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RESEARCH ARTICLE

A new effect residual ratio (ERR) method for the validation of the concentration addition and independent action models

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Abstract
Background, aim, and scope Glutaraldehyde (GA) often acts as an effective sterilant, disinfectant, and preservative in chemical products. It was found that GA had clearly acute toxicity to aquatic organisms. Furthermore, GA in natural environment could not exist as single species but as complex mixtures. To explore the toxicity interaction between GA and the other environmental pollutant, it is necessary to determine the mixture toxicities of various binary mixtures including GA. Two reference models, concentration addition (CA) and independent action (IA), are often employed to evaluate the mixture toxicity, which can be finished by comparing the concentration–response curves (CRCs) predicted by the reference models with the experimental CRC of the mixture. However, the CRC-based method cannot effectively denote the degree of the deviations from the reference models, especially at very low effect levels. Though the model deviation ratio (MDR) can be used to quantitatively evaluate the deviation of a mixture at EC50 level from the reference model, it is difficult to evaluate the deviations at the lower effect levels. Therefore, the primary aim of this study was to develop a new effect residual ratio (ERR) method to validate the deviations from the reference models at various effect levels.

Materials and methods Four chemicals having possible dissimilar mode of actions with GA, acetonitrile (ACN), dodine (DOD), simetryn (SIM), and metham sodium (MET), were selected as another component in the binary mixtures including GA, which constructed four binary mixtures, GA-ACN, GA-DOD, GA-SIM, and GA-MET ones. For each binary mixture, two equipotent mixture rays where the concentration ratios of GA to another mixture component are respectively EC50 and EC5 ones were designed and their toxicities (expressed as a percent inhibition to *Photobacterium phosphoreum*) were determined by microplate toxicity analysis. The observed concentration–response curve (CRC) of a ray was compared with that predicted by CA or IA model to qualitatively assess the toxicity interaction of the mixture ray. To quantitatively and effectively examine the deviations at various effect levels from the reference models, a new concept, ERR at an effect, was defined, and the ERR was employed to evaluate the deviation at various effects with confidence intervals.

Results For three binary mixtures, GA-ACN, GA-DOD, and GA-SIM, the CRCs predicted by IA models were almost located in the 95% confidence intervals of the experimental CRCs for both equipotent mixture rays, which indicated the independent actions between binary mixture components. However, two rays of GA-MET binary mixture displayed a little synergistic action because both CRCs predicted by CA and IA were lower than the experimental CRC. ERR showed the same results as MDR, but ERR results at low effect area were clearer than MDR ones.

Discussion In CRC comparison, the deviation of CA (for GA-ACN, GA-DOD, and GA-SIM combinations) or IA (for GA-MET) model from the experimental values could be obviously observed at medium area of the CRC. However, at very low effect levels, both deviations of CA

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and IA and difference between CA and IA model predictions were not very apparent. Thus, it was difficult to confirm which model, CA or IA, had better predicted power at very low effect levels. MDR in many literatures often refers to a ratio at EC50 level. It was also difficult to reflect not only the deviation fact at the other ECx but also the deviation uncertainty. After we extended the definition of MDR to all ECx and examined the 95% confidence intervals based on observation, the plot of the redefined MDRs at many effect levels could better explain the deviations of CA or IA model from the observation. However, MDRs at very low effect levels did not still reflect the high uncertainty there. The ERRs defined in our paper could explicitly explain the degree of deviation from the reference models and especially reflect the high uncertainty at very low effects. It could be said that the ERR is a better indicator than MDR.

Conclusions The new ERR validation method developed in our laboratory could provide us with the information about the toxicity interaction between the mixture components and quantitatively assess the accuracy of the reference models (CA or IA) at whole effect levels. The ERR method conquered the invalidation of the classical CRC comparison method on the deviation decision at low effect levels and also got the advantage over the MDR methods.

Recommendations and perspectives It holds promise to become an effective method of hazard and risk assessments of chemical mixtures by well characterizing the uncertainty at very low effect levels.

Keywords Effect residual ratio · Model deviation ratio · Concentration addition · Independent action · *Photobacterium phosphoreum* · Glutaraldehyde

1 Background, aim, and scope

Glutaraldehyde (GA) is a colorless liquid with a pungent odor. Products based on GA are effective sterilants and disinfectants for medical devices that cannot be steam sterilized, particularly heat-sensitive for lensed instruments that are commonly subjected to high-level disinfection between patient uses (Russell 1994). Furthermore, GA has been used as a preservative in chemical products such as fabric softeners, antiperspirants, and fixatives for biological specimens (Andersen 1996). It was indicated that the GA had clearly acute toxicity to aquatic organisms (Leung 2001). For example, the acute EC50 values of GA to *Raphidocelis subcapitata* (Chen et al. 2005), *Daphnia magna* (Boillot and Perrodin 2008), and *Pseudokirchneriella subcapitata* (Sano et al. 2005) are 13.20, 20.03, and 1.0 mg/L, respectively.

It is well known that GA does not exist as single species but as complex mixtures in real environment. Thus, it is necessary to explore whether other environmental pollutants show influence on the toxicity of GA. Previous studies were done in this aspect. For example, Boillot determined the combined effects of GA and three surfactants on *D. magna* (Boillot and Perrodin 2008). The combined effects of GA and surfactant mixtures on aquatic organisms were also experimentally determined by Emmanuel et al. (2005). Two reference models, concentration addition (CA) and independent action (IA), are often employed to evaluate the mixture toxicity, which can be finished by comparing the concentration–response curves (CRCs) predicted by the reference models with the experimental CRC of the mixture (Arrhenius et al. 2004; Faust et al. 2001; Goldoni and Johansson 2007; Greco et al. 1995; Henry and Black 2007; Junghans 2004; Backhaus et al. 2000; Faust et al. 2003; Zhang et al. 2008). However, the CRC comparison method cannot significantly characterize whether the deviation of CRC predicted by CA or IA model from the observed CRC truly exist at low effect levels. Moreover, the model deviation ratio (MDR) reported in the literatures can be used to quantitatively evaluate the degree of deviation from the reference model, CA and IA, which mainly focus on the deviation at 50% effect level (Belden et al. 2007; Trimble et al. 2009) and at 10% effect level (Belden and Lydy 2006). In this paper, a new concept, effect residual ratio (ERR) at a certain effect level, was firstly defined to quantify the deviation of a reference model from the experimental data. To test the validity of the new ERR, we selected four chemicals having possible dissimilar mode of actions from GA, acetonitrile (ACN), dodine (DOD), simetryn (SIM), and metham sodium (MET), as another mixture component in the binary combinations including GA. For each of four binary combinations, GA-CAN, GA-DOD, GA-SIM, and GA-MET, two equipotent concentration ratio mixtures (mixture ratio of EC50 and EC5) were designed. Using *Photobacterium phosphoreum* as indication organism and the percent luminescence inhibition ratio of a toxicant or a mixture to the *P. phosphoreum* as a toxicity endpoint, the toxicities of four single chemicals and their binary mixtures were determined by the microplate toxicity analysis (MTA; Liu et al. 2006), which was proved to be a high precision bioassay and used in modeling the toxicity of many multiple component mixtures (Liu et al. 2009; Zhang et al. 2008; Zhu et al. 2009a).

To examine the combined toxicity of the mixtures along the CRCs, each equipotent mixture was extended to a CRC by using the fixed mixture ratio ray procedure (Altenburger et al. 2000; Gennings et al. 2004a, b). To examine the uncertainty of the mixture toxicity, we also calculated the observation-based 95% confidence intervals (OCI) of the mixtures' CRC under study (Zhu et al. 2009b).

2 Materials and methods

2.1 Chemicals, mixtures, and apparatus

The physicochemical properties of five test chemicals, GA, ACN, DOD, SIM, and MET, purchased from Chem-Service were listed in Table 1. For each chemical a stock solution was prepared in 3% NaCl solution and stocked in the dark at 4°C.

Four combinations, GA-ACN, GA-DOD, GA-SIM, and GA-MET, were designed and two equivalent effect concentration ratio (EECR) mixtures were set for each combination. Each EECR mixture, EC50 or EC5 mixture, was then extended as a CRC, which is called a ray using a fixed mixture ratio method.

Glomax-Multi detection system from Turner BioSystems Inc., USA was used to determine the relative light units (RLUs) of *P. phosphoreum* exposed to a chemical or mixture.

2.2 Testing organism and culture

The freeze-dried *P. phosphoreum* as testing organism was purchased from the Institute of Soil Science, Chinese Academy of Sciences, Nanjing, People's Republic of China and stored at −20°C.

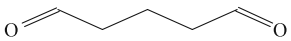
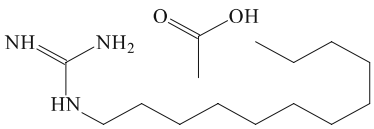
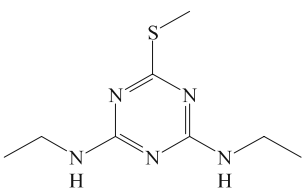
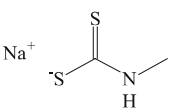
The luminescence medium consists of 0.5% yeast extract, 0.5% tryptone, 0.3% glycerol, 3% NaCl, 0.5% Na_2HPO_4, and 0.1% KH_2PO_4. The pH of the medium was adjusted to 7.0±0.5 with 1 mol/L NaOH.

Before each test, the bacteria were inoculated from a stock culture to a fresh agar and cultured at 20±0.5°C for 24 h. Then the cells were incubated in liquid culture medium with a rotary shaker (125 rpm) at 20±0.5°C for 12–15 h for the toxicity test.

2.3 Toxicity test

The MTA (Liu et al. 2009; Zhang et al. 2008; Zhu et al. 2009a) was used to determine the toxicities of single chemicals or binary mixtures on *P. phosphoreum*. Twelve different concentration gradients and 24 controls were set for each CRC by selecting an appropriate dilution factor (Liu et al. 2009). In 24 wells of the second and third rows in a microplate, 100 μL Milli-Q water was added as 24 controls. To minimize any foreseeable interference that can be associated with toxicity testing conducted in microplate (e.g., volatility of test substance), 12 wells of the fourth rows were not used. In the wells of the fifth, 12 different toxicant volumes derived from a certain dilution factor were added and the Milli-Q water was supplied up to a total volume of 100 μL. In the same way, the sixth and seventh rows were arranged. Then 100 μL bacterial suspensions were added into each test well to make the final test volume be 200 μL. After 15 min, the RLUs of all test solutions were determined on Glomax-Multi detection system.

Table 1 The physiochemical properties of glutaraldehyde (GA) and the other four chemicals

Substance	CAS RN[a]	Structural Formula	Purity	Molecular Weight	Stock (mol/L)
Glutaraldehyde (GA)	111-30-8		50±1%	100.12	2.81E-3
Acetonitrile (ACN)	75-05-8		99.8%	41.05	1.92
Dodine (DOD)	2439-10-3		97.5%	287.51	1.50E-4
Simetryn (SIM)	1014-70-6		99.2%	213.30	5.95E-4
Metham sodium (MET)	137-42-8		99.5%	129.17	1.68E-3

Furthermore, the microplate toxicity test had to be repeated three times at least to ensure the toxicity test precision.

The toxicity endpoint (biomarker) was expressed as a percent inhibition on bioluminescence, noted as E.

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

where I_0 was an average of the RLU of *P. phosphoreum* exposed to the controls (24 parallels) and I, an average of the RLU of the bacteria exposed to the test toxicant or mixture.

Logit function with two parameters (position parameter α and shape β) was used to fit the concentration (C)–inhibition (E) data of the chemicals on *P. phosphoreum* determined by MTA procedure.

2.4 CA and IA prediction model

The CA and IA models (Zhang et al. 2008) were used to predict the mixture toxicity to assess possible joint action between mixture components. The CA model can be rewritten as follows when it was used in prediction.

$$\mathrm{EC}_{x,\mathrm{mix}} = \left(\sum_{i=1}^{n} \frac{p_i}{\mathrm{EC}_{x,i}} \right)^{-1} \quad (2)$$

where $\mathrm{EC}_{x,\mathrm{mix}}$ is the effect concentration of the mixture eliciting x% effect, $\mathrm{EC}_{x,i}$ denotes the concentration of the ith component when exists individually and elicits the same effect (x%) as the mixture; p_i is the molar concentration ratio of the ith component in the mixture.

The IA predictive model was written as follows.

$$x\% = 1 - \prod_{i=1}^{n} \left(1 - F_i\left(p_i\left(\mathrm{EC}_{x,\mathrm{mix}}\right)\right)\right) \quad (3)$$

where x% is the overall effect caused by the total concentration c_{mix} of a mixture; F_i is the function that depicts the concentration–response curve of the ith component.

2.5 MDR and ERR

The MDR, model deviation ratio, is often defined as a ratio of the effect concentration predicted by a reference model to that observed (Belden et al. 2007; Belden and Lydy 2006; Trimble et al. 2009). In the present paper, the MDR concept defined in effect level was extended to many effect levels along a concentration–response curve. On the other hand, to more explicitly depict the deviation ratio, we proposed a relative model deviation ratio (rMDR) to replace the MDR. The rMDR was defined as a ratio of the difference between the effect concentration (ECx) at a certain effect level (x) predicted by a reference model and that observed to the ECx observed Eq. 4. It was found that both MDRs and rMDRs at many low effect levels could still not explicitly explain the degree of deviation of the reference models. Thus, we defined a new concept, called ERR. The new ERR was defined as a ratio of the difference between the effect (E) at a certain effect level (x) predicted by a reference model and that observed to the observed effect level Eq. 5.

$$\mathrm{rMDR} = \frac{\mathrm{ECx}_{\mathrm{Prd}} - \mathrm{ECx}_{\mathrm{Obs}}}{\mathrm{ECx}_{\mathrm{Obs}}} \times 100\% \quad (4)$$

$$\mathrm{ERR} = \frac{E_{\mathrm{Prd}} - E_{\mathrm{Obs}}}{E_{\mathrm{Obs}}} \times 100\% \quad (5)$$

where $\mathrm{ECx}_{\mathrm{Prd}}$ and $\mathrm{ECx}_{\mathrm{Obs}}$ are the effect concentration predicted by the CA or IA model and one observed at a certain effect level (x), respectively. $\mathrm{E}_{\mathrm{Prd}}$ and $\mathrm{E}_{\mathrm{Obs}}$ are the effect value predicted by the CA or IA model and one observed at a certain concentration level (ECx), respectively.

It s well known that any toxicity experiment has certain uncertainty. To properly characterize such uncertainty so as to rationally depict the deviation of the mixture toxicity observed from that predicted by the reference model, CA or IA, we also defined the 95% confident intervals, $\mathrm{rMDR}_{\mathrm{OCI}}$, and $\mathrm{ERR}_{\mathrm{OCI}}$. Based on observation of rMDR and ERR, the $\mathrm{rMDR}_{\mathrm{OCI}}$ and $\mathrm{ERR}_{\mathrm{OCI}}$ can be written as follow

$$\mathrm{rMDR}_{\mathrm{OCI}} = \frac{\mathrm{ECx}_{\mathrm{L}} - \mathrm{ECx}_{\mathrm{Obs}}}{\mathrm{ECx}_{\mathrm{Obs}}} \times 100\% \quad (6)$$

$$\mathrm{ERR}_{\mathrm{OCI}} = \frac{E_{\mathrm{L}} - E_{\mathrm{Obs}}}{E_{\mathrm{Obs}}} \times 100\% \quad (7)$$

where $\mathrm{rMDR}_{\mathrm{OCI}}$ and $\mathrm{ERR}_{\mathrm{OCI}}$ are rMDR and ERR based on the confidence intervals of mixture toxicity observed,

Table 2 The concentration–response models, two effect concentrations, and their 95% confidence intervals of five chemicals

Substance	α	β	RMSE	R	EC50 (mol/L)	EC5 (mol/L)
GA	22.16	6.22	0.027	0.998	2.74E−4 (2.44E−4,3.10E−4)	9.20E−5 (0,1.31E−4)
ACN	2.37	6.69	0.015	0.999	0.44 (0.40,0.50)	0.16 (0,0.22)
DOD	23.55	5.18	0.023	0.997	2.84E−5 (2.52E−5,3.25E−5)	7.68E−6 (0,1.16E−5)
SIM	20.54	5.45	0.023	0.996	1.70E−4 (1.53E−4,1.91E−4)	4.91E−5 (0,7.04E−5)
MET	29.15	7.65	0.033	0.997	1.55E−4 (1.37E−4,1.78E−4)	6.38E−5 (0,8.95E−5)

RMSE root mean square error

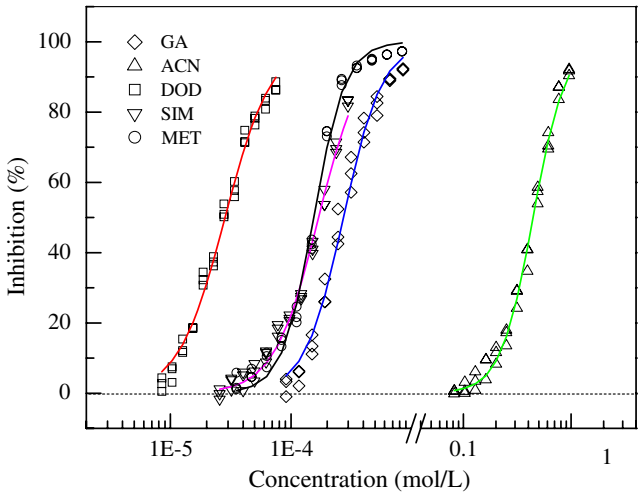


Fig. 1 Concentration–response curves (CRCs) of glutaraldehyde (GA) and the other four chemicals. The *solid lines* refer to CRCs fitted by Logit function

respectively. ECx_L and E_L are an effect concentration and effect corresponding to the upper or lower limits of conference intervals, respectively.

3 Results

3.1 Toxicity of five test chemicals

It is found that the Logit can excellently describe the concentration–response relationships of the chemicals with the correlation coefficients of $R>0.995$ and the root mean square errors of <0.033 (Table 2). From Fig. 1, the shapes of the CRCs fitted were relatively similar but not strictly parallel. Two classical effect concentrations, EC50 and EC5, and their 95% confidence intervals (OCI) calculated from the fitted Logit function were listed in Table 2.

If EC50 is taken as a toxicity index, the toxicity of the test chemicals can be ranked as follows: DOD>MET≈SIM>GA>ACN.

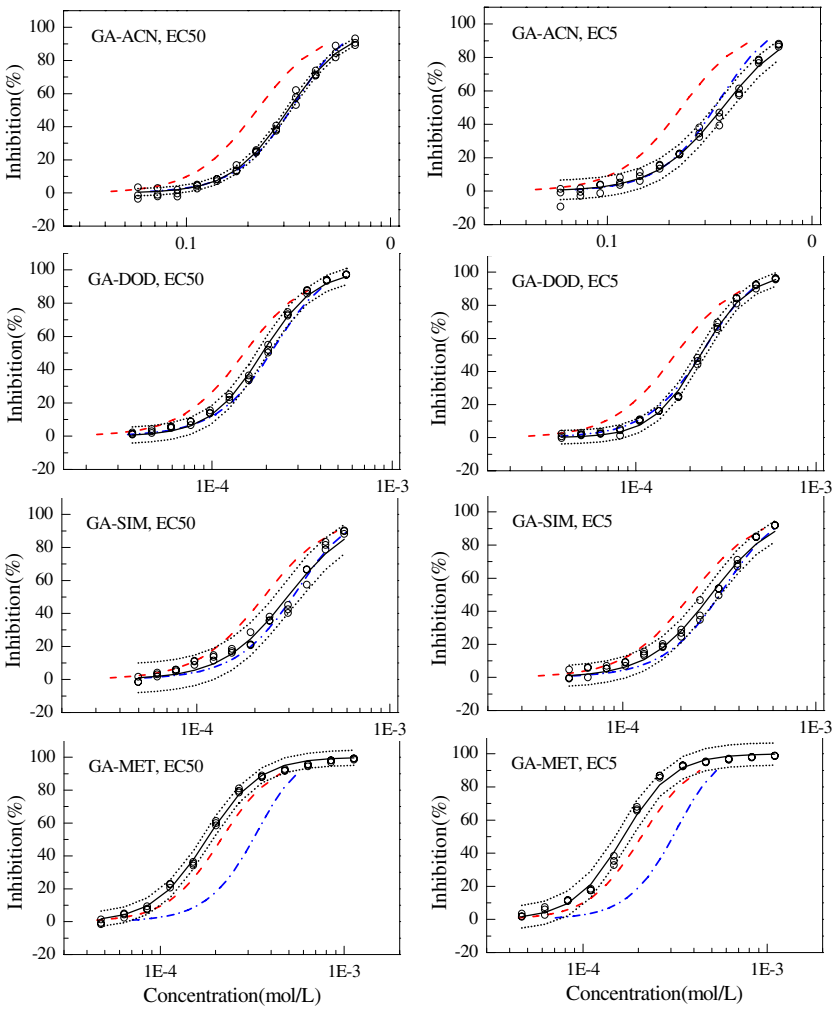


Fig. 2 The concentration–response relationships of eight mixture rays set in four binary combinations. *Circle* refers to the toxicity observed; *red dash line* to CRC predicted by CA; *dash dot line* to CRC predicted by IA; *solid line* to CRC fitted; *short dot line* to *upper* and *lower* limits of 95% confidence intervals observed

3.2 Mixture toxicity and interaction

The total concentration (C)–response (E) scatter points and fitted CRCs of all eight binary mixture rays were shown in Fig. 2 together with the CRCs predicted by the CA and IA models. From Fig. 2, for six binary mixture rays set in three combinations (GA-ACN, GA-DOD, and GA-SIM), the CRCs predicted by IA were almost completely located between the upper limit and lower one of the 95% OCI, whereas, those from CA were located higher than the upper limit of OCI observed, which implied that the GA has a possible dissimilar mode of action from ACN, DOD, or SIM. However, for two mixture rays of GA-MET combination, the CRCs predicted by both IA and CA were lower than the lower limit of OCI observed (Fig. 2). It could be observed that the experimental CRC was significantly deviated from the CRC predicted by IA and somewhat deviated from the curve from CA. The find above implied a possible synergistic interaction between GA and MET.

The MDRs at 11 effect concentrations (EC5, EC10,..., EC85) and ERRs at 11 effects (5, 10, 15,..., 85%) deviated from the reference models (CA and IA) and the 95% OCI observed were shown in Fig. 3 (rMDR) and Fig. 4 (ERR), and several classical MDRs and ERRs as examples were listed in Table 3. From Figs. 3 and 4, in contrast with the CRC comparison method shown in Fig. 2, both plot of rMDR and ERR at a certain effect level more distinctly showed the same toxicity action rules, especially at low concentration range. That is, for the former six mixture rays in three binary combinations (GA-ACN, GA-DOD, and GA-SIM), the rMDRs from IA were almost completely located between the upper limit and lower one of the 95% OCI, whereas, those from CA were located below the lower limit of OCI observed (a few low concentration point exception), which revealed an independent interaction between the mixture components with possible dissimilar modes of action. However, for two mixture rays of GA-MET combination, most rMDRs from both IA and CA were higher than the upper limit of OCI observed, which implied a possible synergistic interaction between GA and MET. ERR results showed the same toxicity action rule as MDR, but ERR results at low effect area are clearer than MDR ones (see Figs. 3 and 4).

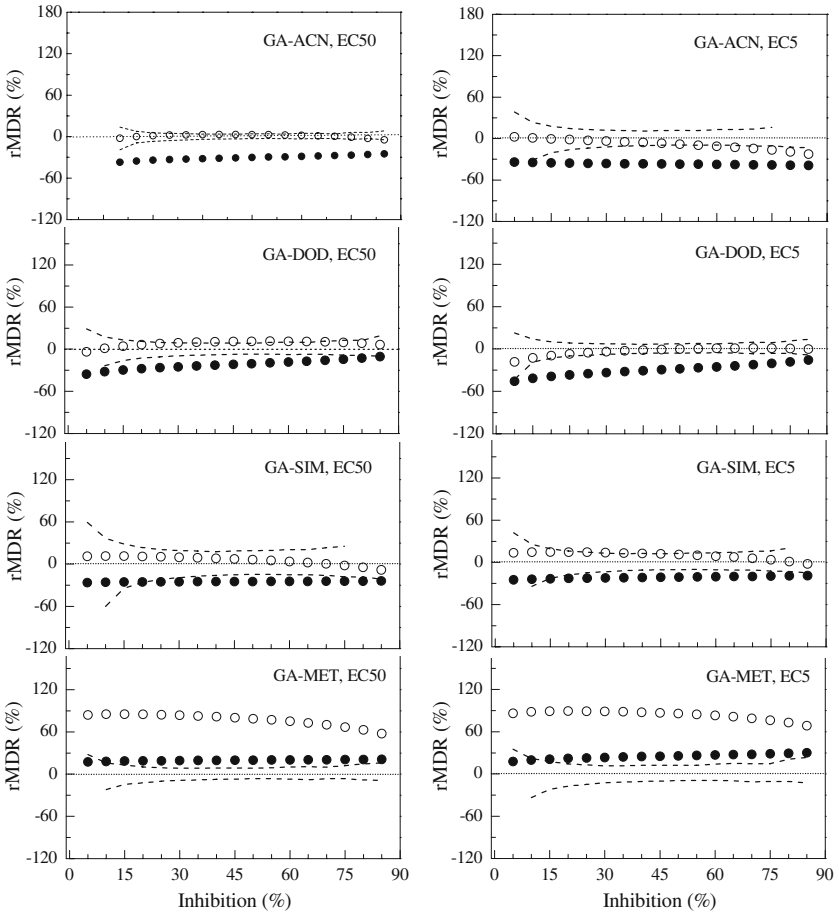


Fig. 3 The relative model deviation ratio (rMDR) of the CA (*filled circle*) and IA (*circle*) from observed values as well as the upper and lower limits of 95% confidence intervals (*dash line*)

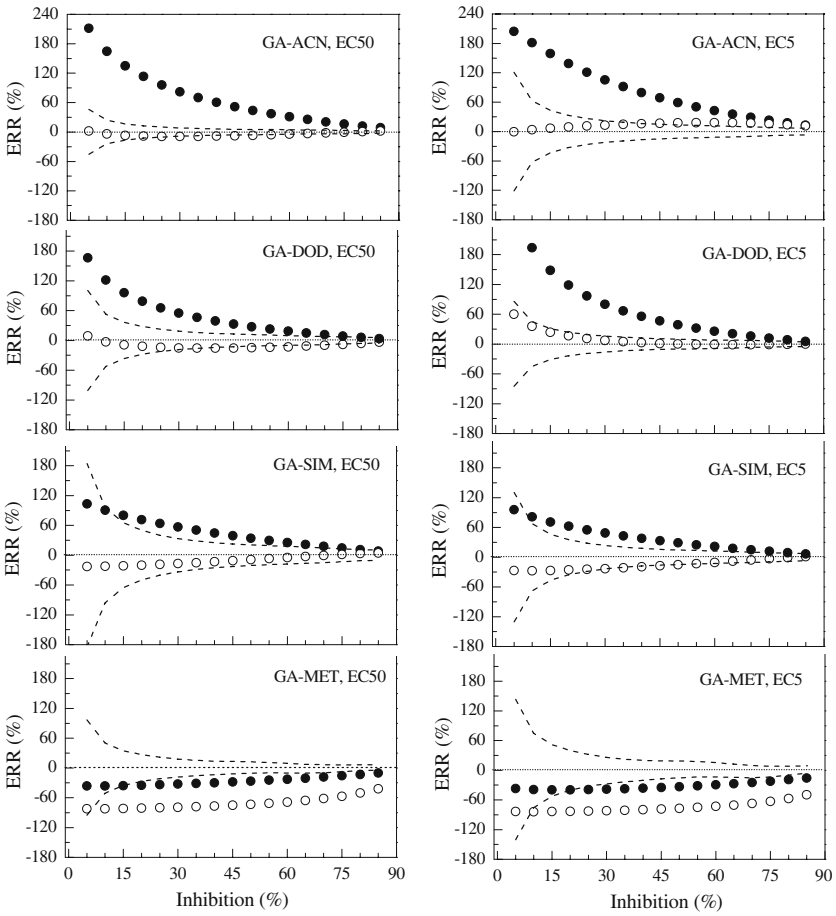


Fig. 4 Effect residual ratio (ERR) of the CA (*filled circle*) and IA (*circle*) from observed values as well as the *upper* and *lower* limits of 95% confidence intervals (*dash line*)

4 Discussions

4.1 Effect of other four chemicals on the toxicity of GA

In real environment, wildlife is not exposed to only one single contaminant, but simultaneously to multiple mixtures of numerous chemicals. Thus, it is necessary to determine potential influence of other pollutants on the adverse effect of GA. The results of the experiments indicated that the addition of ACN, DOD, and SIM almost had no effect on the toxicity of GA because the concentration–response curves of these mixtures observed could be predicted by IA model. However, when GA mixed with MET, toxicity of the mixture observed was apparently higher than those predicted by CA and IA models. In other words, there was probably synergistic effect between GA and MET.

4.2 Validity of CA and IA based on CRCs

For the compounds chosen in this study, GA was a saturated five-carbon dialdehyde, which readily cross-linked with amine groups on outer cell walls or cell membranes of bacteria (Sano et al. 2005). ACN was an organic solvent, which had no specific acting sites on bacteria. DOD is a kind of fungicide and it disturbs the cell membrane permeability and inhibits its respiration (Mitjans and Vinardell 1999). SIM belonged to a selective systemic conductive triazine herbicide that inhibited photosynthesis. Therefore, GA had a possible dissimilar mode of action with that of ACN, DOD, or SIM. Like the experimental results depicted in Fig. 2, the CRC predicted by IA was remarkably close to the CRC of the mixture rays of the GA-ACN, GA-DOD, or GA-SIM observed. Likewise, ERRs of IA were located between the lower and upper limits of the 95% confidence intervals of observed ERR (Fig. 4). This also demonstrated the mixtures, GA-ACN, GA-DOD, and GA-SIM, could have dissimilar modes of action and different target sites.

Although previous studies and three binary mixtures (GA-ACN, GA-DOD, and GA-SIM) under study had demonstrated the excellent prediction of IA for dissimilar-acting mixtures, there were some cases having deviations from IA model (Arrhenius et al. 2006; Manzo et al. 2008) and so did our GA-MET combination. For two mixture rays of GA and MET (a kind of effective soil fumigation), the CRC observed was not only higher than that predicted by IA but also higher than that predicted by CA and closer to the CRC predicted by CA model, displaying a little synergistic interaction (Fig. 2). It should be indicated that

Table 3 The values of rMDRs and ERRs of eight binary mixture rays at several effects such as 5%, 10%, 50%, and 80%

Binary combination	Mixture ray	Reference model	*E*=5%		*E*=10%		*E*=50%		*E*=80%	
			rMDR	ERR	rMDR	ERR	rMDR	ERR	rMDR	ERR
GA -ACN	EC50	CA	−37.1	211.8	−35.3	164.8	−29.7	44.0	−25.9	12.4
		IA	−2.4	2.1	0.03	−4.1	2.7	−6.7	−2.6	0.9
		OCI	(−18.9,13.8)	(−45.5,45.9)	(−9.0,7.5)	(−23.9,24.0)	(−2.8,3.9)	(−5.4,5.1)	(−2.9,6.0)	(−3.2,2.7)
	EC5	CA	−34.0	204.6	−34.8	181.6	−37.1	59.2	−38.5	17.9
		IA	2.6	−0.6	0.9	3.7	−8.2	17.8	−19.2	14.1
		OCI	(−,38.5)	(−121.1,120.8)	(−30,23.3)	(−62.9,63.0)	(−9.4,11.8)	(−13.6,13.6)	(−12.3, −)	(−7.2,7.6)
GA -DOD	EC50	CA	−35.6	166.3	−32.0	121.6	−20.5	27.5	−12.7	6.0
		IA	−3.8	9.2	1.5	−3.1	11.2	−14.9	8.3	−5.8
		OCI	(−,29.0)	(−100.9,100.53)	(−23.4,17.4)	(−52.5,53.1)	(−7.0,8.6)	(−11.5,11.9)	(−9.0,12.6)	(−6.1,6.8)
	EC5	CA	−45.5	280.3	−41.5	194.1	−28.0	38.7	−18.3	8.6
		IA	−18.2	60.1	−12.7	35.8	−0.1	0.2	0.7	−0.5
		OCI	(−42.9,22.6)	(−85.2,86.1)	(−18.5,14.2)	(−45.0,45.2)	(−5.8,7.6)	(−10.1,9.7)	(−6.1,11.5)	(−5.2,5.6)
GA -SIM	EC50	CA	−26.2	103.1	−25.8	90.5	−24.7	34.3	−24.1	11.2
		IA	11.3	−22.7	11.6	−22.5	6.3	−9.0	−4.7	3.2
		OCI	(−,59.2)	(−184.4,184.2)	(−60.0,36.7)	(−95.7,95.7)	(−14.6,19.1)	(−20.7,20.7)	(−18.2, −)	(−11.2,11.4)
	EC5	CA	−24.9	95.8	−23.9	81.4	−21.0	29.1	−19.3	9.2
		IA	13.7	−26.8	14.4	−27.3	11.0	15.3	1.1	−0.8
		OCI	(−,42.1)	(−130.4,130.4)	(−34.1, 25.7)	(−67.8,67.8)	(−10.2,12.5)	(−14.8,14.6)	(−12.3,16.2)	(−8.2,8.1)
GA -MET	EC50	CA	17.3	−36.3	18.0	−36.3	19.9	−26.3	20.7	−13.1
		IA	84.1	−82.1	85.2	−82.0	78.8	−73.3	62.8	−50.5
		OCI	(−,27.8)	(−95.0,97.1)	(−21.8,15.9)	(−50.9,50.4)	(−6.4,8.5)	(−10.5,12.4)	(−8.2,14.8)	(−5.2,6.7)
	EC5	CA	17.6	−37.1	19.8	−39.1	25.8	−33.2	29.3	−19.2
		IA	85.9	−83.5	88.2	−83.8	85.8	−76.9	73.1	−57.1
		OCI	(−,35.3)	(−140.4,143.5)	(−33.5,22.2)	(−75.2,75.0)	(−9.5,12.4)	(−15.0,18.7)	(−10.4,20.9)	(−9.6,8.4)

CA concentration addition, *IA* independent action, *OCI* confidence intervals based on observation, *rMDR* relative model deviation ratio, *ERR* effect residual ratio

both CA and IA models failed to predict accurately mixture toxicity mainly because the concept of CA and IA assume a noninteractive type of joint action (Goldoni and Johansson 2007; Greco et al. 1995).

4.3 ERRs method

Generally, the CRC comparison method based on Fig. 2 can clearly explain the deviation of CA or IA model from the observation at medium and high effect levels. However, at very low effect levels, the CRC comparison cannot significantly decide whether the deviations are or not. For example, the CRCs predicted by CA and IA for the former four mixture rays in Fig. 2 were almost location between the upper and lower limits of 95% OCI at very low effect levels. However, it was very distinctly seen from Fig. 4 that the four CRCs predicted by CA were obviously located above the upper limits, while those predicted by IA were located between the upper and lower limits, which implied that the new ERR method developed in this paper could effectively depict the deviations of CA and IA at all effect area especially very low effect levels. The reason why the ERR can accurately evaluate the toxicity interaction (Fig. 4 and Table 3) is that the ERR is a kind of relative deviation between the effects predicted and observed and the ERR is defined at effect levels of 0–1 range rather than the concentration levels. The relative deviation (ERR) has higher sensitivity at the low effect levels than the absolute deviation in the CRC comparison and can distinctly distinguish from the small deviation in low effect area. For example, for the EC5 mixture ray of GA-DOD binary combination, the absolute deviations of CA and IA at 5% effect were −4.17E−5 and −1.67E−5 mol/L (Fig. 2), respectively, almost having no difference, while the ERRs of CA and IA at 5% effect were 280.3% and 60.1% (Table 3), respectively, displaying significant difference. The rMDR extended in our paper is also a kind of relative deviation defined at the concentration level, and the rMDR is better than the CRC comparison (comparison of Fig. 3 with Fig. 2). The rMDRs of CA and IA at 5% effect were −45.5% and −18.2% (Table 3), respectively. However, the rMDR based on the concentration ratio still difficultly explicitly explain the degree of deviation of the reference models from the observation.

On the other hand, ERRs can clearly characterized different degree of deviation of the reference model at different effect levels. For example, for the EC5 mixture ray of GA-ACN binary combination, the effects predicted by CA model at 5% and 80% effect levels were 15.23% and 94.33%, respectively. The absolute deviations of CA model at 5% and 80% effect levels were 10.23% and 14.33%, respectively, having no significant difference. However, the relative deviations (ERRs) of CA model at 5% and 80% effect levels were 204.6% and 17.9% (Table 3), respectively, displaying a very significant difference. Thus, ERR can be regarded as an effective method to sensitively evaluate the toxicity interaction between pollutants at whole effect ranges.

5 Conclusions

Results showed that if the EC50 was specified as a toxicity index the toxicity of the five substances could be ordered as follows: DOD>MET≈SIM>GA>ACN. It was found that six mixture rays for three binary combinations, GA-ACN, GA-DOD, and GA-SIM, can be predicted by IA model, which implies that GA has possible dissimilary mode of action with ACN, DOD, or SIM. For two mixture rays of GA and MET, the CRC observed was significantly higher than that predicted by IA and was closer to the CRC predicted by CA model. Whether the toxicity action between GA and MET is a concentration addition or the other interaction such as synergism needs to be further study.

ERR defined at the effect level is a kind of relative deviation concept and can sensitively depict the deviation of CA and IA from observation at various effect levels especially at low effect levels. It has been proved that the ERR is a significant improvement over the classical methods based on CRC comparison or MDRs. It could be promised that the ERR will become an effective method of hazard and risk assessments of chemical mixtures.

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