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The combined toxic effects of nonpolar narcotic chemicals to *Pseudokirchneriella subcapitata*

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ABSTRACT

This paper presents the toxicity data of 10 nonpolar narcotic chemicals on *Pseudokirchneriella subcapitata* (green algae) assessed by a new algal toxicity testing technique conducted under air-tight environment. Based on DO production, median effective concentration (EC50) varies from 1.73 mg/L (1-octanol) to 8040 mg/L (2-propanol). The endpoint of algal growth rate reveals similar sensitivity as that from DO production. Compared to literature data, *Pseudokirchneriella subcapitata* and *Nitrosomonas* are apparently more sensitive to nonpolar narcotics than other organisms such as minnow, daphnia, and *Tetrahymena pyriformis*. Furthermore, good correlations between toxic effects observed from *Pseudokirchneriella subcapitata* and other aquatic organisms were found. Hence, algal toxicity test can be considered as a surrogate test for estimating the toxicity of nonpolar chemicals to fathead minnow, Microtox, activated sludge, *Daphina magna*, and *Tetrahymena pyriformis*. The combined effects of 13 binary mixtures of nonpolar chemicals were investigated using both additive-index method and isobologram analysis. Overall speaking, the joint actions between these chemicals are strictly additive. Model analyses indicate that these compounds act on identical reaction sites or receptors, which verify that these chemicals are of the same toxicity mechanism (narcosis).

1. Introduction

The development of the narcosis quantitative structure–activity relationships (QSARs) has led to a general classification of organic chemicals. Toxicity of organic compounds can be compiled into two general categories: reversible nonspecific toxicity (i.e. narcosis) and irreversible specific toxicity (Schultz et al., 1998). Approximately 70% of all industrial organic chemicals are thought to exhibit a narcosis mode of toxic action (Bradbury and Lipnick, 1990). Historically, narcotic effects are estimated by the ability of a compound to interact with cellular membranes as quantitated by the 1-octanol:water partition coefficient (Kow). Narcotic effects are thought to result in noncovalent interactions including the disruption of van der Waals interactions between lipid and/or protein components within the membrane (Franks and Lieb, 1990).

The toxicity of nonpolar narcotics is directly related to the chemical's hydrophobicity or the 1-octanol:water partition coefficient, log Kow (Konemann, 1981). Narcosis type toxicity is also called the baseline toxicity (Konemann, 1981; Schultz et al., 1998; McFarland, 1970; Liao et al., 1996; Cronin and Schultz, 1997). Previous studies showed that the combined effects of mixtures of narcotic chemicals can be described by the concentration addition (CA) model: Broderius and Kahl (1985) evaluated the joint effects on fathead minnow, and reported that acute toxicity of binary and multiple-component mixtures containing up to 21 constituents to be simply additive, with the sum of toxic units ranging from 0.87 to 1.23. Wolf et al. (1988) reached the similar conclusions regarding

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the joint effects of organic chemicals on *Daphnia magna*. Broderius et al. (1995) evaluated the toxicity of binary mixtures of 46 industrial chemicals on fathead minnow and concluded that the chemical acted primarily by simple addition. According to the test results based on fish, water flea, and bacteria, most researchers have concluded that the joint action mode of nonreactive chemicals to be not significantly different from simple addition (Konemann, 1981; Hermens and Leeuwangh, 1982; McCarty et al., 1992; Chen and Chiou, 1995; Van et al., 1996).

Research efforts on multiple toxicity have existed for several decades. The major aims of these studies were to explore ways to predict and to identify hazardous combinations of chemicals relevant to humans and the environment. 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, that unified the above basic modes in a general model based on a bivariate normal distribution of the action tolerances. Their model has a noninteractive nature, meaning that the response of one toxicant does not affect the combination of another with receptors or the intrinsic activity of the other (Plackett and Hewlett, 1967). Christensen and Chen (1985) further expanded the model to introduce *n* toxicants and an arbitrary tolerance distribution.

Current algal toxicity test protocols (American Society for Testing and Materials (ASTM), 1994; International Organization for Standardization (ISO), 1987; United States Environmental Protection Agency (US EPA), 1996; Organization for Economic Cooperation and Development (OECD), 1984) are mainly batch tests in nature. These tests are considered as unsuitable for assessing the effects of volatile compounds due to their open test environment and vigorous mixing usually applied during the tests (European Centre for Ecotoxicology and Toxicology of Chemicals (ECETC), 1996). Several studies solved the above problem by adopting the closed system and providing large headspace for additional carbon supply (Herman et al., 1990; Brack and Rottler, 1994; Galassi and Vighi, 1981; Halling-Sørensen et al., 1996). Large headspace may cause a significant portion of the volatile compound to partition from the aqueous phase into the headspace until equilibrium is reached. Mayer et al. (2000) pointed out that the exposure concentration might thus be altered significantly. Therefore, they proposed the use of a closed test system with no headspace and an enriched bicarbonate buffer in the growth medium. However, enriched carbonate buffer may also result in increased ionic strength and lower test sensitivity (Brack and Rottler, 1994; Lin et al., 2005). To overcome this, we have proposed a closed-system algal toxicity test technique with no headspace and with a low bicarbonate-buffer content. The experimental design is quite simple and the test revealed satisfactory sensitivities to both metallic and organic toxicants (Chen et al., 2005; Lin et al., 2005).

Due to the aforementioned limitations of conventional algal toxicity test protocols, the combined effects of organic toxicants on phytoplankton have rarely been investigated. The objective of this study was to evaluate the mixture toxicity and the joint action modes of nonpolar narcotics using the air-tight algal toxicity test method developed previously (Chen et al., 2005, Lin et al., 2005). Furthermore, the toxicity of various mixtures of nonpolar chemicals was analyzed using a noninteractive multiple toxicity model to gain a theoretical insight for the observed phenomena.

2. Theory

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

$$Q = \Pr[(\delta_1)^{1/\lambda} + (\delta_2)^{1/\lambda} \leqslant 1], \quad (1)$$

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 of an individual organism to toxicant I, and λ is the similarity coefficient for the action of two toxicants on two biological systems or receptors. 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), while, $\lambda = 0$ indicates that two toxicants act on different biological systems (independent joint action). The nonresponse probability Q can be calculated by integrating the bivariate normal density function which describes the distribution of tolerances Z_1 and Z_2, where Z_1 and Z_2 are defined by the concentration–response relationships for the two toxicants applied individually. 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. Detailed description of numerical integration of Eq. (1) can be found in Christensen and Chen (1985).

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

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

where z_i denotes the toxicant concentration. The concentrations z_1 and z_2 are combined to produce exactly 50% response. A toxic unit (TU) for toxicant *i* is defined as $TU_i = z_i/EC50_i$, where $EC50_i$ is the (effective) concentration of toxicant *i* alone, giving 50% response. Simple addition (CA) is characterized by $M = 1$. The condition of $M > 1$ represents antagonism and $M < 1$ indicates synergism. Through probit analysis, *M* and its 95% confidence interval (CI) can be obtained. Mixtures that results in 95% CI for *M* that overlaps 1 are judged to be additive; those with 95% CI that do not overlap 1 are either antagonistic or synergistic in toxicity.

3. Materials and methods

3.1. Algal incubation

The alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*, UTEX 1648) was grown in a 4-L

transparent chemostat incubator. The growth medium was continuously supplied by a variable-speed pump. Air agitation was used to achieve adequate mixing. The chemostat reactors were placed in a constant-temperature room at $24\pm1\,^{\circ}C$. Light intensity was set at $65\,\mu E\,m^{-2}\,s^{-1}$ ($\pm10\%$). Growth medium composition is basically the same as that described by the EPA bottle technique (US EPA, 1996). However, according to our previous work (Chen and Lin, 1997), $NaNO_3$, K_2HPO_4, and EDTA contents were reduced to 12.75, 0.52 mg/L, and 30 μg/L, respectively. The dilution rate (D) for the chemostat was set at 0.25/day.

3.2. *Toxicity Testing*

After the algal incubator has reached the steady state, toxicity testing was conducted by transferring adequate amounts of algal suspension, dilution water (with growth medium), and toxicants into 300-mL BOD bottles. The BOD bottles were completely filled up with no headspace left. Water seal was provided to ensure a closed test environment. The bottles were then placed on an orbital shaker operated at 100 rpm. Temperature and light intensity were kept the same as the algal incubator. US EPA (1996) bottle medium with no EDTA content was used for toxicity testing. The dilution water was stripped by nitrogen gas to reduce the dissolved oxygen level. In addition, the N_2 gas contained 0.5% carbon dioxide as an extra carbon source. The DO level at the beginning of the test was approximately 1–3 mg/L. Two response endpoints were used to evaluate the toxicity of toxicants; dissolved oxygen production (ΔDO) and algal growth rate based on cell density. The median effective concentration (EC50) was defined as the toxicant concentration that reduced the final DO or algal growth rate to half of that obtained by the control. Probit analyses were applied to obtain the concentration–response relationships and EC50 values. The initial inoculated cell density was 15,000 cells/mL and the duration of the test was 48 h. A detail description of the test method and the concept of experimental design can be found from the author's previous work (Lin et al., 2005).

Mixture toxicity tests were conducted at equitoxic ratio, which means that $(z_1/EC50_1) : (z_1/EC50_2) = 1 : 1$. 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% 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 $1 \times EC50_1$ and $1 \times EC50_2$, respectively. Thirteen binary mixtures were tested including aromatic mixtures, aliphatic mixtures and aromatic–aliphatic mixtures. However, eventually, no distinct difference in terms of the combined toxic effects was found among the three types of mixtures.

Three aromatic chemicals and seven aliphatic compounds were tested in this study. All chemicals used were of 99% purity (reagent grade) except for benzene (95% purity). Tests were performed in triplicate. Stock solutions of toxicants were prepared in foil-wrapped glass containers. Before commencing the experiment, stock solution was freshly prepared and its concentration was analyzed using a HPLC analyzer. The analytical results were used to define the nominal concentrations for various treatments. Cell density was measured by electronic particle counting (Coulter Multisizer).

4. Results and discussion

EC50 values based on either DO production or algal growth rate as determined by the BOD-bottle test are summarized in Table 1. As for aromatics, the effective concentration (EC50) based on DO varies from 11.7 to 22.2 mg/L. EC50 s based on growth rate are between 14.4 and 28.7 mg/L. The growth rate endpoint is slightly less sensitive than DO. The differences between the two response endpoints, however, are insignificant. Furthermore, considering the case of chlorobenzene,

Table 1 – 48 h-EC50 and 95% confidence interval (CI) values for nonpolar narcotics

	Solubility (g/L)	Testing range (mg/L)	DO		Growth rate	
Toxicant			EC50[a]	95% CI	EC50	95% CI
Aromatics						
Benzene	2.0	10.2–75.0	20.9	16.2–35.9	28.7	25.5–32.9
Toluene	0.573	3.10–29.5	22.2	15.4–39.2	26.3	21.6–43.9
Chlorobenzene	0.40	3.01–30.0	11.7	9.07–13.8	14.4	10.8–17.9
Aliphatics						
Methanol	1000	0.50–60.4	3.01[b]	1.91–5.57	>60.4	—
Ethanol	792	2.01–29.9	8.09[b]	6.41–12.0	>29.9	—
1-Propanol	271.5	1750–10050	6370	5510–8230	9170	7940–11400
2-Propanol	402.4	3500–14000	8040	6530–10350	10500	9780–11300
1-Octanol	0.766	0.050–12.0	1.73	0.81–8.16	9.85	3.98–18.4
Acetone	219.9	2520–13600	5640	4380–6410	7270	5260–8590
2-Octanone	0.884	10.3–49.4	32.9	32.0–34.0	41.2	39.2–43.9

[a] EC50: mg/L.
[b] Data may be related to metabolic oxidation.

chlorine substituent on the aromatic-ring compound has doubled the inhibitory effects as compared to the toxicity of benzene. Kong et al. (1998) has reported that the activity of an enzyme system (Glucose-6-phosphate dehydrogenase, G6PDH) for photosynthesis was strongly inhibited by chlorinated benzene.

For aliphatics, EC50 based on DO production varies from 1.73 to 8040 mg/L. The most toxic compound to *Pseudokirchneriella subcapitata* is 1-octanol and the least toxic substance is 2-propanol. The difference in toxicity among this category of chemicals is almost four orders of magnitude. Considering the growth rate endpoint, EC50 is within the range of 9.85–10,500 mg/L. Three compounds, i.e., methanol, ethanol and 1-octanol, revealed much stronger inhibitory effects on DO production than that on algal growth rate. In particular, as shown in Fig. 1, methanol displayed no significant effects on algal growth. However, strong inhibitory effects on DO production were observed. When the methanol concentration was increased from 10 to 60 mg/L, a moderate hormesis phenomenon was observed as the final cell densities for treatments were even higher than that for the controls. In the mean time, the inhibition rate based on DO production was 100%. Therefore, it appears that methanol is quite toxic to algal photosynthesis reactions but displays only minor inhibitory effect on algal growth. Ethanol also revealed a similar pattern as that observed from methanol. Within the range of 10–30 mg/L of ethanol, enhancement of algal growth was observed while inhibitory effect on the DO endpoint has reached 100%.

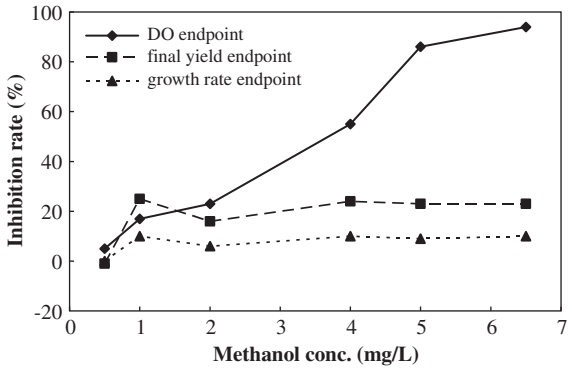

Fig. 1 – The inhibition rates of methanol according to different endpoints.

Gustavson et al. (1998) reported that alkanol (C_n–OH) toxicity increases with the chain length—the number of carbon atoms. From Table 1, we may find that the above conclusion is valid for the observed toxicity based on algal growth rate when considering C_1–OH (methanol), C_2–OH (ethanol), C_3–OH (propanol) and C_8–OH (octanol). However, the toxicity of C_1–OH (methanol) and C_2–OH (ethanol), based on the DO endpoint, is two to three orders of magnitude higher than that for the C_3–OH (propanol). It seems that the chain-length phenomenon for alkanol toxicity is not applicable to algal photosynthesis reaction. Although alkyl alcohols have a designated general mode of narcosis *I*, this refers only to physiological manifestation of the toxicity and not to any specific molecular mechanism. The excess toxicity revealed by methanol and ethanol may be related to the enhancement of membrane penetration due to small molecular size. Akers et al. (1999) have made similar observations based on the effects of halogenated aliphatic chemicals to the ciliate *Tetrahymena pyriformis*.

The above theory of the enhancement of membrane penetration still cannot satisfactorily explain the low toxic effects for methanol or ethanol as revealed by the growth rate endpoint. Previous report based on mammalian testing indicated that methanol can be transformed into formaldehyde through metabolic oxidation (Manaham, 1992). Theoretically, 32 mg/L of methanol may produce 30 mg/L of

Table 2 – Comparison of species sensitivities to nonpolar narcotics

Toxicant	*Pseudokirchne subcapitata* log(1/EC50)	Fathead minnow[a] log(1/LC50)	Activated sludge[a] log(1/EC50)	*Nitrosomonas*[a] log(1/EC50)	*Tetrahymena*[a] log(1/IGC50)	*Daphnia magna*[b] log(1/LC50)	Microtox[c] log(1/EC50)
Aromatics							
Benzene	0.57	0.49	−1.10	0.78*	−0.28	−0.54	−0.004
Toluene	0.62	0.43	−0.50	0.04	0.16	−0.57	0.71*
Chlorobenzene	0.98	—	−0.14	2.20*	0.09	−1.26	0.95
Chlorobenzene							
Aliphatics							
1-Propanol	−2.02	−1.88	−2.26	−1.21*	−1.68	−2.16	−2.10
2-Propanol	−2.13	—	–	–	−1.99*,[b]	−2.77	−2.47
1-Octanol	1.88*	0.97	−0.17	0.29	0.36	1.22	1.61
Acetone	−1.99	−2.09	−2.92	−1.32*	−2.04	−2.46	−2.39
2-Octanone	0.59	—	—	—	—	—	1.02*

Unit for EC50 or LC50: mmol/L.
[a] Ren and Frymier (2003).
[b] Kaiser and Esterby (1991).
[c] Chen and Chiou (1995).
* Most sensitive species.

formaldehyde and cause a DO depletion of 16 mg/L. However, the formation of 30 mg/L of formaldehyde should also produce significant toxic effect on algal growth because the median effective concentration for formaldehyde is only 4.25 mg/L (Chen et al., 2005). Though the above biological pathway is not likely to take place in our algal toxicity tests, the possibility of transformation of methanol into other nontoxic chemical forms through metabolic oxidation cannot be completely ruled out. Therefore, for methanol and ethanol, further investigation regarding their toxicity and pathway is still necessary.

In Table 2, EC50 values based on the DO endpoint are compared with literature data derived from toxicity tests using other aquatic organisms. Figures in Table 2 are in terms of Log(1/EC50) or Log(1/LC50). Hence, greater values stand for higher toxicity or better sensitivity for the test organism. For aromatic compounds, *Nitrosomonas* is the most sensitive species among all the aquatic organisms in Table 2. *Daphnia magna*, *Pseudokirchneriella subcapitata* (algae), fathead minnow, and the luminescent bacteria (Microtox test) reveal similar sensitivities to aromatic compounds. On the other hand, considering aliphatics, *Pseudokirchneriella subcapitata* appeared to be more sensitive than other organisms, except for *Nitrosomonas*. Activated sludge, in contrast, is the least sensitive species among all the test organisms in Table 2.

Based on the DO data, in Fig. 2, the species sensitivity for various aquatic organisms was compared. A data point located below the diagonal line indicates that, the sensitivity of a specific test organism is lower than *Pseudokirchneriella subcapitata*. For the eight compounds tested by this study (methanol and ethanol were not included), one may find that most of the test organisms are relatively insensitive as compared to *Pseudokirchneriella subcapitata* except for *Nitrosomonas*. Therefore, *Pseudokirchneriella subcapitata* and *Nitrosomonas* are the most vulnerable species in the aquatic environment subject to the adverse effects of nonpolar chemicals.

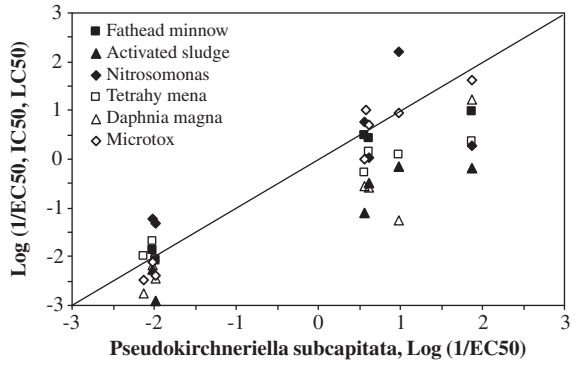

Fig. 2 – Comparisons of species sensitivity.

Good correlations between toxic effects observed from *Pseudokirchneriella subcapitata* and other aquatic organisms were found from the linear regression. As shown in Table 3, EC50 values for fathead minnow, Microtox, activated sludge, *Daphina magna*, and *Tetrahymena* correlated well with EC50 s from *Pseudokirchneriella subcapitata* (DO endpoint). The correlation coefficients (R^2) range from 0.85 to 0.97. Hence, algal toxicity test can be considered as a surrogate test for estimating the toxicity of nonpolar chemicals to these aquatic species. The reason for the above good correlations could be due to the simple and nonspecific toxicity mechanism (narcosis). *Nitrosomonas*, however, did not show good linear relationship with algal toxicity and the R^2 value is only 0.59.

The toxic effects of binary nonpolar mixtures are shown in Table 4, in terms of the additive index (*M* or ΣTU_i), 95% CI for *M*, and toxic ratio (TU_1/TU_2). These tests were conducted at equitoxic ratio ($TU_1/TU_2 = 1$) based on the EC50 (DO endpoint) values for the two test compounds. The additive index varies from 0.76 to 1.31. The majority of index values are close to one indicating that these toxicants acted additively. Judging from the 95% CI for *M*, all the mixtures displayed additive joint action mode based on the DO endpoint. For the growth rate endpoint, toxic ratios for binary mixtures of toxicants are irregular and the additive indices vary from 0.75 to 1.37. Judging from the observed joint action modes, most mixtures were still additive except for one case (1-octanol and acetone) which was found to be slightly synergistic.

Four binary-mixtures were chosen for more detailed isobologram analyses (Fig. 3). For each specific mixture, different pairs of the correlation coefficient (ρ) and the similarity coefficient (λ) were used to construct the isoboles at 50% response level. By comparing the calculated isobole and the actual data points, the optimum values for ρ and λ

Table 3 – Correlations between EC50 values derived from BOD bottle test (independent variable) and other aquatic organisms (dependent variable)

Toxicity assay	*n*	R^2	*a**	*b**	SE	*F*
Fathead minnow	5	0.97	−0.26	0.82	0.29	97.63
Microtox	8	0.97	−0.14	1.05	0.33	179.19
Activated sludge	6	0.91	−1.19	0.68	0.39	40.5
Daphnia magna	7	0.85	−0.99	0.76	0.60	27.33
Tetrahymena	7	0.96	−0.58	0.63	0.23	128.02
Nitrosomonas	6	0.59	0.13	0.62	0.94	5.83

* *a* is slope, *b* is intercept of the equation $\text{Log}(1/Y) = a + b\,\text{Log}(1/X)$, where *X* is the EC50 for *P. subcapitata* (based on DO) and *Y* represents the EC50 for the other assays.

Table 4 – The combined effects of mixtures of nonpolar narcotics

Toxicant 1	Toxicant 2	DO			Growth rate		
		ΣTU_i	95%CI	TU_1/TU_2	ΣTU_i	95%CI	TU_1/TU_2
Benzene	1–Octanol	0.76 (+)	1.03–0.51	1/1	0.86 (+)	1.08–0.55	4.15/1
Benzene	2-Octanone	0.98 (+)	1.17–0.73	1/1	1.13 (+)	1.42–0.72	0.91/1
Toluene	Chlorobenzene	0.91 (+)	1.01–0.80	1/1	1.17 (+)	1.40–0.90	1.04/1
Toluene	2-Propanol	1.31 (+)	1.47–1.16	1/1	1.05 (+)	1.19–0.92	1.09/1
Toluene	1-Octanol	0.91 (+)	1.09–0.73	1/1	0.89 (+)	1.14–0.60	4.8/1
Toluene	Acetone	0.90 (+)	1.40–0.61	1/1	1.08 (+)	1.43–0.61	1.09/1
Toluene	2-Octanone	1.12 (+)	1.56–0.78	1/1	1.37 (+)	1.88–0.82	1.06/1
Chlorobenzene	1-Octanol	0.96 (+)	1.17–0.76	1/1	0.96 (+)	1.05–0.87	4.63/1
Chlorobenzene	2-Octanone	0.98 (+)	1.25–0.67	1/1	1.22 (+)	1.63–0.72	1/1
2-Propanol	Acetone	1.26 (+)	2.16–0.82	1/1	1.19 (+)	1.51–0.91	0.98/1
1-Octanol	2-Propanol	1.00 (+)	1.15–0.87	1/1	0.92 (+)	1.47–0.64	0.23/1
1-Octanol	Acetone	1.28 (+)	1.74–0.89	1/1	0.75 (S)	0.91–0.58	0.23/1
1-Octanol	2-Octanone	1.01 (+)	1.32–0.63	1/1	0.98 (+)	1.15–0.78	0.22/1

TU, toxic unit; CI, 95% confidence interval; (+), simple addition; (s), synergism.

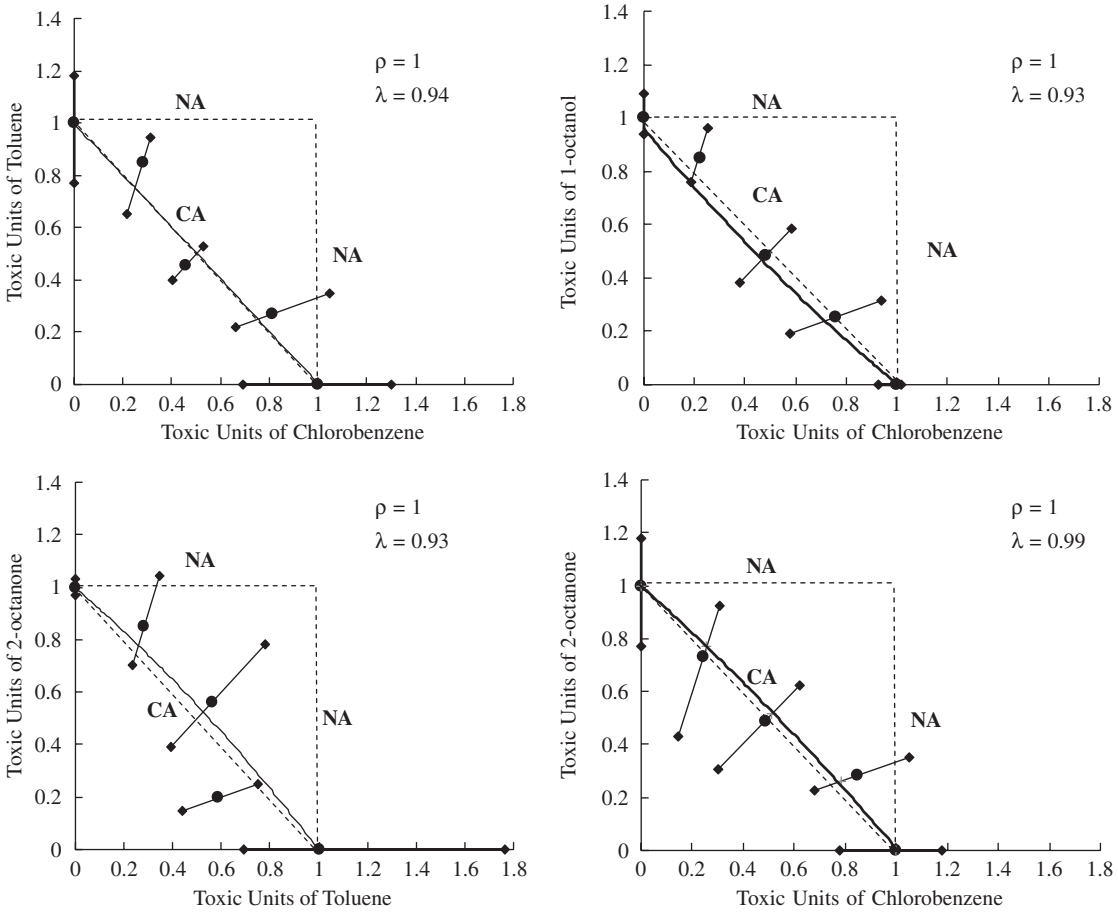

Fig. 3 – Isobolograms for the combined toxic effects of nonpolar narcotics (based on DO endpoint).

can then be determined using the minimum-χ^2 approach. For a pure-culture microorganism, ρ is expected to approach unit (1 or –1) due to the genetic simplicity of the culture. The author's previous work has also demonstrated that the optimum ρ values for *Escherichia coli* and activated sludge (mixed culture) are 1 and 0, respectively (Lin and Chen, 1996). In Fig. 3, the optimumρ values for the four binary mixtures are all equal to 1, which is consistent with the above theoretical expectation. On the other hand, the similarity coefficient (λ) varied from 0.93 to 0.99. This indicates that the two toxicants

in a specific mixture have almost identical reaction sites (or receptors). Such a conclusion also agrees with the theoretical expectation that toxicants having the same toxicity mechanism (narcotic mode of action) should act on the same reaction sites of a biological system. Overall, the isobolograms in Fig. 3 depict strict additivity between toxicants indicating that CA is the joint action mode for nonpolar narcotics.

5. Conclusions

This paper presents the toxicity data of 10 nonpolar narcotic chemicals on *Pseudokirchneriella subcapitata* (green algae) assessed by a new algal toxicity testing technique conducted under air-tight environment. Based on DO production, median effective concentration (EC50) varies from 1.73 mg/L (1-octanol) to 8040 mg/L (2-propanol). The endpoint of algal growth rate reveals similar sensitivity as that from DO production. Compared to literature data, *Pseudokirchneriella subcapitata* and *Nitrosomonas* are apparently more sensitive to nonpolar narcotics than other organisms such as minnow, daphnia, and *Tetrahymena pyriformis*. Furthermore, good correlations between toxic effects observed from *Pseudokirchneriella subcapitata* and other aquatic organisms were found. Hence, algal toxicity test can be considered as a surrogate test for estimating the toxicity of nonpolar chemicals to fathead minnow, Microtox, activated sludge, *Daphina magna,* and *Tetrahymena*. The combined effects of 13 binary mixtures of nonpolar chemicals were investigated using both additive-index method and isobologram analysis. Overall speaking, the joint actions between these chemicals are strictly additive. Model analyses indicate that these compounds act on identical reaction sites or receptors, which verify that these chemicals are of the same toxicity mechanism (narcosis).

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