicals and used to replace traditional volatile organic solvents in industrial processes. Yet the potential effects of ILs on the toxicities of chemicals such as pesticides had been poorly studied. The aim of this paper is to determine the joint toxicity between IL and pesticide. Desmetryn (DES) and dichlorvos (DIC) were chosen as representatives of pesticides and 1-butyl-2,3-dimethylimidazolium chloride (IL1) and 1-butyl-pyridinium bromide (IL2) as those of ILs. The toxicities of the pesticides and ILs as well as their binary mixtures on *Vibrio-qinghaiensis* sp.-*Q67* were determined using the microplate toxicity analysis. A simplified central composite design (SCCD) was employed to design the concentration distribution of components in binary mixtures to effectively detect the possible toxic interactions between pesticide and IL over the whole concentration range. Results showed that all the binary mixtures between pesticide and IL exhibited a similar toxicity action rule, i.e., displayed a synergistic interaction in a high concentration region, an additive action in a medium concentration region, and an antagonistic interaction in a low concentration region. The reason how to produce the toxic interaction is still under study.

1. Introduction

Ionic liquids (ILs), being a relatively recent magical chemical due to their unique properties, have a large variety of applications in all areas of the chemical industries [1]. The areas of application include catalysis [2], extraction [3,4], synthesis [5], dissolution [6,7], nuclear industry [8], food science [9], etc. Non-volatility and nonflammability are their common characteristics giving them an advantageous edge in various applications. Because they are nonvolatile, ILs are relatively benign to the atmosphere, but their impacts on aquatic organisms and communities are largely unknown [10]. It has been shown that some ILs react with water, whereas others, including imidazolium- and pyridinium-based ILs, are water stable. It is important to make the toxicity of IL itself and its impact on the toxicity of other pollutants in aquatic environment clear. Kulacki and Lamberti examined the toxicity of imidazolium ILs to freshwater algae [11]. Arning et al. used qualitative and quantitative structure–activity relationships to explore the inhibitory effects of cationic head groups, functionalised side chains and anions of ILs on acetylcholinesterase [12]. Matzke et al. studied the influence of anion species on the toxicity of 1-alkyl-3-methylimidazolium ILs observed in an ecotoxicological test battery [13]. Also, we systematically determined the toxicities of 12 ILs in a variety of concentrations on *Vibrio-qinghaiensis* sp.-*Q67* (Q67) and found that four of 12 ILs had relative high toxicity [14].

However, whether ILs have an impact on the toxicity of the other pollutants possibly coexisted with them in aquatic environment are almost completely unknown. The major purpose of this paper is to try to explore possible toxic interaction between pesticides and ILs. To do this, it is very important to use a rational experimental design to effectively detect the possible interaction in all concentration range of two components, pesticide and IL. Central composite design (CCD) is one of the most useful response surface exploration method, and widely used in the optimization of many procedures [15–19]. CCD derived from the $(n \times n)$ design can almost cover any possible combinations. In general, CCD is constructed in such a way that $(2^n + 2n + 1)$ experiments are required, where n is the number of factors to be studied, 2^n means the number of the experiments for boundary points, $2n$ means the number of the experiments for axial spots, and the number “1” means the experiment for a central point [19]. In this paper, various binary mixtures between one IL and one pesticide are designed using a simplified central composite design (SCCD) method directly taken from the literature [20]. In our preliminary test, it is found that there could be synergistic interaction between some ILs and insecticides. To validate this, we selected desmetryn (DES) and dichlorvos (DIC) as representatives of pesticides and 1-butyl-2,3-dimethylimidazolium chloride (IL1) and 1-butyl-pyridinium bromide (IL2) as those of ILs to explore the toxic interactions of four pesticide and IL com-

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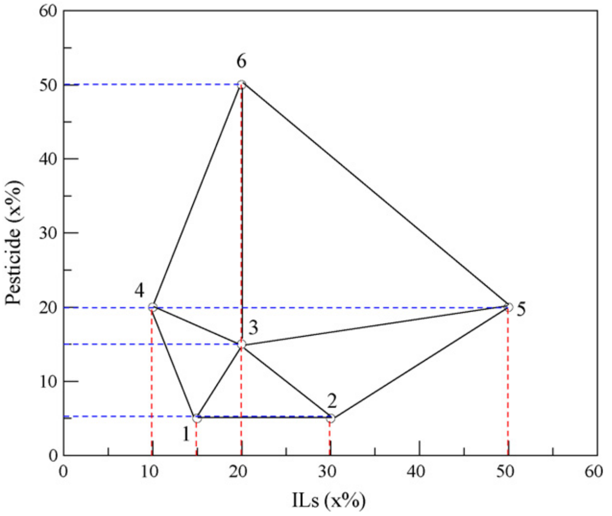


Fig. 1. The distribution of effect concentrations in six binary mixtures (nos. 1–6) between pesticide and IL designed using SCCD.

binations, DIC and IL1, DES and IL1, DIC and IL2, and DES and IL2. The toxicity data of the pesticides and ILs as well as their binary combinations to *Vibrio-qinghaiensis* sp.*-Q67* (Q67) were determined by using the microplate toxicity analysis (MTA) previously developed in our laboratory [21–24]. The concentration compositions of six binary mixtures (six points shown in Fig. 1) for each pesticide–IL combination such as DIC–IL one were designed by the above SCCD procedure. To examine the toxic interaction along the concentration–response curve (CRC), each of six binary mixtures, each point, was extended into a concentration–response curve by using the fixed concentration ratio ray procedure [25–27]. Comparing the total concentration–response curve (t-CRC) of the mixture observed experimentally to the CRC predicted from the concentration addition (CA) or independent action (IA) model [28,29], it is found that all the binary mixtures between pesticide and IL exhibited a similar toxicity action rule, i.e., displayed a synergistic interaction in a high concentration region, an additive action in a medium concentration region, and an antagonistic interaction in a low concentration region.

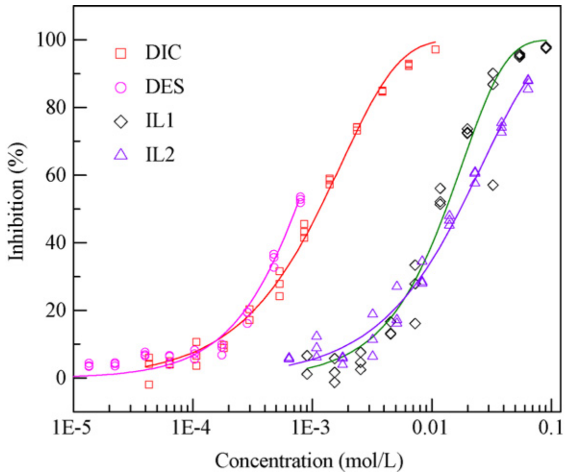


Fig. 2. The concentration–response relationships of DIC (□), DES (○), IL1 (◊), and IL2 (△) to Q67.

Table 2
The Weibull-type concentration–response models and their statistics as well as medium effect concentrations of dichlorvos (DIC), desmetryn (DES), and two ILs.

Brief	n^a	α	β	RMSE	R	EC_{50} (CI)^b
DIC	12	5.65	2.05	0.0198	0.9986	1.16E−3 (9.18E−4, 1.43E−3)
DES	12	7.94	2.66	0.0233	0.9916	7.54E−4 (6.32E−4, 8.85E−4)
IL1	10	4.64	2.65	0.0522	0.9903	1.31E−2 (1.14E−2, 2.51E−2)
IL2	10	3.05	1.92	0.0341	0.9934	1.73E−2 (7.60E−3, 2.20E−2)

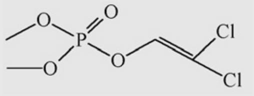
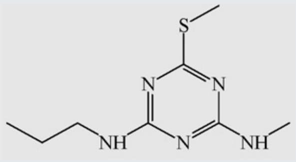
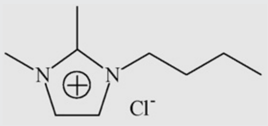
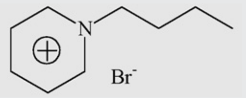
[a] Number of concentration gradients.
[b] CI refers to 95% confidence interval.

2. Materials and methods

2.1. Chemicals

Desmetryn (DES) was purchased from Sigma–Aldrich (Germany). 1-Butyl-2,3-dimethylimidazolium chloride (IL1) and 1-butyl-pyridinium bromide (IL2) were purchased from ACROS (USA). Dichlorvos (DIC) was purchased from DIKMA Company. These test chemicals together with their structures, formula, CAS register numbers, and other related parameters, were listed in Table 1.

Table 1
Some physicochemical properties of dichlorvos, desmetryn, and two ionic liquids.

Compound (abbr.)	Formula	CAS-RN	Stucture	Purity (%)	Molecular weight
Dichlorvos (DIC)	$C_4H_7O_4Cl_2P$	62-73-7		99.2	220.98
Desmetryn (DES)	$C_8H_{15}N_5S$	1014-69-3		98.1	213.30
1-Butyl-2,3-dimethylimidazolium chloride (IL1)	$C_9H_{17}ClN_2$	98892-75-2		>98.0	188.70
1-Butyl-pyridinium bromide (IL2)	$C_9H_{14}NBr$	874-80-6		>98.0	216.12

2.2. Cell culture

The freeze-dried luminescent bacterium *Q67* was supplied by East China Normal University [30]. The culture medium consists of 13.6 mg KH_2PO_4, 35.8 mg $Na_2HPO_4 \cdot 12H_2O$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.61 g $MgCl_2 \cdot 6H_2O$, 33.0 mg $CaCl_2$, 1.34 g $NaHCO_3$, 1.54 g NaCl, 5.0 g yeast extract, 5.0 g tryptone, 3.0 g glycerin, and 1000 mL Milli-Q water and adjusted to pH 8.5 ± 0.5. Before each test, the bacteria were inoculated from a stock culture, which was maintained on *Q67* culture medium agar at 4 °C, to a fresh agar plate and cultured at 22 ± 1 °C for 24 h. The cells were further grown in a liquid culture medium by shaking (120 rev/min) at 22 ± 1 °C for 18 h until the final relative light unit (RLU) reached about 2.0×10^5 for the toxicity tests [21,22,31].

2.3. Toxicity test

According to the microplate toxicity analysis (MTA) developed in previous study [22–24], the toxicity of any component (DES, DIC, IL1, or IL2) or any binary mixture of pesticide and ionic liquid on *Q67* was determined on the SpectraMax M5 reader (Molecular Devices Inc., USA) with 96-well microplate. An appropriate dilution factor was selected after some preliminary experiments to make the response (inhibition) values equably locate in the range from the maximum inhibition to minimum inhibition. To construct a concentration–response curve (CRC) of a chemical or a total concentration–response curve (t-CRC) of a binary mixture, 12 different test concentrations (total concentrations for the mixtures) in three parallels and 12 controls in a 96-well microplate were arranged and the microplate test was repeated three times. The procedure in detail was as follows: in 12 wells of the first row in the microplate, added 100 μL Milli-Q water as 12 controls. In 12 wells of the second row, added, respectively, 12 different toxicant volumes derived by an appropriate dilution factor and supplied Milli-Q water 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 and fourth row. And then 100 μL bacterial suspension was added into each test well to make the final test volume 200 μL. The relative light units of *Q67* in various wells in the test microplate were then determined using the reader after 15 min exposure to the toxicants at 22 ± 1 °C. The toxicity of each substance or mixture is expressed as an inhibition ratio (E of x) as follows:

$$E\ (\%) = x = \frac{I_0 - I}{I_0} \times 100 \tag{1}$$

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

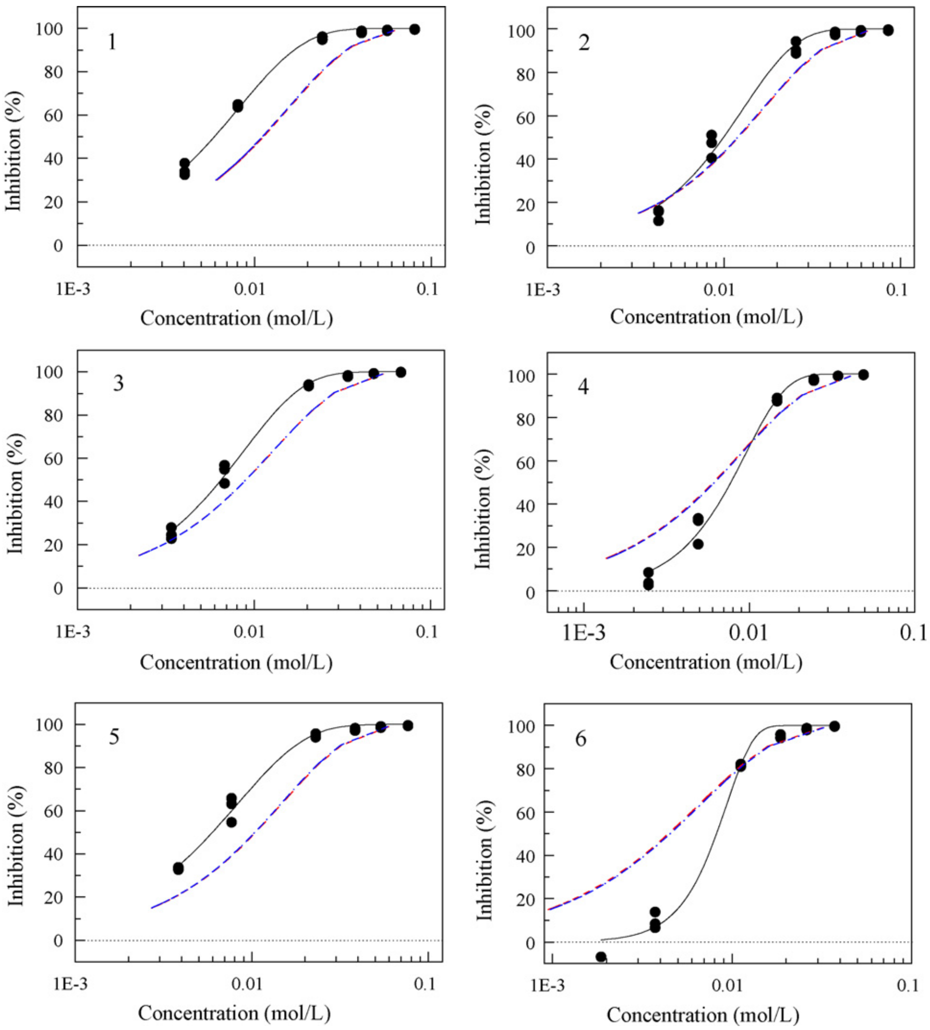


Fig. 3. Comparison of the experimental points (●) and CRC (solid line) observed with the CA-CRC (dash line) and IA-CRC (dash dot line) predicted by CA and IA models for 24 binary mixture ratios. ((a) DIC–IL1 combination; (b) DES–IL1 combination; (c) DIC–IL2 combination; (d) DES–IL1 combination).

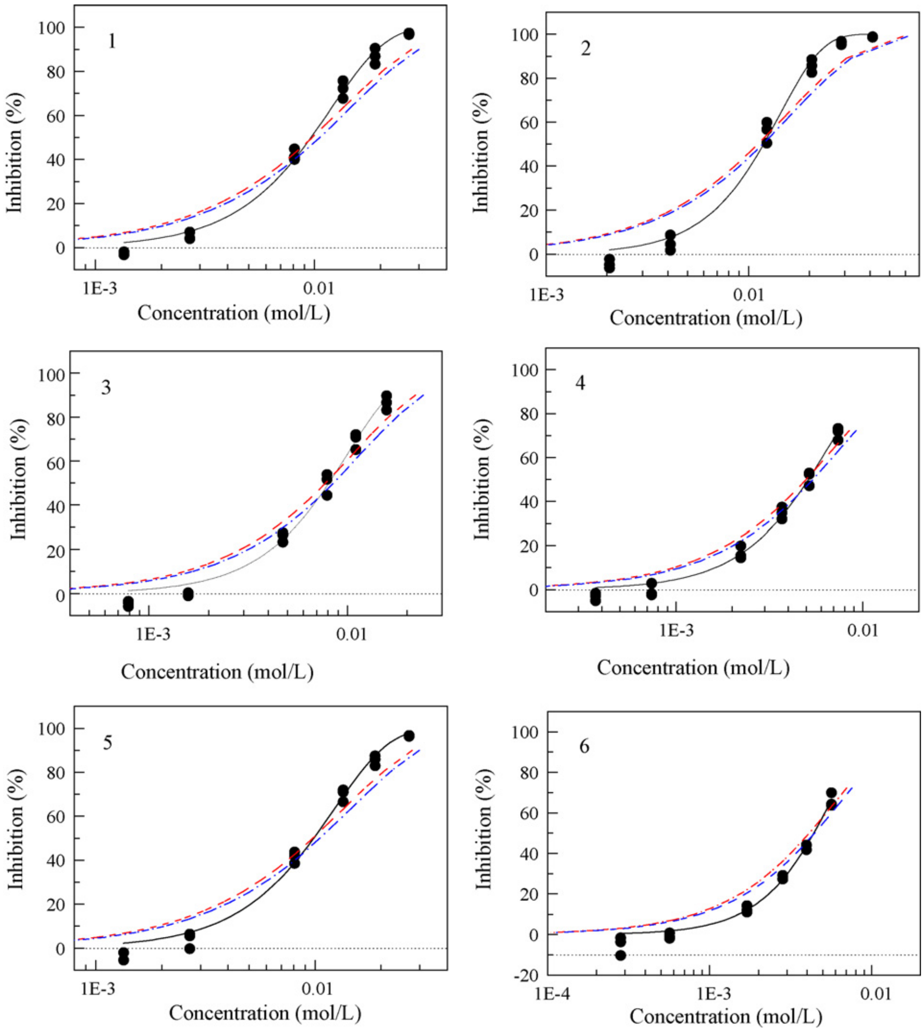


Fig. 3. (*Continued*)

2.4. Binary mixture design

To effectively examine the interactions between various concentration pairs of pesticide and IL, a simplified central composite design (SCCD) is employed to design the concentration compositions in various binary mixtures between pesticide and IL. The SCCD used in this study is directly taken from Table 4 in the literature [20] and it is derived from CCD procedure of two-factor with five-level to save the experimental cost. Fig. 1 shows the distribution of various effect concentrations between IL and pesticide from the SCCD. Six points (nos. 1, 2, 3, . . ., 6) in Fig. 1 represented the effect concentrations of six binary mixtures between one pesticide and one IL. In this paper, there are in all 24 binary mixtures consisted of four combinations of DIC and IL1, DIC and IL2, DES and IL1, and DES and IL2. The concentration ratios of pesticide to IL are nos. 1 (EC_5:EC_{15}), 2 (EC_5:EC_{30}), 3 (EC_{15}:EC_{20}), 4 (EC_{20}:EC_{10}), 5 (EC_{20}:EC_{50}), and 6 (EC_{50}:EC_{20}), respectively. Then, each of six mixture points depicted in Fig. 1 was expanded into a total concentration–response curve (also called a ray) using a fixed concentration ratio ray design [25–27].

2.5. Concentration–response curve fitting

To quantitatively describe various effect concentrations (EC_x), especially at low effect, the observed concentration-effect data were fitted by a non-linear function, Weibull with two parameters (α and β) [32]. The fitted goodness is characterized by using the relationship coefficient (R) and the root mean square error (RMSE) between the effects (luminescence inhibition) observed and predicted by the Weibull function. The Weibull function is written as follows:

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

where α and β are the location and slope parameters depicting a CRC model, E was the effect or response to *Q67*, and c was the test concentration of single substance or mixture.

3. Results and discussion

3.1. Toxicity of single pesticide or ionic liquid on Q67

It has been shown that the concentration–response data of DIC, DES, IL1, and IL2 are well fitted to Weibull model. The fitted concentration–response curves (CRCs) are shown in Fig. 2. The corresponding fitted parameters (α and β) and some statistics (RMSE and R) are given in Table 2. It should be indicated that two points located at the low concentration area for IL1 and IL2 in Fig. 2 display a little curl upwards. Only 10 points ($n = 10$) were, therefore, used in the Weibull function fit procedure. From Table 2, the R's between the responses observed and fitted by Weibull function are higher than 0.990 and the RMSE are lower than 0.025, which indicates a good significance statistically.

Various EC_x required in SCCD procedure, such as EC_{50}, EC_{20}, EC_{10}, and EC_5 of pesticide (DIC or DES) and EC_{50}, EC_{30}, EC_{20}, EC_{15}, and EC_{10} of IL1 or IL2 (from Fig. 1), can be easily computed from the fitted Weibull function and the EC_{50} values and their 95% confidence interval (CI) were listed in Table 2.

From Fig. 2, the toxicities of two pesticides are obviously higher than those of two ILs. Also, the values of EC_{50}'s in Table 2, about 10^{-3} for DES and DIC and 10^{-2} for IL1 and IL2, explain it. Then, how does IL affect the toxicity of DES or DIC or how much is there combined toxicity when IL entering into environment is mixed with pesticide? To address this problem, it is necessary to examine the combined toxicities in binary mixtures between ILs and pesticides with various concentration compositions.

3.2. *Toxic interaction between pesticide and ionic liquid*

The combined toxicities of 24 binary mixture rays designed by using SCCD procedure and fixed concentration ratio ray design were determined using the microplate toxicity analysis and the toxicity data for each ray were then fitted to the Weibull function. Here, each ray includes six concentration–response points with a fixed concentration ratio of pesticide and IL. The fitted results and some statistics of various mixture rays were listed in Table 3. It has been shown that 24 mixture rays exhibited a good statistical significance with the relationship coefficient of >0.99, which explains that all binary combinations of pesticide and IL have good total concentration–response relationship.

Comparing the t-CRC observed experimentally with the t-CRC predicted by the concentration addition (CA) and/or independent action (IA), it is possible to derive toxicity interaction information about antagonistic, synergistic, CA, or IA between IL and pesticide. The t-CRCs observed and t-CRCs predicted by CA (CA-CRC) and IA (IA-CRC) of 24 binary mixture rays of four combinations: DIC–IL1, DES–IL1, DIC–IL2, and DES–IL2, between ILs and pesticides are shown in Fig. 3 together with the observed CRCs of single pesticide and IL. To explain the toxic interaction between pesticide and IL, two cross-points, CP_{CA} between t-CRC observed and CA-CRC predicted by CA model and CP_{IA} between t-CRC and IA-CRC predicted by IA model (seeing Fig. 4), were obtained from Fig. 3 and listed in Table 3.

From Fig. 3a, three mixture rays (nos. 1, 3, and 5 in Fig. 1) of six t-CRCs observed are, on the whole, higher than both the CA-CRCs and the IA-CRCs, displaying a synergism interaction and there being no cross-point (CP) between t-CRC observed and CA-CRC or IA-CRC predicted. For the other three mixture rays (nos. 2, 4, and 6 in Fig. 1) in Fig. 3a, there are two almost equal cross-points, CP_{CA} and CP_{IA} (seeing Table 3), displaying a synergism interaction in a higher concentration range than the concentration of the CP_{IA} or CP_{CA}, and an antagonism interaction in the concentration range lower than the concentration of CP_{IA} or CP_{CA}. The values of the concentration and inhibition (%) in all cross-points were listed in Table 3.

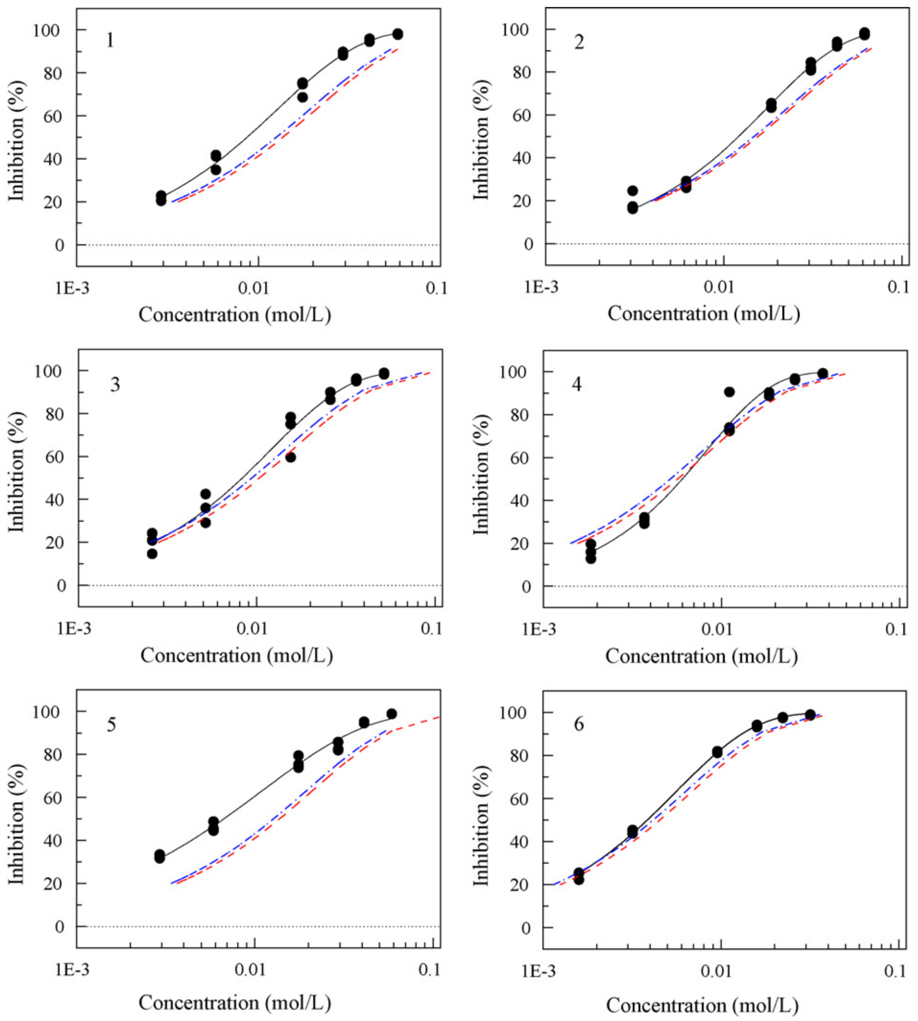


Fig. 3. (*Continued*)

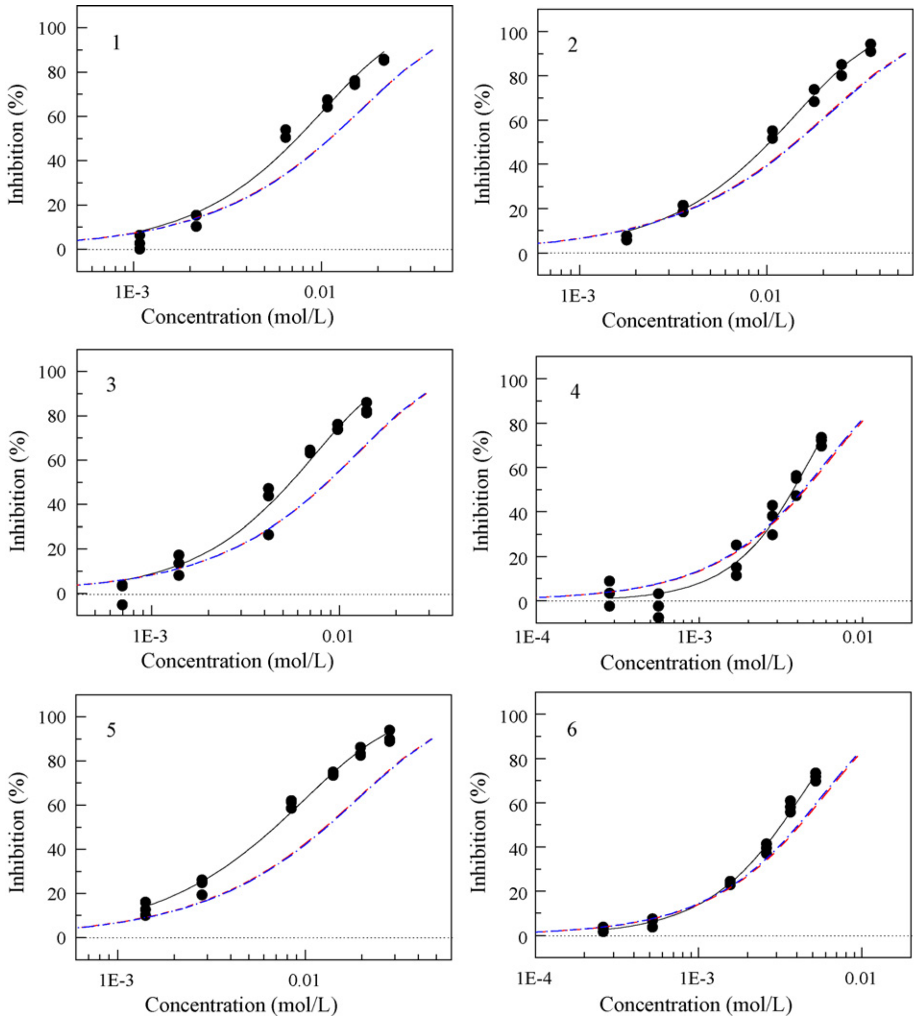


Fig. 3. (*Continued*).

From Table 3 and Fig. 3b, for the DES–IL1 combination, all the t-CRC of six mixture rays had a cross-point not only with CA-CRC but also with IA-CRC. Different from the DIC–IL1 combination, the CA-CRCs are higher than the IA-CRC. Thus, all six mixture rays display a synergism interaction in a higher concentration range than the concentration of the CP_{CA}, an addition action in the concentration range higher than the concentration of CP_{IA} but lower than that of CP_{CA}, and an antagonism interaction in the concentration range lower than the concentration of CP_{IA}.

From Table 3 and Fig. 3c, apart from the mixture ray of no. 3, the toxicity interaction results between DIC and IL2 are similar to those between DIC and IL1. For the ray of no. 3, there is no cross-point between the t-CRC and IA-CRC or CA-CRC in DIC–IL combination, while there is a cross-point between the t-CRC and IA-CRC in DIC

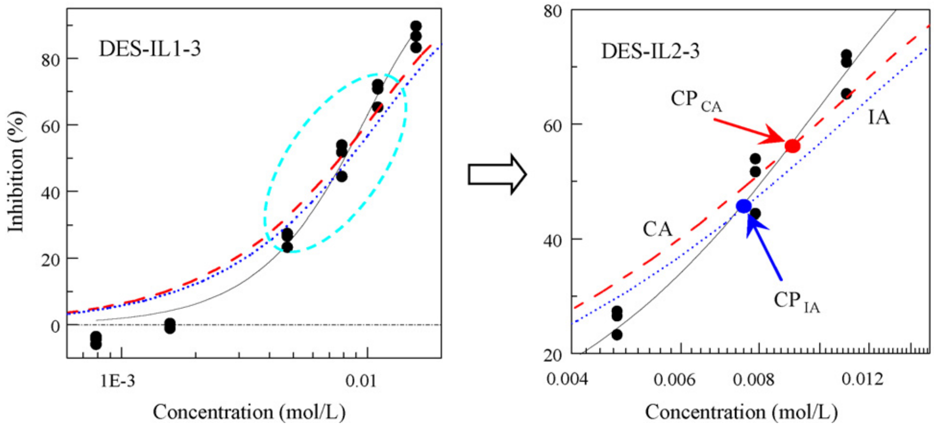


Fig. 4. From the concentration–response relationships of the binary mixture between DES and IL2 to two cross-points, CP_{CA} between t-CRC and CA-CRC as well as CP_{IA} between t-CRC and IA-CRC.

Table 3

Various concentration ratios (CR) of pesticide and IL in the binary mixtures based on the simplified CCD procedure, the Weibull models and their statistics, the cross-points (CP_{CA} or CP_{IA}) between the CRC observed and one predicted by CA or IA.

Binary mixture ray	No. in SCCD	CR_{PEST}	CR_{IL}	α	β	RMSE	R	CP_{CA} (ECx, x%)	CP_{IA} (ECx, x%)
DIC–IL1	1	0.0164	0.9836	5.58	2.67	0.0132	0.9992	–	–
	2	0.0084	0.9916	6.20	3.28	0.0258	0.9974	4.91E−3, 22.72	5.11E−3, 23.52
	3	0.0443	0.9557	5.95	2.89	0.0113	0.9994	–	–
	4	0.1127	0.8873	8.23	4.07	0.0211	0.9988	1.04E−2, 69.23	1.03E−2, 68.52
	5	0.0241	0.9759	5.37	2.58	0.0125	0.9994	–	–
	6	0.1911	0.8089	10.00	4.92	0.0649	0.9959	1.11E−2, 81.25	1.10E−2, 80.23
DES–IL1	1	0.0209	0.9791	7.64	3.97	0.0262	0.9986	9.39E−3, 48.48	8.18E−3, 39.99
	2	0.0107	0.9893	8.64	4.68	0.0345	0.9981	1.29E−2, 55.79	1.19E−2, 50.50
	3	0.0421	0.9579	7.82	3.91	0.0324	0.9975	8.78E−3, 55.89	7.36E−3, 44.68
	4	0.0999	0.9001	8.27	3.77	0.0228	0.9981	5.45E−3, 54.11	4.39E−3, 42.35
	5	0.0211	0.9789	7.52	3.93	0.0301	0.9981	1.01E−2, 51.30	8.81E−2,43.18
	6	0.1333	0.8667	9.25	4.08	0.0263	0.9969	4.95E−3,58.25	4.41E−3, 49.28
DIC–IL2	1	0.0195	0.9805	4.09	2.16	0.0078	0.9996	–	–
	2	0.0078	0.9922	4.06	2.31	0.0174	0.9985	3.09E−3, 16.18	3.89E−3, 20.22
	3	0.0476	0.9524	4.36	2.27	0.0078	0.9997	–	3.28E−3, 24.41
	4	0.1474	0.8526	5.63	2.70	0.0197	0.9983	7.27E−3, 57.71	9.28E−3, 68.90
	5	0.0184	0.9816	3.29	1.68	0.0202	0.9967	–	–
	6	0.2031	0.7969	5.65	2.05	0.0198	0.9986	2.77E−3, 36.98	1.62E−3, 25.46
DES–IL2	1	0.0255	0.9752	5.08	2.56	0.0476	0.9903	–	–
	2	0.0100	0.9900	4.57	2.50	0.0170	0.9987	2.78E−3, 15.20	2.56E−3, 14.63
	3	0.0452	0.9548	5.73	2.70	0.0314	0.9954	7.68E−3, 6.686	7.71E−3, 6.686
	4	0.1312	0.8688	8.53	3.67	0.0261	0.9954	2.68E−3, 33.03	2.78E−3, 34.97
	5	0.0161	0.9839	4.39	2.20	0.0206	0.9977	–	–
	6	0.1423	0.8577	7.11	3.00	0.0128	0.9987	1.11E−3, 15.10	1.09E−3, 16.25

and IL2 combination, displaying a synergism interaction in a higher concentration range than the concentration of the CP_{IA}, an addition action in the concentration range higher than the concentration of CP_{CA} but lower than that of CP_{IA}, and an antagonism interaction in the concentration range lower than the concentration of CP_{CA}. Moreover, all the IA-CRCs in DIC–IL2 combination are significantly higher than the IA-CRCs, which is different from the DIC–IL1 combination where all the IA-CRCs are close to the CA-CRCs (Fig. 3a). Thus, liking the DES–IL1 combination, there is an addition action region in a medium concentration range from the concentration of CP_{CA} to that of CP_{IA}. However, different from the DES–IL1 combination where the IA-CRCs are lower than CA-CRC, the IA-CRCs are higher than CA-CRCs.

For six mixture rays between DES and IL2, apart from the third ray, the toxic interaction of the other five rays is the same as those in DIC–IL1 combination, displaying a synergism interaction in whole concentration range or in a higher concentration range than the concentration of the CP_{CA} ($\approx CP_{IA}$), and an antagonism interaction in the concentration range lower than the concentration of CP_{CA} (Fig. 3d). The third ray in the DIC–IL1 combination has no cross-point with the CA-CRC or IA-CRC, while the ray in the DES–IL2 combination has a cross-point not only with the CA-CRC but also with the IA-CRC.

From above results and analysis on Table 3 and Fig. 3, the rule of toxicity interaction between pesticide and IL is very similar, displaying a synergism interaction in a high concentration range of pesticide or IL, an addition action in a medium concentration range, and an antagonism interaction for the low concentration range.

4. Conclusion

Using the simplified central composite design (SCCD) and fixed concentration ratio ray method to design the binary mixture rays and the microplate toxicity analysis (MTA) to determine the toxicity, it has been found that all the binary mixtures between pesticide and IL exhibited a similar toxicity action rule, displaying a synergistic interaction in a high concentration region, an additive action in a medium concentration region, and an antagonistic interaction in a low concentration region. This is an important finding which will encourage us to further study on the toxic interaction mechanism of pesticides and ILs.

Acknowledgments

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