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An analysis of the combined effects of organic toxicants

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Abstract

This paper presents a basic database for the joint actions of 44 binary mixtures of various organic toxicants on *Escherichia coli*. The multiple toxicity behaviors observed from the *E. coli* organisms were analyzed and compared with previous works based on the Microtox tests. The two kinds of tests produced quite different responses, in terms of the joint action mode and the sum of toxic units, to various organic mixtures. However, detailed analyses with the considerations of the chemical's mechanisms of toxicity and the slope of toxicant's dose–response curve have revealed several general criteria for the prediction of combined effects of organic toxicants. First, for both reactive and non-reactive toxicants, either additive or less than additive (antagonistic) joint actions will be observed for chemicals of the same mechanism of toxicity. Second, the mixture of reactive toxicants with different mechanisms is the only category of organic mixtures associated with frequent observations of synergism. Third, greater-than-additive (synergistic) effects are inherently associated with toxicants having flat dose–response curves. Less than additive effects are, however, mainly related to a chemical's display steep dose–response curves. Model analyses indicate that the observed synergistic effects are due to response addition or response multiplication joint actions. Hence, most of the synergistic joint actions are non-interactive in nature and are governed by the dose–response relationships of individual toxicants.

Keywords: Joint action; Synergism; Toxicity; Combined effect; Mechanism

1. Introduction

The fundamental development of multiple toxicity theory were made by Bliss (1939), who defined two basic reaction modes for joint toxicity: (1) similar joint action; and (2) independent joint action. Hewlett and Plackett (1959) later presented a more comprehensive approach, unifying the above basic modes in a general model based on a bivariate normal distribution of the action tolerances. Their model has a non-interactive nature, meaning that the response of one toxicant does not affect the combination of another with

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receptors or the intrinsic activity of the other (Plackett and Hewlett, 1967). Christensen and Chen (1985) further expanded the model to introduce toxicants and an arbitrary tolerance distribution. Synergism has been considered to be due to some unpredictable interactive joint actions and is described only by interactive multiple toxicity models (Hewlett, 1969; Hewlett and Plackett, 1979; Durkin, 1981). These models are purely empirical and therefore, of limited value compared to non-interactive models which have more of a theoretical foundation. However, Christensen and Chen (1985, 1989) demonstrated that synergistic effects might occur even when the joint actions between two toxicants are non-interactive. The necessary conditions for such occurrences are two toxicants exhibiting rather flat dose–response curves and independent joint actions (response multiplication or response addition) between two toxicants acting jointly. The above findings have been further discussed by phamacologists for its possible applications in devising drugs to achieve better therapeutic effects (Unkelbach and Pöch, 1988; Pöch et al., 1990).

The development of the narcosis quantitative structure–activity relationships (QSARs) has led to a general classification of organic chemicals into non-reactive and reactive types. Reactive toxicants have been further divided into four different categories according to their mechanisms of toxicity (Lipnick, 1991). The QSARs have been applied to discriminate between chemicals having similar and dissimilar mechanisms of toxicity. Chemicals belonging to the same QSAR group are considered as having the same mechanism and their combined toxic effects have been found to be additive (Konemann, 1981; Hermens et al., 1984a,b, 1985; Broderius and Kahl, 1985; Prakash et al., 1994). However, data based on binary mixtures of organic toxicants indicates that a considerable proportion of mixtures of reactive toxicants displayed greater than additive effects. The majority of synergistic joint actions observed were related to reactive toxicants having different mechanisms of toxicity and flat concentration–response curves (Chen and Yeh, 1996; Chen and Huang, 1996).

Escherichia coli is probably one of the most familiar and well-explored freshwater microorganisms to biologists and toxicologists. The objective of this study was to provide a basic database for the joint actions of organic toxicants revealed by *E. coli* organisms. In addition, by comparing the mixture toxicity behaviors between the luminescent bacteria (Chen and Chiou, 1995; Chen and Yeh, 1996; Chen and Huang, 1996) and *E. coli*, general guidelines for predicting the multiple toxicity of organic mixtures can be established.

2. Theory

Considering the quantal response of organisms to two toxicants. The non-response probability Q can be expressed in the following form according to Hewlett and Plackett (1959) or Christensen and Chen (1985):

$$Q = \Pr\left[(\delta_1)^{1/\lambda} + (\delta_2)^{1/\lambda} \leq 1\right] \quad (1)$$

where Pr is the probability, δ_i is the z_i/Z_i, z_i is the concentration of toxicant i, Z_i is the concentration tolerance (random variable) of individual organisms to toxicant I and λ is the similarity coefficient for the action of two toxicants on two biological systems (enzyme systems or other receptors). The distribution of the tolerance Z_i is described by the probit function (Finney, 1971). The similarity coefficient λ measures the degree of similarity between the actions of two toxicants: $\lambda = 1$ indicates two toxicants act on the same biological system (similar joint action), meanwhile, $\lambda = 0$ indicates that two toxicants act on different biological systems (independent joint action). The non-response probability Q can be calculated by integrating the bivariate normal density function, which describes the distribution of tolerances Z_1 and Z_2. The correlation coefficient ρ of the bivariate density function measures the degree of linear association between the two variables, Z_1 and Z_2. $\rho = 1$ (or -1) indicates that Z_1 and Z_2 are fully correlated with each other. $\rho = 0$ means no correlation existed between the two variables.

Unique cases of joint action modes, e.g. con-

centration addition (CA), response multiplication (RM), no addition (NA) and response addition (RA), can be generated by the above non-interactive model. Table 1 gives the definitions (in terms of ρ and λ) and the equations for calculating the combined responses, with respect to different joint action modes. The CA mode is a special case for similar joint actions and the combined toxic effect is additive. NA, RM and RA are cases of independent joint actions. As frequently assumed, a mixture acting via CA is more toxic than acting via RM or NA (Finney, 1971; Shelton and Weber, 1981). Naturally, the CA model has been recommended for the prediction of the combined effects of mixtures of toxicants (EIFAC, 1980).

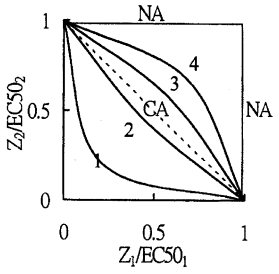
Hypothetical cases are used to illustrate the influence of dose–response curves on combined toxic effects when two toxicants act via RM or RA mode. Fig. 1 depicts the isoboles of different types of dose–response curves based on the RA mode ($\rho = -1$ and $\lambda = 0$). For each binary mixture of toxicants, the dose–response relationships in terms of the intercept (α) and the slope (β) are also given in Fig. 1. The probit models for toxicant 1 and toxicant 2 define variables Z_1 and Z_2 in Eq. (1). Isobolograms were constructed by integrating Eq. (1) to find various combinations of z_1 and z_2, which resulted in exactly 50% survival (or 50% inhibition). Detailed description of the numerical integration can be found in Christensen and Chen (1985). The isoboles for CA and NA modes are also displayed for comparison. It is clear that toxicants having flat dose–response curves (small β values) tend to act synergistically while those having steep dose–response curves (large β values) may produce antagonistic effects. Toxicants acting via RM mode will produce similar isoboles as those in Fig. 1. However, for a particular hypothetical case, the RA isobole always located below the RM isobole, indicating that RA mode results in more severe toxic effects than that by the RM mode. (Christensen and Chen, 1985, 1989).

3. Materials and methods

E. coli ATCC 25922 strain was incubated at 37°C in growth medium with shaker at 100 rpm. The growth medium was prepared by dissolving 8 g of nutrient broth in 1 l of deionized water and with a pH of the solution of 6.8. The bacterial culture was incubated until its optical density (OD) reached 1.0 absorbance at 600 nm (approx. equal to 1.5×10^9 cells/ml) and then, was harvested by centrifuging at 4000 *g* for 5 min. The *E. coli* settlement was resuspended with aerated deionized water (pH = 6.5–7.0) to adjust the absorbance of *E. coli* suspension equal to 1.0. The above *E. coli* suspension was then spiked with the desired amounts of nutrient broth and toxicants. All tests were performed at COD = 180 mg/l. The specific oxygen uptake rate (SPOUR) was determined in a 300-ml BOD bottle using a DO electrode. On-line readings were directly stored in a computer to calculate the corresponding SPOUR values. Test temperature was maintained at 37°C by a water bath. The inhibition rate (%) equals to $(1 - A/A_0) \times 100$. A_0 denotes the original SPOUR determined by blank tests (controls) and A represents the SPOUR for treatments with toxicant spiking. The test duration was ap-

Table 1
Definitions of basic modes of action

Parameter		Type of action	Abbreviation	Response	Effect
ρ	λ				
1	1	Concentration addition	CA	–	Additive
0	0	Response multiplication	RM	$1\text{-}(1 - P_1)(1 - P_2)$	–
1	0	No addition	NA	Max (P_1,P_2)	Antagonistic
−1	0	Response addition	RA	Min (1,$P_{1+}P_2$)	–



Probit model : $Y=\alpha+\beta\log(z)$

	Toxicant1		Toxicant2		
Case	α_1	β_1	α_2	β_2	Effect
1	5	1	5	1	S*
2	5	2	5	2	S
3	5	3	5	3	A
4	5	4	5	4	A

*S: synergism; A: antagonism.
α : intercept, β : slope.

Fig. 1. Isobolograms for two toxicants acting via response addition mode.

proximately 10 to 15 min where a straight-line relationship in DO readings can be easily identified. Median effective concentration (EC50) was defined as the toxicant concentration causing 50% reduction on SPOUR and was calculated using the probit model (Finney, 1971). Eighteen organic chemicals, including both the reactive and non-reactive types, were tested (listed in Table 2). The concentrations of organic toxicants were checked using a total organic carbon (TOC) or HPLC analyzer, before mixing with the growth medium. All chemicals used were of reagent grade.

The additive index (M), or the sum of toxic units, that determines the type of joint action for a specific binary mixture of toxicants was defined by the following equation:

Table 2
Data from individual toxicity tests

Chemicals	n*	*E. coli*				Microtox	
		EC50 (mg/l)	S.D. (mg/l)	C.V.(%)	slope	EC50 (mg/l)	slope
(1) Reactive toxicants							
Lactonitrile	3	107.5	21.96	20.43	3.86	695	0.65
Acetonitrile	6	22 760	5158	22.66	1.53	17 500	4.70
Malononitrile	4	2939	724.0	24.64	1.76	244	1.57
Acrolein	3	8.550	2.430	28.42	3.41	0.16	1.51
Acrylamide	3	48 840	7205	14.75	3.23	9950	1.99
Formaldehyde	4	87.94	12.00	13.65	2.14	5.60	1.55
Butyraldehyde	5	1165	292.4	25.10	1.85	150	1.15
Glutardialdehyde	3	148.9	38.51	25.86	2.66	3.95	1.51
Acetaldehyde	4	1952	309.8	15.89	2.96	328	1.76
Allyl alcohol	3	6519	1836	28.16	2.31	850	1.17
Propargyl alcolhol	3	6556	1901	29.00	2.53	2070	1.78
2-*butyn*-1,4-diol	3	108 400	10 360	9.550	2.67	772	1.66
p-nitroso-*N*,*N*-Dimethylaniline	3	7.990	2.032	25.43	3.37	0.096	1.88
(2) Non-reactive toxicants							
Phenol	3	1984	237.8	11.98	1.63	22.1	1.57
Ethyl acetate	3	28 750	5752	20.01	3.20	1860	1.25
Methanol	3	38 800	4764	12.12	2.46	48 269	3.74
Acetone	3	22 190	5504	24.80	3.70	14 283	2.84
Benzene	3	1121	330.0	29.44	3.81	78.0	1.37

*n: sample size. S.D.: standard deviation of EC50. C.V.: percent coefficient of variation of EC50.

$$M = \frac{z_1}{EC50_1} + \frac{z_2}{EC50_2} \quad (2)$$

where z_i denotes the toxicant concentration. Combining z_1 and z_2 resulted in exactly a 50% response. Simple addition is characterized by $M = 1$. $M > 1$ represents antagonism and $M < 1$ indicates synergism. Mixture toxicity tests were conducted at equitoxic ratio, which means that $(z_1/\mathrm{EC50}_1)$: $(z_2/\mathrm{EC50}_2) = 1{:}1$. Mixtures of the two toxicants with different sums of toxic units (say, 4, 2, 1, 0.5, 0.25, etc.) were tested. Based on the observed inhibition rates, M and its 95% confidence interval (CI) at 50% response could be determined using probit analysis. For instance, if the toxicant concentration causing 50% response was found to be 2 toxic units, z_1 and z_2 were equal to $1 \times \mathrm{EC50}_1$ and $1 \times \mathrm{EC50}_2$, respectively. A stringent criterion was applied to determine the joint action modes. Mixtures that resulted in 95% CI for M that overlapped 1 were judged to be additive, those with 95% CI that did not overlap 1 were either antagonistic or synergistic in toxicity.

4. Results and discussion

Table 2 summarizes results of *E. coli* tests on individual toxicants, as mean EC50 values, the standard deviation of the EC50, coefficient of variation (CV) and the probit slope of the dose–response curve. CV values are within the range of 10 to 30% of the EC50. Based on the EC50 values, lactonitrile, acrolein, formaldehyde and *p*-nitroso-*N*,*N*-dimethylaniline are considerably more toxic than other toxicants. With respect to data generated by our previous studies (Chen and Huang, 1996; Chen and Yeh, 1996), the luminescent bacteria test (Microtox test) is obviously more sensitive than the *E. coli* test. In addition, *E. coli* organisms reveal considerably steeper concentration–response curves (or, larger probit slopes) than that by the luminescent bacteria, except for cases of acetonitrile and methanol. For reactive toxicants, a generally good correlation ($R^2 = 0.78$) exists between the two sets of EC50 values for *E. coli* and the luminescent bacteria. Analysis of non-reactive toxicants indicates an even better correlation, with $R^2 = 0.83$.

Table 3 summarizes the combined effects of 44 mixtures of organic toxicants observed from the *E. coli* tests. These mixtures of toxicants are divided into three categories, as shown in Table 3, according to the type of toxicants and the mechanism of toxicity. Results from the Microtox test are also listed for comparison. Experience from our previous studies (Chen and Chiou, 1995; Chen and Yeh, 1996) using the Microtox test indicates that, for toxicants of the same mechanism of toxicity, the joint actions are most likely to be additive or antagonistic. Synergistic action rarely occurred. For category (1) and (2), the observed effects are either additive or less than additive. Thus, data based on the *E coli* tests generally agree with the above conclusions.

Organic mixtures containing reactive toxicants with different mechanisms are listed in the third category. Reactive toxicants have been divided into four different categories according to their mechanisms of toxicity (Lipnick, 1991). In the third category, the mechanisms for toxicants acting jointly are all different from each other. Based on our previous Microtox test results (Chen and Yeh, 1996; Chen and Huang, 1996), reactive toxicants with different mechanisms are the only type of mixtures that are associated with frequent observations of synergistic effects. Most of our experimental works were therefore focused on these mixtures. For the *E. coli* tests, similarly, greater than additive effects (synergism) were observed only in this category of organic mixtures. The three mixtures revealed synergistic effects are acetonitrile–acetaldehyde, malononitrile–acetaldehyde and malononitrile–butyraldehyde.

We may also find that, for a total of 44 organic mixtures listed in Table 3, there are only six mixtures that result in identical joint effects on the *E. coli* and luminescent bacteria. Correlation analyses using the additive index (M) values derived by the two different tests also indicates that these M values are poorly correlated with a R^2 equal to only 0.096. It seems that different types of organisms will have completely different responses to mixtures of toxicants. However, the

Table 3
Joint actions of organic toxicants

Toxicant	Toxicant	*E. coli*			Microtox	
		M	95% C.I.	Mode	*M*	Mode
(1) Non-reactive toxicants (same mechanism)						
Phenol	Methanol	4.21	8.23–3.19	A	–	–
Phenol	Acetone	1.86	2.63–1.60	A	1.19	+
Ethyl acetate	Methanol	2.02	3.05–1.71	A	–	–
Ethyl acetate	Acetone	0.820	1.14–0.69	+	–	–
Benzene	Phenol	1.73	1.96–1.60	A	0.87	+
Methane	Acetone	0.780	1.08–0.573	+	–	–
Benzene	Ethyl acetate	1.66	1.82–1.57	A	–	–
(2) Reactive toxicants (same mechanism)						
Acrolein	Acrylamide*	1.64	2.69–1.31	A	1.23	A
Formaldehyde	Glutardialdehyde*	1.55	1.84–1.40	A	1.25	A
Glutardialdehyde	Butyraldehyde	1.16	1.63–0.824	+	0.9	S
Butyraldehyde	Acetaldehyde	1.08	2.12–0.792	+	1.15	A
Allyl alcohol	Propargyl alcohol	1.02	1.57–0.852	+	–	–
Lactonitrile	Acetnotrile	1.00	1.19–0.87	+	2.25	A
Propargyl alcohol	2-butyn-1,4-diol	0.698	1.08–0.515	+	–	–
(3)Reactive toxicants (different mechanisms)						
Lactonitrile	Formaldehyde	1.74	2.22–1.34	A	0.92	+
Lactonitrile	Acetaldehyde	1.76	2.45–1.27	A	1.10	+
Lactonitrile	Butyraldehyde	1.49	1.82–1.30	A	–	–
Lactonitrile	Glutardialdehyde	1.34	1.51–1.25	A	–	–
Lactonitrile	Propargyl alcohol	1.41	1.55–1.31	A	–	–
Lactonitrile	PND	1.49	1.82–1.28	A	1.10	+
Lactonitrile	Acrylamide	1.67	2.28–1.37	A	0.79	S
Lactonitrile	Allyl alcohol	1.17	1.56–0.849	+	0.24	S
Acetonitrile	Acrylamide*	2.41	2.87–2.19	A	1.11	A
Acetonitrile	Butyraldehyde	0.990	1.58–0.680	+	1.59	A
Acetonitrile	Glutardialdehyde	1.37	1.53–1.26	A	–	–
Acetonitrile	Formaldehyde	1.22	1.64–0.902	+	1.47	A
Acetonitrile	Acetaldehyde	0.696	0.95–0.535	S	1.62	A
Acetonitrile	PND*	1.98	3.02–1.44	A	2.17	A
Malononitrile	Formaldehyde	1.11	1.76–0.837	+	0.06	S
Malononitrile	Acetaldehyde*	0.587	0.823–0.475	S	0.13	S
Malononitrile	Propargyl alcohol	1.13	1.65–0.819	+	–	–
Malononitrile	Acrylamide	1.05	1.19–0.837	+	1.17	A
Malononitrile	Butyraldehyde*	0.417	0.616–0.350	S	0.37	S
Malononitrile	Glutardialdehyde	1.17	1.71–0.789	+	–	–
Glutardialdehyde	Acrylamide	1.03	1.62–0.836	+	1.20	A
Glutardialdehyde	Propargyl alcohol	0.868	1.16–0.675	+	–	–
Acetaldehyde	Acrylamide	1.48	2.61–1.14	A	–	–
Acrolein	Butyraldehyde	1.28	2.55–0.913	+	1.35	A
Acrolein	Allyl alcohol	1.88	2.91–1.38	A	1.13	+
Propargyl alcohol	Acrylamide	1.75	2.57–1.35	A	–	–
Propargyl alcohol	Butyraldehyde	0.924	1.36–0.804	+	–	–
PND	Acrylamide	0.852	1.44–0.927	+	–	–
PND	Butyraldehyde	1.17	2.13–0.788	+	1.76	A
PND	Allyl alcohol	1.39	1.50–1.23	A	–	–

M: additive index, 95% CI = 95% confidence intervals, A = antagonism, + = addition, S = synergism. PND: *p*-nitroso-*N*,*N*-dimethylaniline. * Cases showing identical joint action mode.

Table 4
The numbers of cases showing various joint action modes with respect to different probit slopes from the Microtox and *E. coli* tests

Slope		Microtox			E. coli		
		Small*	Median	Large	Small*	Median	Large
Small	Synergism	9†	3	0	1	2	0
	Addition	10	13	0	1	5	4
	Antagonism	12	8	5	0	1	3
Median	Synergism	–	0	0	–	0	0
	Addition	–	3	0	–	1	2
	Antagonism	–	0	3	–	0	7
Large	Synergism	–	–	0	–	–	0
	Addition	–	–	0	–	–	1
	Antagonism	–	–	0	–	–	2

* Probit slope < 2.0 (small), > 2.0 and < 3.0 (median), > 3.0 (large). †: from Chen and Yeh (1996).

two types of organisms also reveal similar multiple-toxicity-behaviors: (1) combined effects from toxicants of the same mechanism will be either additive or less-than-additive; and (2) reactive toxicants of different mechanisms are the only category mixtures associated with frequent observations of synergism.

It is reasonable to assume that independent joint actions (e.g. NA, RM or RA) may take place for two toxicants having different mechanisms of toxicity. Analyses were thus performed by summarizing the numbers of cases showing various joint action modes with respect to different magnitudes of slopes. The magnitude of the probit slope has been divided into three classes, i.e. small (< 2.0), medium (> 2.0 and < 3.0) and large (> 3.0). Table 4 summarizes the statistics drawn from results based on our Microtox tests (Chen and Yeh, 1996) and the *E. coli* tests. For both test organisms, synergistic effects are related to at least one toxicant which has a small slope and none of these cases contains any steep-slope chemical (say, slope > 3.0). Furthermore, joint actions between chemicals associated with steep slope are either additive or antagonistic. By referring to Fig. 1, the above phenomena indicate that the observed synergistic effects could be due to RA or RM joint action mode. The observed antagonistic effects from toxicants associated with small probit slopes may be due to toxicants acting via the NA mode.

Based on the RA mode, combined effects of hypothetical examples for toxicants having various dose–response curves (as characterized by the probit slope) are shown in Table 5. All the synergistic effects appear at the top left-hand-side corner of the table. However, at the right-hand-side of Table 5, combined effects related to steep-slope chemicals are either additive or antagonistic. Broderius et al. (1995) also observed

Table 5
Joint action mode and sum of toxic units for binary mixtures of toxicants predicted by RA model ($\rho = -1$, $\lambda = 0$)

Probit	slope β	Small		Median	Large
		1	2	3	4
Small	1	S* (0.42)	S (0.69)	S (0.87)	+ (1.02)
	2	–	+ (0.91)	+ (1.06)	A (1.17)
Median	3	–	–	A (1.18)	A (1.28)
Large	4	(Symmetrical)	–	–	A (1.35)

* Modes of joint action: A = antagonism, + = addition, S = synergism.

that, based on results from fish tests, dissimilar chemicals with very steep concentration-response curves generally showed less-than-additive combined effects. However, no specific conclusion regarding synergism was drawn from their study. The above phenomena shown in Table 5 are identical to the experimental observations drawn from the *E. coli* and Microtox tests (Table 4). We may thus, conclude that the observed synergistic effects in Table 4 are most likely due to RA or RM joint actions. If interactive joint actions are the main cause for greater-than-additive effects, then, the observed synergistic effects should have been evenly distributed in Table 4 instead of gathering at the top left-hand-side corner. The apparent influence of the steepness of dose–response curves on the joint action mode suggests that most of the synergistic effects are non-interactive in nature.

Table 6 compares the predicted effects, based on the RA model, with the actual observed effects of cyanogenic toxicants with another reactive chemical of different mechanism of toxicity. From a total of 26 cases drawn from the luminescent bacteria (Microtox tests) and the *E. coli* tests, sixteen agree with the experimental observations (62%). We should also bear in mind that actual experimental observations consist of the effects of both interactive and non-interactive actions. Model predictions, however, consider only the effects of non-interactive joint actions. In other words, discrepancies between experimental observations and theoretical predictions could be due to interferences from interactive joint actions. Furthermore, our previous studies (Chen and Chiou, 1995; Chen and Yeh, 1996) have identified a unique complex joint action occurred when the probit slopes of two toxicants are significantly different in magnitude. The complex joint action, which is strongly antagonistic, may produce additional masking effect to make the model prediction become less accurate. Yet, among the eight observed synergistic actions in Table 6, our model has successfully predicted six of them (75%). For a total of 24 synergistic effects observed from our *E. coli* and Microtox tests (data from this study; Chen and Yeh, 1996; Chen and Huang, 1996), the RA model has achieved more than 90% of successful predictions (22 cases). Such a good accuracy suggests that the non-interactive force is the robust factor for synergistic joint actions.

For toxicants having dissimilar mechanisms, it is apparent that the model tends to overestimate

Table 6
Prediction of the toxic effects of mixtures containing cyanogenic toxicants based on the RA mode

	E. coli			Microtox		
	Slopes S_1, S_2	Observed M^a/Effect[b]	Predicted M/Effect	Slopes S_1, S_2	Observed M/effect	Predicted M/Effect
Lactonitrile allyl alcohol	(3.86,2.31)	1.17/+	1.20/A	(0.65,1.17)	0.24/S	0.35/S[†]
Lactonitrile formaldehyde	(3.86,2.14)	1.74/A	1.18/A[†]	(0.65,1.55)	0.92/+	0.46/S
Lactonitrile acetaldehyde	(3.86,2.96)	1.76/A	1.26/A[†]	(0.65,1.79)	1.10/+	0.52/S
Lactonitrile PND*	(3.86,3.37)	1.49/A	1.30/A[†]	(0.65,1.88)	1.10/+	0.55/S
Lactonitrile acrylamide	(3.86,3.23)	1.67/A	1.29/A[†]	(0.65,1.99)	0.79/S	0.56/S[†]
Acetonitrile acrylamide	(1.53,3.23)	2.41/A	1.02/+	(4.70,1.99)	1.11/A	1.24/A[†]
Acetonitrile formaldehyde	(1.53,2.14)	1.22/+	0.86/S	(4.70,1.55)	1.47/A	1.17/A[†]
Acetonitrile acetaldehyde	(1.53,2.96)	0.70/S	0.98/+	(4.70,1.79)	1.62/A	1.23/A[†]
Acetonitrile PND*	(1.53,3.37)	1.98/A	1.04/+	(4.70,1.88)	2.17/A	1.23/A[†]
Malononitrile formaldehyde	(1.76,2.14)	1.11/+	0.90/+[†]	(1.57,1.55)	0.06/S	0.73/S[†]
Malononitrile acetaldehyde	(1.76,2.96)	0.59/S	1.02/+	(1.57,1.79)	0.13/S	0.78/S[†]
Malononitrile Butyraldehyde	(1.76,1.85)	0.42/S	0.84/S[†]	(1.57,1.15)	0.37/S	0.62/S[†]
Malononitrile acrylamide	(1.76,3.23)	1.05/+	1.06/+[†]	(1.57,1.99)	1.17/A	0.83/S

Additive index $M = (Z_1/EC50_1) + (Z_2/EC50_2)$, A: antagonism, +: simple addition, S: synergism. *PND: *p*-nitroso-*N*,*N*-Dimethylaniline. [†]: predicted effect agrees with the observed effect.

the toxicity of some mixtures because we cannot tell exactly what kind of joint action (RA, RM or NA) may take place. However, we think the value of model analyses lies in the ability of forecasting the unexpected hazards from mixtures of toxicants that produce synergistic effects. Based on the predictions in Table 6, using *E. coli* as an example, acetonitrile and malononitrile have higher tendencies to display greater-than-additive effects than the lactonitrile. Similarly, lactonitrile and malononitrile could be more harmful to the luminescent bacteria because the observed slopes are small. Mixtures containing acetonitrile, on the other hand, are not likely to produce synergistic effects to the luminescent bacteria judging from the steep probit slope. The experimental observations apparently verify the above inferences.

Table 6 also reveals an important fact that a specific mixture of toxicants may produce entirely different combined effects on different species of microorganisms (or organisms), depending on the dose–response curves depicted by the organisms. Such a conclusion highlights the importance of the dose–response relationship in multiple toxicity studies.

5. Conclusions

This study demonstrates that, for chemicals of the same mechanism of toxicity (reactive or non-reactive), either additive or less than additive joint actions will be observed. Synergistic effects are most likely to be observed from mixtures containing reactive toxicants having different mechanisms of toxicity. The fact that synergism occurs only when toxicants display rather flat dose–response curves suggests the existence of response addition or response multiplication joint actions. We may also conclude that different organisms will respond to the stresses from mixtures of toxicants in quite different manners. Less synergistic effects will be encountered for organisms that generally reveal steep dose–response curves (e.g. the *E. coli* organism). However, organisms that always depict moderate or flat dose–response curves (for example, the luminescent bacteria) are most vulnerable to the potential hazard caused by synergistic joint actions.

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References

Bliss CI. The toxicity of poisons applied jointly. Ann. Appl. Biol. 1939;26:585–615.

Broderius SJ, Kahl MD. Acute toxicity of organic chemical mixtures to the fathead minnow. Aquat. Toxicol. 1985;6:307–322.

Broderius SJ, Kahl MD, Hoglund MD. Use of joint toxic response to define the primary mode of toxic action for diverse industrial organic chemicals. Environ. Toxicol. Chem. 1995;14:1591–1605.

Chen CY, Chiou YS. Toxicity of binary mixtures of organic chemicals. Environ. Toxicol. Water. Qual. 1995;10:97–106.

Chen CY, Yeh JT. Toxicity of binary mixtures of reactive toxicant. Environ. Toxicol. Water. Qual. 1996;11:83–96.

Chen CY, Huang CF. Toxicity of organic mixtures containing cyanogenic toxicants. Environ Toxicol Chem 1996;15:1464–1469.

Christensen ER, Chen CY. A general non-interactive multiple toxicity model including probit, logit and Weibull transformations. Biometrics 1985;41:711–725.

Christensen ER, Chen CY. Modeling of combined toxic effects of chemicals. Hazard Assessment of Chemicals 1989;6:125–186.

Durkin PR. Approach to the analysis of toxicant interactions in the aquatic environment. In: Branson DR, Dickson KL, editors. Fourth Conference. Aquatic Toxicology and Hazard Assessment, ASTM STP 737. ASTM: Philadelphia, 1981:388–407.

European Inland Fisheries Advisory Commission (EIFAC). Report on combined effects on freshwater fish and other aquatic life of mixtures of toxicants in water. EIFAC Technical Paper No. 37. Rome: Food and Agriculture Organization of the United Nations, 1980.

Finney DJ. Probit Analysis. London: Cambridge University Press, 1971.

Hermens J, Canton H, Janssen P, De Jong R. Quantitative structure-activity relationships and toxicity studies of mixtures of chemicals with anaesthetic potency: acute lethal and sublethal toxicity to *Daphnia magna*. Aquat Toxicol 1984a;5:143–154.

Hermens J, Canton H, Steyger N, Wegman R. Joint effects of a mixture of 14 chemicals on mortality and inhibition of reproduction of *Daphnia magna*. Aquat Toxicol 1984b;5:315–322.

Hermens J, Broekhuyzen E, Canton H, Wegman R. Quantitative structure-activity relationships and mixturetoxicity studies of alcohols and chlorohydrocarbons: Effects on growth of *Daphnia magna*. Aquat Toxicol 1985;6:209–217.

Hewlett PS. Measurement of the potencies of drug mixtures. Biometrics 1969;25:477–487.

Hewlett PS, Plackett RL. A unified theory for quantal responses to mixtures of drugs: non-interactive action. Biometrics 1959;15:591–610.

Hewlett PS, Plackett RL. The Interpretation of Quantal Responses in Biology. London: Edward Arnold, 1979.

Konemann H. Quantitative structure-toxicity relationships in fish toxicity studies. Part I: Relationship for 50 industrial pollutants. Toxicology 1981;19:209–221.

Lipnick RL. Outliers: their origin and use in the classification of molecular mechanisms of toxicity. Sci Total Environ 1991;109/110:131–153.

Plackett RL, Hewlett PS. A comparison of two approaches to the construction of models for quantal responses to mixtures of drugs. Biometrics 1967;23:27–44.

Pöch G, Reiffenstein RJ, Unkelbach HD. Application of the isobologram technique for the analysis of combined effects with respect to additivity as well as independence. Can J Physiol Pharmacol 1990;68:682–688.

Prakash J, Nirmalakhandan N, Sun B, Peace J. Toxicity of binary mixtures of organic chemicals to microorganisms. Water Res 1994;30:1459–1463.

Shelton DW, Weber LJ. Quantification of the joint effects of mixtures of hepatoxic agents: evaluation of a theoretical model in mice. Environ Res 1981;26:33–41.

Unkelbach HD, Pöch G. Comparison of independence and additivity in drug combinations. Arzneim-Forsch/Drug Res (I) 1988;38:1–6.