

Combining the uniform design-based ray procedure with combination index to investigate synergistic lethal toxicities of ternary mixtures on *Caenorhabditis elegans*

Han-Xiao Tang,^a Shu-Shen Liu,^{*a} Kai Li^a and Li Feng^a

(Key Laboratory of Yangtze River Water Environment, Ministry of Education, College of Environmental Science and Engineering, Tongji University, Shanghai 200092)

Supporting Information

Contents

- Table S1 The properties of five chemicals and the concentrations of stocks
- Table S2 Fitting regression coefficients (α and β), statistics (R^2 and RMSE), LC_{50} and its 95% confidence intervals, and pLC_{50} of five chemicals
- Table S3 The concentration ratios (p_i) of various components in 20 rays from Table 1 and concentrations of stocks
- Table S4 The concentration ratios (p_i) and concentrations (C_i) of various components and stocks for four fixed NOEC ratio rays
- Table S5 Toxicological interactions of 20 rays designed by UD-Ray at five effects
- Table S6 Toxicological interactions of four fixed NOEC ratio rays at five effects
- Figure S1 Molecular structures of two pesticides and two ionic liquids
- Figure S2 Concentration-response relationships of 20 rays designed by UD-Ray in four mixture system
- Figure S3 Concentration-response relationships of four fixed NOEC ratio rays

Table S1 The properties of five chemicals and the concentrations of stocks*

Abbr.	Chemical	CAS RN	Solubility (g/L)	MW (g/mol)	Purity (%)	Stock (mol/L)
CuSO ₄	Copper sulfate	7758-98-7	203	159.61	99.0	1.2531E-02
DIC	Dichlorvos	62-73-7	8	220.98	98.8	3.6202E-02
GLY	Glyphosate	1071-83-6	10.5	169.07	99.7	2.3659E-02
[hmim]Br	1-hexyl-3-methylimidazolium bromide	85100-78-3	Soluble	247.18	99.0	4.0456E-02
[omim]Cl	3-methyl-1-octylimidazolium chloride	64697-40-1	Soluble	230.78	99.0	4.3331E-02

*MW: Molecular weight

Table S2 Fitting regression coefficients (α and β), statistics (R^2 and RMSE), LC₅₀ and its 95% confidence intervals, and pLC₅₀ of five chemicals

Chemical	Function	Regression coefficients and statistics				LC ₅₀ and its 95% confidence intervals (mol/L)			pLC ₅₀
		α	β	R^2	RMSE	Lower limit	Fitted value	Upper limit	
CuSO ₄	Logit	23.96	7.68	0.9946	0.0170	7.186E-04	7.589E-04	8.036E-04	3.120
DIC	Weibull	12.75	5.84	0.9796	0.0422	4.961E-03	5.676E-03	6.433E-03	2.246
GLY	Weibull	6.79	2.58	0.9802	0.0352	1.317E-03	1.683E-03	2.137E-03	2.774
[hmim]Br	Logit	14.46	6.50	0.9828	0.0373	5.154E-03	5.962E-03	6.974E-03	2.225
[omim]Cl	Weibull	7.11	2.74	0.9753	0.0400	1.434E-03	1.868E-03	2.391E-03	2.729

Table S3 The concentration ratios (p_i) of various components in 20 rays from Table 1 and concentrations of stocks

No.	Mixture ray	p_i (%)				Stock (mol/L)
		CuSO ₄	DIC	GLY	[hmim]Br [omim]Cl	
1	CuSO ₄ -DIC-[hmim]Br-R1	0.0465	0.4302		0.5233	2.603E-02
2	CuSO ₄ -DIC-[hmim]Br-R2	0.0606	0.6085		0.3310	2.450E-02
3	CuSO ₄ -DIC-[hmim]Br-R3	0.0696	0.3195		0.6109	2.239E-02
4	CuSO ₄ -DIC-[hmim]Br-R4	0.0774	0.5027		0.4199	2.198E-02
5	CuSO ₄ -DIC-[hmim]Br-R5	0.0612	0.4578		0.4809	2.391E-02
6	CuSO ₄ -DIC-[omim]Cl-R1	0.0771	0.7130			0.2099 2.408E-02
7	CuSO ₄ -DIC-[omim]Cl-R2	0.0847	0.8505		0.0648	2.250E-02
8	CuSO ₄ -DIC-[omim]Cl-R3	0.1243	0.5704		0.3053	1.903E-02
9	CuSO ₄ -DIC-[omim]Cl-R4	0.1167	0.7582		0.1251	1.924E-02
10	CuSO ₄ -DIC-[omim]Cl-R5	0.0914	0.6836		0.2250	2.224E-02
11	CuSO ₄ -GLY-[hmim]Br-R1	0.0725		0.1129	0.8146	2.195E-02
12	CuSO ₄ -GLY-[hmim]Br-R2	0.1108		0.2836	0.6056	1.906E-02
13	CuSO ₄ -GLY-[hmim]Br-R3	0.0970		0.0516	0.8513	1.908E-02
14	CuSO ₄ -GLY-[hmim]Br-R4	0.1280		0.1772	0.6948	1.720E-02
15	CuSO ₄ -GLY-[hmim]Br-R5	0.0903		0.2003	0.7094	2.055E-02
16	CuSO ₄ -GLY-[omim]Cl-R1	0.1894		0.2952		0.5154 1.545E-02
17	CuSO ₄ -GLY-[omim]Cl-R2	0.2312		0.5918		0.1770 1.332E-02
18	CuSO ₄ -GLY-[omim]Cl-R3	0.2508		0.1335		0.6157 1.241E-02
19	CuSO ₄ -GLY-[omim]Cl-R4	0.2893		0.4005		0.3102 1.109E-02
20	CuSO ₄ -GLY-[omim]Cl-R5	0.1761		0.3905		0.4334 1.634E-02

Table S4 The concentration ratios (p_i) and concentrations (C_i) of various components and stocks for four fixed NOEC ratio rays

No	Mixture ray (X-Y-Z-FR)	p_i (%)			C_x (mol/L)	C_y (mol/L)	C_z (mol/L)	Stock (mol/L)
		X	Y	Z				
21	CuSO ₄ -DIC-[hmim]Br-FR	0.0702	0.4596	0.4701	1.596E-03	1.044E-02	1.068E-02	2.272E-02
22	CuSO ₄ -DIC-[omim]Cl-FR	0.1216	0.7955	0.0829	2.273E-03	1.487E-02	1.550E-03	1.869E-02
23	CuSO ₄ -GLY-[hmim]Br-FR	0.1215	0.0653	0.8132	2.089E-03	1.122E-03	1.398E-02	1.720E-02
24	CuSO ₄ -GLY-[omim]Cl-FR	0.4506	0.2421	0.3073	3.423E-03	1.839E-03	2.334E-03	7.597E-03

* X, Y and Z represents three components in ternary mixtures respectively.

Table S5 Toxicological interactions of 20 rays designed by UD-Ray at five effects

No	Mixture system	Ray	x=0.1	x=0.3	x=0.5	x=0.7	x=0.9
1	CuSO ₄ -DIC-[hmim]Br	R1	SYN	SYN	SYN	SYN	SYN
2		R2	SYN	SYN	SYN	SYN	SYN
3		R3	SYN	SYN	SYN	SYN	SYN
4		R4	SYN	SYN	SYN	SYN	SYN
5		R5	SYN	SYN	SYN	SYN	SYN
6	CuSO ₄ -DIC-[omim]Cl	R1	ADD	ADD	SYN	SYN	SYN
7		R2	ADD	ADD	SYN	SYN	SYN
8		R3	ADD	SYN	SYN	SYN	SYN
9		R4	ADD	SYN	SYN	SYN	SYN
10		R5	SYN	SYN	SYN	SYN	SYN
11	CuSO ₄ -GLY-[hmim]Br	R1	ADD	SYN	SYN	SYN	SYN
12		R2	ADD	SYN	SYN	SYN	SYN
13		R3	SYN	SYN	SYN	SYN	SYN
14		R4	ADD	SYN	SYN	SYN	SYN
15		R5	SYN	SYN	SYN	SYN	SYN
16	CuSO ₄ -GLY-[omim]Cl	R1	ANT	ANT	ADD	SYN	SYN
17		R2	ANT	ANT	ADD	ADD	SYN
18		R3	ADD	SYN	SYN	SYN	SYN
19		R4	ANT	ANT	ADD	SYN	SYN
20		R5	ANT	ANT	ADD	ADD	SYN

* ADD, ANT and SYN refer to the additive action, antagonism and synergism, respectively.

Table S6 Toxicological interactions of four fixed NOEC ratio rays at five effects

No	Mixture ray	x=0.1	x=0.3	x=0.5	x=0.7	x=0.9
21	CuSO ₄ -DIC-[hmim]Br-FR	ADD	SYN	SYN	SYN	SYN
22	CuSO ₄ -DIC-[omim]Cl-FR	ADD	SYN	SYN	SYN	SYN
23	CuSO ₄ -GLY-[hmim]Br-FR	ADD	ADD	SYN	SYN	SYN
24	CuSO ₄ -GLY-[omim]Cl-FR	ADD	SYN	SYN	SYN	ADD

* ADD, ANT and SYN refer to the additive action, antagonism and synergism, respectively.

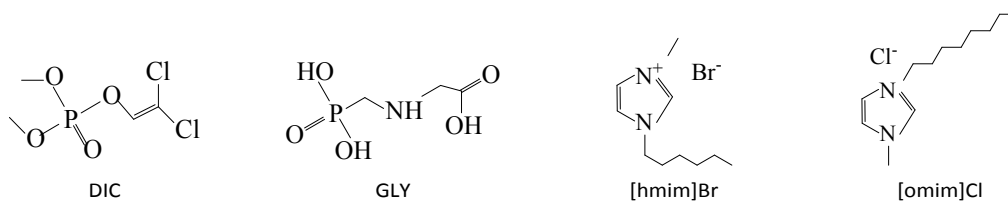


Figure S1 Molecular structures of two pesticides and two ionic liquids

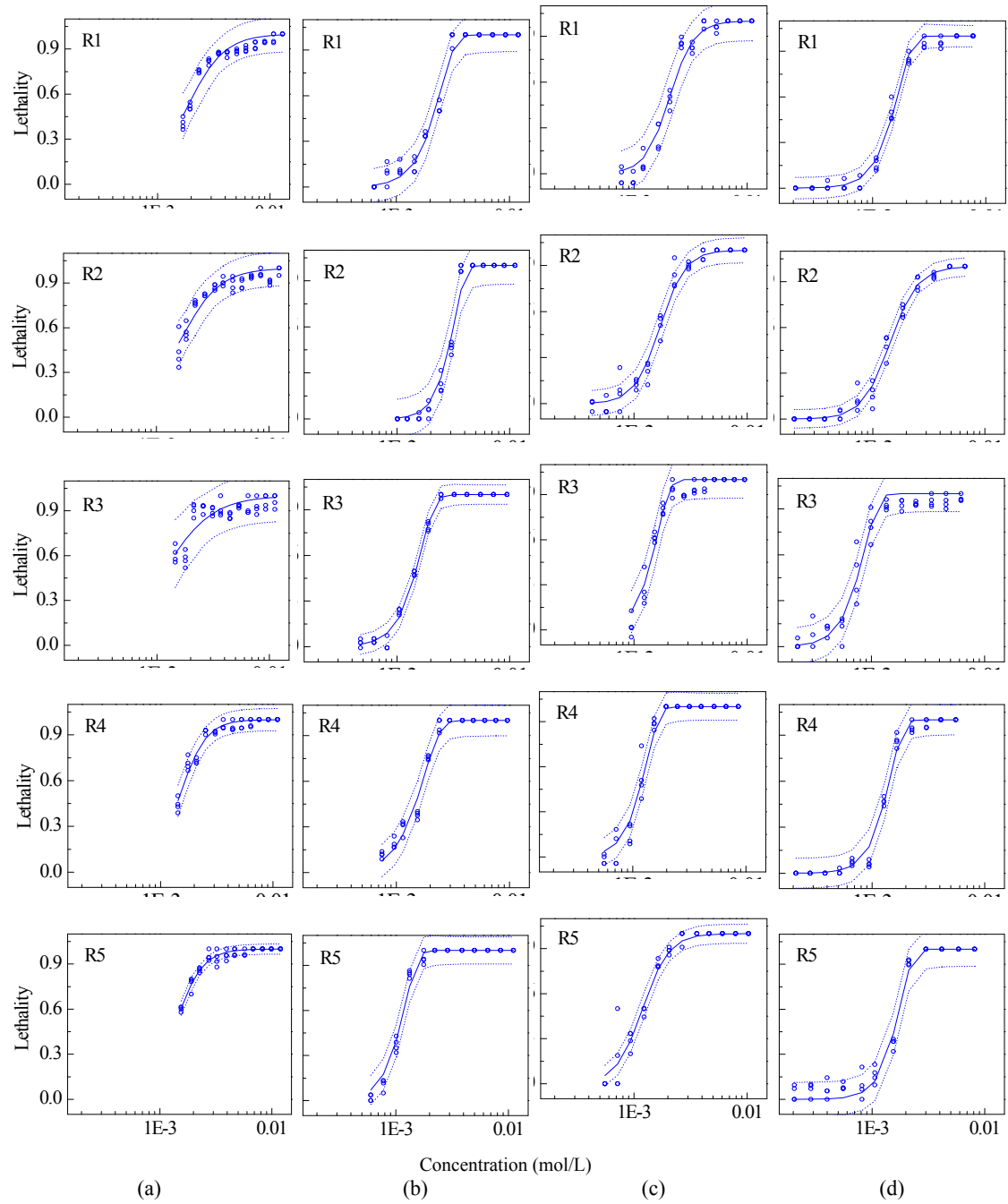
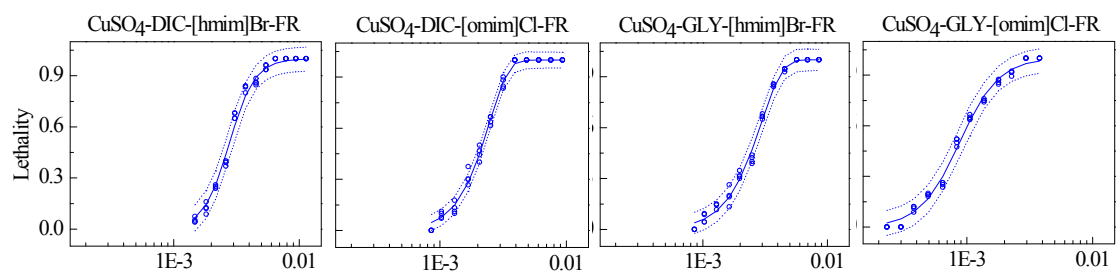


Figure S2 Concentration-response relationships of 20 rays designed by UD-Ray in four mixture system, (a) CuSO_4 -DIC-[hmim]Br system, (b) CuSO_4 -DIC-[omim]Cl system, (c) CuSO_4 -GLY-[hmim]Br system, and (d) CuSO_4 -GLY-[omim]Cl system. (\circ : experimental lethality at 24 h; —: fitted curve; ---: 95% confidence intervals)



Concentration (mol/L)

Figure S3 Concentration-response relationships of four fixed NOEC ratio rays.
(α : experimental lethality at 24 h; —: fitted curve; ---: 95% confidence intervals)