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Estimating low-toxic-effect concentrations in closed-system algal toxicity tests

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ABSTRACT

The no-observed-effect concentrations (NOEC) and EC_{10} values for 108 organic compounds were estimated, using multiple endpoints (i.e., biopopulation, growth rate, and dissolved oxygen production), from previous data obtained by a closed-system algal toxicity test (test alga: *Pseudokirchneriella subcapitata*). These low-toxic-effect concentrations are valuable to risk assessment of chemicals and protection of the aquatic environment as such information is quite scarce in existing toxicological databases. Furthermore, based on limited amount of available data, we found that the risk of organic toxicants to phytoplankton may be severely underestimated by existing databases, which are primarily derived by the conventional batch technique. Good correlation relationships between NOEC (or EC_{10}) and EC_{50} values were established. For polar and nonpolar narcotics, quantitative structure–activity relationships (QSARs) based on hydrophobicity, and/or the lowest unoccupied molecular orbital energy (Elumo) were developed with satisfactory predictive powers. The above statistical relationships can be applied to derive a preliminary estimation for the low-toxic-effect levels for other (or new) organic compounds that has no toxicological data available.

1. Introduction

Ecological risk assessments of chemicals are aimed at estimating low, or no toxic effect levels, which may then be used as input for risk assessments, or the development of environmental quality criteria and guidelines for risk management purposes. The no-observed-effect concentration (NOEC) is a traditional parameter adopted by risk assessment procedures. NOEC is derived by hypothesis testing in which treatment responses are compared with a control response to test the null hypothesis that they are the same. The determination of NOEC is highly dependent on the test design, e.g., the selection of test concentrations and the number of replicates (Kooijman et al., 1996). In the past decade, the relevance and utility of the NOEC has been seriously criticized (Chapman et al., 1996, 1998; Chapman and Chapman, 1997; Moore and Caux, 1997). Previous studies pointed out that, NOEC was highly variable and concluded that EC_{50}, and other point estimates (EC_x), are more consistent parameters (Chapman et al., 1996; Chapman and Chapman, 1997). Moore and Caux (1997), based on the analyses of 198 toxicity data sets, found that most NOECs represent 10–30% reductions from control responses, and suggested that the regression-based approach is a better tool than hypothesis testing for estimating low-toxic effects. The Organization for Economic Cooperation and Development Workshop has therefore recommended replacing the NOEC with a regression-based estimation procedure (Chapman, 1997). However, the use of point estimator also suffers from several shortcomings, such as: estimates of low-toxic effects were often model dependent when an extrapolation beyond the toxicity data was required and, confidence intervals can be quite large, at 5% effect and lower (Moore and Caux, 1997). Isnard et al. (2001) showed that EC_5, and the lowest bound of the confidence interval of the EC_{10} were close to the NOEC and concluded that the EC_x approach would lead to no major changes in the risk assessment procedure. Therefore, they questioned the necessity for replacing the traditional hypothesis testing method by the point estimating approach.

Toxicity testing with microalgae has been used extensively in ecotoxicological studies. The traditional batch tests have been applied by most algal toxicity test protocols (OECD, 1984, 2000; ISO, 1987; US EPA, 1996). These tests have been challenged in regard to their applicability for testing volatile organic toxicants (European Centre for Ecotoxicology and Toxicology of Chemicals. 1996), considering their open test environment and the vigorous mixing provided during testing. Several closed-system tests have been proposed by previous researchers (Herman et al., 1990; Galassi and Vighi, 1981; Halling-Sørensen et al., 1996; Mayer et al., 2000). Most of these closed-system tests are considerably more complicated in experimental design, compared to the conventional batch technique. Furthermore, the enriched bicarbonate buffer, as applied by some of the above researchers, may also

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result in increased ionic strength and lower test sensitivity (Lin et al., 2005). Therefore, algal toxicity data derived from closed-system tests are still quite scarce. The author's recent work has proposed a closed-system algal toxicity test technique, with no headspace and with low bicarbonate buffer content (Lin et al., 2005). The experimental design is simple and the test revealed satisfactory sensitivities to both metallic and organic toxicants. The test technique has been successfully applied to assess the toxicity of aldehydes, chlorophenols, anilines, benzenes, alkanes, alcohols, ketones, and nitriles (Tsai and Chen, 2007). In addition, our results showed that, based on EC_{50} values, conventional algal batch tests tend to underestimate the toxicity of organic compounds. Toxicity observed from the closed-system test is approximately 2- to 380-fold higher than that estimated by conventional batch tests (Tsai and Chen, 2007).

In existing toxicity databases, algal toxicity data for low-toxic-effect levels are still not abundant as compared to those based on the median effective concentration. In addition, most of the above data were derived primarily by conventional batch-type tests (open test systems). The objective of this study is to present low-toxic-effect concentrations (in terms of NOEC and EC_{10}) for 108 organic toxicants on *Pseudokirchneriella subcapitata* (green alga), as obtained from our closed-system tests. Furthermore, correlation relationships were established with respect to EC_{50} values, the 1-octanol:water partition coefficient (K_{ow}), and the lowest unoccupied molecular orbital energies (Elumo), to enhance the predictive capability of low-toxic-effect concentrations for other organic toxicants.

2. Materials and methods

In the present study, 108 sets of raw data including aldehydes, nitriles, anilines, chlorophenols, benzenes, alkanes, alcohols, polycyclic aromatic hydrocarbons, and pesticides from the author's previous works (Chen et al., 2006; Yeh and Chen, 2006; Tsai and Chen, 2007) were analyzed for low-toxic-effect concentrations. These toxicants were divided into three categories, i.e., nonpolar narcosis, polar narcosis, and reactive, according to previous studies (Verhaar et al., 1992; Russom et al., 1997; Akers et al., 1999). The test alga is *P. subcapitata*. All chemicals used were of reagent grade. The toxicant concentrations presented in this work are in the form of nominal concentrations. The differences between the nominal concentration and the actual measured concentration were less than 6% (Tsai and Chen, 2007). All tests were conducted in triplicate with test duration of 48 h. Three different endpoints were used to analyze the toxic effects of various organic compounds: dissolved oxygen (DO) production, algal growth rate (GR), and the net production of algal cell density (final cell density−initial cell density, biopopulation). Toxicity tests were conducted using the 300-ml biochemical oxygen demand (BOD) test bottles, with no headspace left. A water seal was provided to ensure a closed-test environment. More detailed description regarding the test technique can be found in the author's previous work (Lin et al., 2005).

One-tail Dunnett's procedure was applied for the estimation of NOEC and LOEC values at 5% level of significance. NOEC was defined as the toxicant concentration which caused no significant difference compared to the test controls, with respect to all test endpoints (i.e., DO production, growth rate, and biopopulation). The studentized range (*SI*) can be calculated as follows:

$$SI = \frac{Xc - Xi}{Sw\sqrt{(1/nc) + (1/ni)}} \quad (1)$$

where *Xc* and *Xi* are mean observations from controls and treatments, respectively. *Sw* is the square root of the within-group-variance and, *nc* and *ni* are the numbers of replicates for the control and treatment. A specific treatment is considered to be significantly different from the controls if the corresponding *SI* value is greater than the critical value (*T*) specified by the Dunnett's *T* tables.

The EC_{10} value was determined using the best-fit-model approach as described below: Experimental data were fitted into three different dose–response models, i.e., probit, logit, and Weibull. The best-fit model was determined based on the minimum χ^2 values, which calculate the sum of squares of differences between the observation and the model prediction. EC_{10} values were then estimated using the best-fit model. Experimental data were also analyzed by *G* test in order to test the null hypothesis that the fit of the model was adequate (Moore and Caux, 1997). The observed responses were considered as not significantly different from the model estimates if $p > 0.05$. The equation for computing *G* is given by

$$G = 2\sum_{i=1}^{a} f_i \ln\left(\frac{f_i}{\hat{f}_i}\right) \quad (2)$$

where *a* is the number of replicates summed over all treatments, f_i is the observed response for treatment *i*, and $\hat{f}_i$ is the corresponding model estimate. The value of *G* is then compared with the critical value of χ^2 for *a*-*p*-1 degrees of freedom at $\alpha = 0.05$, where *p* is the number of parameters in the model equation.

Regression analyses were performed by using MINITAB (Ver 14.2, MINITAB, State College, PA, USA) to establish prediction models for NOEC and EC_{10}. Leave-one-out cross-validation was carried out to test the significance of each prediction model. The statistical quality was judged by the square of correlation coefficient (r^2), the Fisher criterion (*F*), the root mean square error (*S*), and the cross-validated correlation coefficient (Q^2).

3. Results

Table 1 presents the NOEC, LOEC, and EC_{10} values for 108 organic toxicants. In addition, EC_{50} values, the 1-octanol:water partition coefficient (K_{ow}), the lowest unoccupied molecular orbital energies (Elumo), and literature NOEC values (*P. subcapitata*, *Daphnia magna*, and fathead minnow) were also listed for discussion. The 108 toxicants were divided into three categories, i.e., nonpolar narcotic (NP), polar narcotic (P), and reactive (R), according to each chemical's modes of toxic action. As indicated in Table 1, 36% of the compounds have yielded identical NOEC values for all three test endpoints (i.e., biopopulation, growth rate, and DO production). Also, 58% (63 sets) of data showed that biopopulation and growth rate were equally sensitive in NOEC determination. Overall, biopopulation was found to be the most sensitive endpoint for approximately 80% of the test compounds. The rest of the compounds (ID numbers: 13, 14, 15, 17, 20, 23, 45, 57, 59, 62, 64, 70, 73, 74, 82, 83, 88, 93, 96, 97, and 105), on the other hand, displayed the most severe toxic effects on dissolved oxygen production. Furthermore, regression analyses showed that satisfactory correlation relationships can only be obtained when all data were derived by a single endpoint. Therefore, all NOEC values in Table 1 are based on biopopulation. However, for toxicants exerted stronger toxic effects on DO production, true NOEC values are specified in brackets. Similarly, all EC_{10} values were calculated based on the biopopulation endpoint, using the best-fit model. The percentages of best model fits for the three different models are: Probit 47.2%, Weibull 13.0%, and Logit 39.8%. However, no obvious model preference was found among the above three models.

In Table 1, only 50% of the cases were tested with low-enough concentrations in order to obtain the actual NOECs. The main reason was that these tests were designed to explore the entire concentration–response relationship. Furthermore, our initial focuses were on the response on growth rate (GR) and DO production, instead of biopopulation. In many cases, a NOEC can be determined based on GR, but not for the endpoint of biopopulation.

Table 2 summarizes the ratios between EC_{50}, NOEC, and EC_{10} values. On average, EC_{10} is 1.65 times higher than the NOEC value. Furthermore, the average acute/chronic ratios (ACR) are 5.80 and 4.20, respectively, with respect to NOEC or EC_{10} values. A small fraction of the compounds were excluded from the regression because their ACR^1 (EC_{50}/NOEC) or ACR^2 (EC_{50}/EC_{10}) values are extremely large, as compared to the majority of data. The modes of action and ACR values for these outliers are listed at the bottom of Table 2. Previous studies showed that, with respect to nonpolar narcotic chemicals and algae, ACR is within the range of 3.5–4.5 (Gray and Sova, 1956; McGrath et al., 2004). Furthermore, Roex et al. (2000) concluded that ACRs for polar narcotic compounds and reactive toxicants are approximately 9.8 and

Table 1
NOEC, LOEC, and EC_{10} value for *Pseudokirchneriella subcapitata* (test endpoint: biopopulation).

ID	Chemical	CAS no.	EC_{50} (mg/l)	NOEC (mg/l)	LOEC (mg/l)	EC10 (mg/l)		log K_{ow}	Elumo	Green algae[a]		Water flea[a] (mg/l)	Fathead minnow[a] (mg/l)
										NOEC (mg/l)	Ratio[b]		
Benzene													
1	Benzene (NP)	71-43-2	15.77	5.81[c]	11.6	8.51	(W)	2.13	0.56	8[d]	1.38		10.2
2	Chlorobenzene (NP)	108-90-7	7.83	3.41[e]	4.87	4.03	(P)	2.84	0.15	<100		11	
3	1,2-Dichlorobenzene (NP)	95-50-1	2.85	<1.42[f]	1.42	1.74	(P)	3.43	−0.14	<10		0.63	
4	1,3-Dichlorobenzene (NP)	541-73-1	1.87	<0.7	0.7	0.792	(L)	3.53	−0.16	32	>45.71	0.5	1
5	1,4-Dichlorobenzene (NP)	106-46-7	2.07	<1.06	1.06	1.07	(L)	3.44	−0.22	5.6	>5.28	0.3	0.57
6	1,2,3-Trichlorobenzene (NP)	87-61-6	0.848	0.2[f]	0.390	0.301	(P)	4.05	−0.36	0.22[g]	1.10	0.03	
7	1,2,4-Trichlorobenzene (NP)	120-82-1	0.638	<0.230	0.230	0.191	(L)	4.02	−0.47	0.37[g]	>1.61	0.2	0.21
8	1,3,5-Trichlorobenzene (NP)	108-70-3	1.68	<0.730[c]	0.730	0.398	(L)	4.19	−0.4			0.2	
9	1,2,3,4-Tetrachlorobenzene (NP)	634-66-2	0.572	0.09[f]	0.180	0.216	(L)	4.6	−0.65	<3.2		0.55[h]	0.25
10	1,2,4,5-Tetrachlorobenzene (NP)	95-94-3	1.26	–	–	0.406	(P)	4.64	−0.73				
11	Hexachlorobenzene (NP)	118-74-1	0.226	<0.06[c]	0.06	0.0480	(L)	5.73	−1.04			0.00013	
12	Ethylbenzene (NP)	100-41-4	1.34	<0.66[c]	0.660	0.232	(P)	3.15	0.53	1	>1.52		
13	2-Chloro-*p*-xylene (NP)	95-72-7	2.34	0.87[e] (<0.87)[i]	1.90	0.945	(L)	3.86	0.19				
14	Nitrobenzene (NP)	98-95-3	13.9	0.78[e] (<0.78)[i]	9.37	3.23	(L)	1.85	−1.07	3.2	4.10	2.6	38.3
15	2-Chlorotoluene (NP)	95-49-8	10.7	9.18[e] (<4.94)[i]	11.3	7.37	(P)	3.42	0.18			0.14	
16	Toluene (NP)	108-88-3	14.2	3.1[f]	7.23	5.17	(W)	2.73	0.52	10[j]	3.23	1	5.44
17	4-Chlorotoluene (NP)	106-43-4	10.9	5.48[e] (<5.48)[i]	11.0	6.51	(P)	3.33	0.14				
18	2,4-Dichlorotoluene (NP)	95-73-8	3.53	<1.88[f]	1.88	1.96	(P)	4.24	−0.15				
19	2,6-Dinitrotoluene (NP)	606-20-2	2.20	<0.28[e]	0.280	0.0410	(L)	2.1	−1.75			0.06	
Alkanes													
20	1,1-Dichloroethane (NP)	75-34-3	42.9	33.38 (<27.82)[i]	38.9	36.5	(P)	1.79	1.79				
21	1,2-Dichloroethane (NP)	107-06-2	155	64.0[c]	118	95.8	(P)	1.48	1.13				20[k]
22	1,1,1-Trichloroethane (NP)	71-55-6	47.4	<17.6[c]	17.6	10.0	(L)	2.49	−0.27	<125		1.3	
23	1,1,2-Trichloroethane (NP)	79-00-5	105	<39.7[f]	39.7	54.1	(P)	2.07	0.32				9.4[k]
24	1,1,1,2-Tetrachloroethane (NP)	630-20-6	8.05	<5.31[c]	5.31	2.60	(L)	3.03	−0.48				
25	1,1,2,2-Tetrachloroethane (NP)	79-34-5	13.7	<**4.3**[f]	4.30	4.14	(P)	2.39	−0.07	<10			2.4[k]
26	Pentachloroethane (NP)	76-01-7	5.61	1.22[f]	2.44	2.50	(L)	3.05	−0.68	10	8.20		1.1[k]
27	Hexachloroethane (NP)	67-72-1	0.469	<0.17[c]	0.170	0.107	(L)	3.82	−0.97	<5.6			0.54[k]
28	Methylene chloride (NP)	75-09 2	33.1	<17.7[c]	17.7	9.26	(P)	1.25	0.6	56	>3.16		
29	Chloroform (NP)	67-66-3	22.9	<4.98[c]	4.98	1.71	(L)	1.97	−0.3			6.3	
30	Tetrachloromethane (NP)	56-23-5	23.6	<9.46[c]	9.46	8.25	(L)	2.83	−1.12				37.1
31	1,2-Dichloropropane (NP)	78-87-5	34.4	<9.82[c]	9.82	5.65	(L)	1.98	1.12				81[k]
32	1,3-Dichloropropane (NP)	142-28-9	19.9	0.10	0.940	0.0780	(L)	2	1.02	<5.6			57[k]
33	1-Chlorobutane (NP)	109-69-3	37.6	19.3 (<19.3)[i]	29.2	21.0	(L)	2.64	1.51				
34	cis-1,2-Dichloroethylene (NP)	156-59-2	59.7	<44.9[c]	44.9	42.0	(L)	1.86	0.38				
35	trans-1,2-Dichloroethylene (NP)	156-60-5	36.4	<30.0[c]	30.0	23.6	(L)	2.09	0.34				
36	Trichloroethylene (NP)	79-01 6	26.2	<9.75[e]	9.75	4.88	(L)	2.42	−0.06			2.3	
37	Tetrachloroethylene (NP)	127-18-4	10.6	<4.31[e]	4.31	2.20	(P)	3.4	−0.44	<500		0.4	0.84[k]
Phenol													
38	Phenol (P)	108-95-2	10.9	<8.48[e]	8.48	3.89	(P)	1.46	0.4			0.16	20.2
39	2-Chlorophenol (P)	95-57-8	8.63	4.93[c]	12.3	3.38	(P)	2.15	0.03			0.3	
40	4-Chlorophenol (P)	106-48-9	8.81	<5.00[e]	5.00	4.21	(L)	2.39	0.1	<0.6		0.63	
41	2,3-Dichlorophenol (P)	576-24-9	1.23	<0.500	0.500	0.413	(L)	2.84	−0.26				
42	2,4-Dichlorophenol (P)	120-83-2	2.43	<0.97	0.97	0.771	(P)	3.06	−0.24			0.21	0.29[l]
43	2,4,6-Trichlorophenol (P)	88-06-2	–	<0.500[c]	0.500	–	(L)	3.67	−0.05			0.5	0.97
44	2,3,4,6-Tetrachlorophenol (P)	58-90-2	0.0120	<0.100[c]	0.100	2.65E-05	(W)	4.45	−0.75			0.25	
45	Pentachlorophenol (P)	87-86-5	0.007	0.001 (<0.001)[i]	0.002	0.001	(P)	5.12	−0.98	0.04	40.00	0.05	0.056
46	2-Nitrophenol (P)	88-75-5	1.09	<0.12[c]	0.120	0.0530	(L)	1.79	−1.19				
47	3-Nitrophenol (P)	554-84-7	6.72	0.990[f]	1.98	2.20	(P)	2	−1.17				
48	4-Nitrophenol (P)	100-02-7	0.258	<0.15[c]	0.150	0.07	(L)	1.91	−1.07			1.3[m]	
49	2,4-Dimethylphenol (P)	105-67-9	13.5	<6.27[c]	6.27	3.70	(L)	2.3	0.4				20[l]
50	2,4-Dinitrophenol (P)	51-28-5	0.941	0.510[c]	1.02	0.388	(L)	1.67	−1.89				

Aniline												
51	3-Chloroaniline (P)	108-42-9	9.61	<5.01[c]	5.01	5.28	(P)	1.88	−0.02		0.013	
52	4-Chloroaniline (P)	106-47-8	1.49	<0.99[c]	0.990	0.319	(L)	1.83	−0.02		0.01	
53	2,4-Dichloroaniline (P)	554-00-7	3.39	0.509[f]	1.02	1.02	(P)	2.78	−0.04		0.015	
54	2,5-Dichloroaniline (P)	95-82-9	5.94	0.990[f]	2.99	2.79	(P)	2.75	−0.03			
55	2,6-Dichloroaniline (P)	608-31-1	16.3	4.0 0[f]	7.99	6.22	(P)	2.2	−0.03			
56	3,4-Dichloroaniline (P)	95-76-1	2.03	0.398	0.800	0.919	(P)	2.69	−0.03		0.006	
57	3,5-Dichloroaniline (P)	626-43-7	3.98	0.975[e] (<0.975)[i]	4.88	1.71	(P)	2.9	−0.03			
58	2,4,5-Trichloroaniline (P)	636-30-6	1.17	0.190	0.560	0.320	(L)	3.45	−0.04			
59	2,4,6-Trichloroaniline (P)	634-93-5	3.46	0.290[e] (<0.290)[i]	0.890	0.714	(P)	3.69	−0.04			
60	3,4,5-Trichloroaniline (P)	634-91-3	2.74	0.202[c]	0.870	0.408	(W)	3.32	−0.04			
61	2,3-Dimethylaniline (P)	87-59-2	45.4	0.973[e]	9.73	8.73	(W)	1.81	−0.004		0.1	
62	3,4-Dimethylaniline (P)	95-64-7	6.94	1.22[e] (<1.22)[i]	5.45	2.92	(L)	1.86	−0.005		0.01	
63	2-Bromoaniline (P)	615-36-1	11.3	<0.990	0.990	3.14	(L)	2.11	−0.02		0.08	
Aldehyde												
64	Formaldehyde (R)	50-00-0	2.55	1.06[e] (<1.06)[i]	2.02	0.801	(P)	0.35	0.79			
65	Acetaldehyde (R)	75-07-0	0.0170	<0.310[f]	0.310	5.54E-6	(P)	−0.22	0.84			
66	Propionaldehyde (R)	123-38-6	12.9	<9.31[c]	9.31	4.93	(P)	0.59	0.87			
67	Butyraldehyde (R)	123-72-8	11.95	<0.49[f]	0.490	0.001	(P)	0.83	0.87			
68	Glutaraldehyde (R)	111-30-8	3.04	<1.25[c]	1.25	0.390	(P)	−0.61	0.78			
69	2-Pyridinecarboxaldehyde (R)	1121-60-4	16.9	6.87[e]	13.7	5.25	(W)	1.81	−0.69			
70	3-Pyridinecarboxaldehyde (R)	500-22-1	30.0	0.530[e] (0.120)[i]	1.01	1.08	(W)	1.29	−0.78			
71	4-Pyridinecarboxaldehyde (R)	872-85-5	11.5	0.990[f]	4.95	2.37	(W)	1.35	−0.74			
72	2-Hydroxybenaldehyde (R)	90-02-8	3.12	<0.995[c]	0.995	1.24	(W)	0.44	−0.41		0.38	
73	3-Hydroxybenaldehyde (R)	100-83-4	34.9	12.0[e] (<12.0)[i]	21.0	11.6	(L)	0.29	−0.54			
74	4-Hydroxybenaldehyde (R)	123-08-0	2.54	0.980[e] (<0.330)[i]	1.30	0.330	(P)	0.43	−0.45			
Nitrile												
75	Acetonitrile (NP)	75-05-8	5.51E+03	<3.17E+03[f]	3.17E+03	2.85E+03	(W)	−0.39	−0.01		160	
76	Chloroacetonitrile (R)	107-14-2	11.5	<1.60[f]	1.60	2.15	(W)	0.45	−0.06			
77	Dichloroacetonitrile (R)	3018-12-0	1.64	<1.33[c]	1.33	0.425	(P)	0.93	−0.07			
78	Trichloroacetonitrile (R)	545-06-2	0.02	0.006[f]	0.0120	0.008	(P)	2.09	−0.09			
79	Bromoacetoitrile (R)	590-17-0	0.07	<0.025	0.0250	0.0240	(L)	0.36	−0.074			
80	Propionitrile (NP)	107-12-0	127.72	14.9[f]	59.7	22.5	(W)	0.14	−0.01			
81	3-Chloropropionitrile (R)	542-76-7	160	64.0[c]	160	114	(P)	0.34	−0.03			
82	Butyronitrile (NP)	109-74-0	724.2	366[e] (91.4)[i]	549	213	(P)	0.66	−0.01			
83	Isobutyronitrile (NP)	78-82-0	728.5	186 (<186)[i]	371	416	(P)	0.44	−0.01			
84	4-Chlorobutyronitrile (R)	628-20-6	526.2	225[f]	449	336	(P)	0.56	−0.02			
85	Benzonitrile (NP)	100-47-0	23.3	12.8[c]	21.3	8.35	(W)	1.56	−0.07			
86	Malononitrile (NP)	109-77-3	12.4	0.480	0.970	0.603	(P)	−1.2	−0.03			
Alcohol												
87	1-Propanol (NP)	71-23-8	4.95E+03	<1.75E+03[c]	1.75E+03	1.77E+03	(P)	0.25	3.63			
88	2-Propanol (NP)	67-63-0	8.47E+03	3.50E+03[e] (<3.50E+03)[i]	5.00E+03	5.40E+03	(P)	0.05	3.49			
89	1-Octanol (NP)	111-87-5	1.85	<0.05[e]	0.05	0.067	(P)	−0.24	3.34		1	1.19
Ketone												
90	Acetone (NP)	67-64-1	5.28E+03	2.52E+03[c]	5.72E+03	3.04E+03	(P)	3	0.84		3110	
91	2-octanone (NP)	111-13-7	32.2	10.3[f]	24.7	21.3	(L)	2.37	0.87			
Pesticides												
92	Atrazine (R)	1912-24-9	0.078	<0.02	0.02	0.0180	(P)	2.82	−0.48	0.076	>3.8	
93	MCPA (R)	94-74-6	3.12	0.8[e] (<0.5)[i]	1.60	1.35	(W)	2.52	−0.40			
94	Parathion (R)	56-38-2	0.93	0.350[e]	0.7	0.0570	(L)	3.73	–		0.125	
95	Dichlorvos (R)	62-73-7	0.606	<0.1[c]	0.1	0.175	(P)	0.6	0.02		0.000109	0.07
96	Malathion (R)	121-75-5	2.32	1.20[e] (0.5)[i]	2	1.22	(P)	2.29	−2.64		0.0003	
97	Fenthion (R)	55-38-9	1.05	0.3 (<0.3)[i]	0.6	0.360	(P)	4.08	–			
PAHs												
98	Benzanthrone (NP)	82-05-3	0.048	<0.0138[e]	0.0138	0.005	(P)	4.81	−1.27			
99	Fluoranthene (NP)	206-44-0	0.033	<0.0160[c]	0.016	0.009	(L)	4.93	−0.93	32	>2000	0.0014	0.0104
100	Anthracene (NP)	120-12-7	0.402	<0.2[c]	0.20	0.106	(P)	4.45	−0.84			
101	Benzo[*a*]anthrace (NP)	56-55-3	0.008	<0.006[c]	0.006	4.81E-04	(L)	5.53	−0.81			
102	Phenanthrene (NP)	85-01-8	0.124	<0.06[f]	0.06	0.0530	(P)	4.35	−0.41		0.048	

Table 1 (*continued*)

ID	Chemical	CAS no.	EC_{50} (mg/l)	NOEC (mg/l)	LOEC (mg/l)	EC10 (mg/l)		log K_{ow}	Elumo	Green algae[a]		Water flea[a] (mg/l)	Fathead minnow[a] (mg/l)
										NOEC (mg/l)	Ratio[b]		
103	Acridine (NP)	260-94-6	0.524	<0.380[c]	0.380	0.242	(L)	3.32	−1.04			0.625	
104	Benzo[*b*]fluorene (NP)	243-17-4	0.043	<0.04[c]	0.04	0.002	(L)	5.19	−0.49				
105	Dibenzo[*b,i*]anthracene (NP)	135-48-8	0.194	0.04[e] (<0.04)[i]	0.08	0.0300	(P)	6.32	−1.52				
106	Perylene (NP)	198-55-0	0.053	<0.03[c]	0.03	0.003	(L)	6.11	−1.15				
107	Benzo[*b*]chrysene (NP)	214-17-5	0.005	<0.00125[f]	0.0013	0.001	(L)	6.54	−0.99				
108	Napthalene (NP)	91-20-3	2.51	<1.28[f]	1.28	0.855	(L)	3.32	−0.27			0.6[n]	0.45[k]

Mode of action: (NP): nonpolar narcosis, (P): polar narcosis, (R): reactive.

[a] ECOTOX reference number—green algae: *Pseudokirchneriella subcapitata* (69631, 69630, 45196, 18440, 12735, 9607) water flea: *Daphnia magna* (61182, 56378, 56345, 56306, 54868, 20489, 20249, 20061, 17138, 14533, 13070, 12872, 10553, 8764, 6628, 6449, 847, 707, 489, 249, 212, 6) fathead minnow: *Pimephales promelas* (57532, 20588, 20456, 17138, 16510, 14078, 12124, 3910, 3783).

[b] $NOEC/NOEC_{closed}$.

[c] Identical NOEC values for all three end points (i.e., biopopulation, growth rate, and DO production).

[d] Mayer et al. (2001).

[e] Identical NOEC values for biopopulation and growth rate endpoints.

[f] Identical NOEC values for biopopulation and DO endpoints.

[g] Calamari et al. (1983).

[h] Hermens et al. (1985).

[i] NOEC values based on the DO endpoint.

[j] European Commission—Joint Research Centre Institute for Health and Consumer Protection European Chemicals Bureau (2003).

[k] Di Toro et al. (2000).

[l] Holcombe et al. (1982).

[m] Kühn et al. (1989).

[n] European Commission—Joint Research Centre Institute for Health and Consumer Protection European Chemicals Bureau (2003).

Table 2
Ratio between EC_{50}, NOEC, and EC_{10}.

	EC_{10}/NOEC		ACR^1 (EC_{50}/NOEC)[a]		ACR^2 (EC_{50}/EC_{10})[b]	
	n	Mean (95%CI*)	*n*	Mean (95%CI)	*n*	Mean (95%CI)
Overall	50	1.65 (1.29–2.00)	48	5.80 (3.66–7.93)	100	4.20 (3.42–4.98)
Nonpolar	23	1.47 (1.15–1.78)	22	4.94 (2.49–7.40)	56	4.50 (3.23–5.78)
Polar	14	2.33 (1.27–3.39)	14	9.09 (3.18–15.01)	24	4.39 (2.74–6.03)
Reactive	13	1.23 (0.87–1.58)	12	3.53 (2.05–5.00)	20	4.49 (2.94–6.04)

* CI: confidence intervals.

[a] Outliers: 1,3-dichloropropane (NP;199), 3-pyridinecarboxaldehyde (R;56.6).

[b] Outliers: 1,3-dichloropropane (NP;255.1), 2,3,4,6-tetrachlorophenol (P;4000), acetaldehyde (R;2833.3), butraldeyde (R;11950), 2,6-dinitrotoluene (NP;53.7), 3-pyridinecarboxaldehyde (R;27.7), 1-octanol (NP;27.6).

17.3, respectively. In the present study, ACR^1 and ACR^2 values for nonpolar chemicals generally agree with previous conclusions. The ACR^1 for polar narcotics is also quite similar to the previous findings (McGrath et al., 2004). However, both ACR^1 and ACR^2 for reactive toxicants are apparently lower than literature values. The reasons causing the above discrepancies could be related to the removal of outliers from regressions and/or the relatively small sample size.

4. Discussion

Correlation relationships between low-toxic-effect levels and EC_{50} values were analyzed and the results are displayed in and Fig. 1(a) and (b). Excellent linear relationships can be observed from the diagrams, with r^2 values equal to 0.88 and 0.84 (based on NOEC and EC_{10}, respectively). These two equations indicate that NOECs and EC_{10}s can be estimated from EC_{50} values with very good accuracies. Only a few data points, mainly the outliers in Table 2, were located a little further from the regression line. Furthermore, as evidenced by the cross-validation with Q^2 equal to 0.883 and 0.838.

A good correlation relationship can also be obtained between LOEC and EC_{50} values, as shown by Eq. (3) with $r^2 = 0.92$ and $Q^2 = 0.91$. The slopes for all three equations are close to 1, indicating that the three regression lines are parallel. From the intercept values of the three equations, we may conclude that $LOEC > EC_{10} > NOEC$.

$$\log(1/\mathrm{LOEC}) = 1.0002\log(1/\mathrm{EC}_{50}) + 0.3292$$
$$n = 106,\ r^2 = 0.917,\ Q^2 = 0.914,\ S = 17.06,\ F = 1152.5 \qquad (3)$$

Quantitative structure–activity relationships (QSARs) for chemicals of different modes of action (nonpolar, polar, and reactive) were developed for estimations of NOECs and EC_{10}s, as shown in Table 3. Fig. 2(a) and (b) describes the relationships between the toxicity of nonpolar narcotic compounds and the logarithm of 1-octanol:water partition coefficient ($\log K_{ow}$). Both NOEC and EC_{10} can be successfully estimated by K_{ow}. The QSARs provide satisfactory fitting for the observed toxicity and the predictive powers are high ($Q^2 = 0.86$ and 0.85, respectively). Several statistical outliers were identified by the above QSARs for NOEC and EC_{10}. Most of these outliers revealed excess toxicity than that estimated by the baseline toxicity relationships ($\log K_{ow}$), except for acetone (ID90). In Table 1, one may find that 1,3-dichloropropane (ID32) and 1,2-dichloropropane (ID31) have similar $\log K_{ow}$ values; however, the former is approximately 10 times more toxic than the latter. Abe et al. (2000) made similar observations from *D. magna* and concluded that *meta*-substitution could be the key factor causing the higher observed toxicity. For malonoitrile (ID86), previous research (Russom et al., 1997) has suggested that

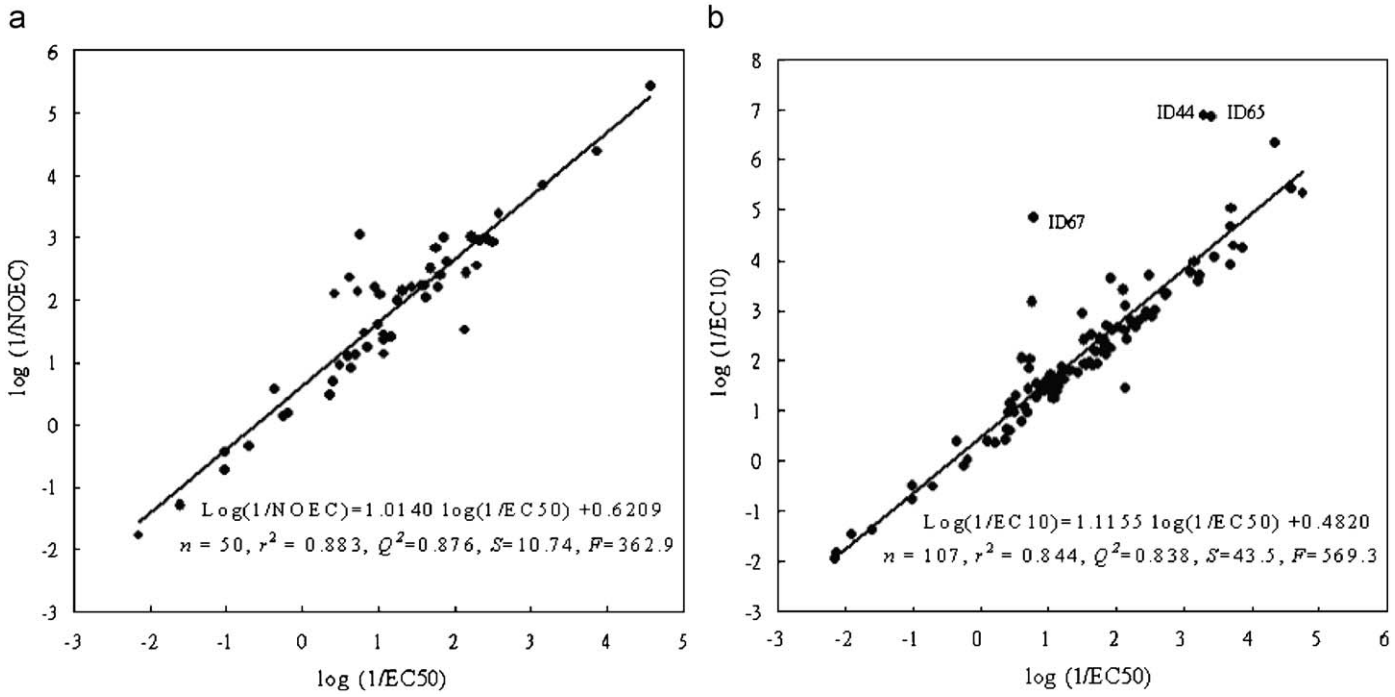

Fig. 1. Correlation relationships for NOEC and EC_{10} with EC_{50} values: (a) NOEC vs. EC_{50} and (b) EC_{10} vs. EC_{50}.

Table 3
QSARs for nonpolar narcotic, polar narcotic, and reactive toxicants.

Chemical	Endpoint	QSAR	n	R^2	S	F	Q^2	Outlier's ID
Non-polar narcosis	NOEC	$\log(1/\text{NOEC}) = 0.8624\log K_{ow} - 1.0633$	18	0.897	3.53	138.7	0.857	14,32,80,86,90
Polar narcosis	NOEC	$\log(1/\text{NOEC}) = 0.8786\log K_{ow} - 0.5927E_{LUMO} + 0.0188$	15	0.876	1.47	42.84	0.572	
Halogenated nitriles	NOEC	$\log(1/\text{NOEC}) = 2.2283\log K_{ow} - 4.717E_{LUMO} - 0.639$	6	0.876	2.16	28.30	–	
Non-polar narcosis		$\log(1/\text{EC10}) = 0.9349\log K_{ow} - 1.0380$	53	0.859	20.03	310.8	0.845	19,32,86,89,90,101
Polar narcosis	EC_{10}	$\log(1/\text{EC10}) = 0.9599\log K_{ow} - 1.3208E_{LUMO} - 0.360$	24	0.766	9,86	72.15	0.595	47
Halogenated nitriles		$\log(1/\text{EC10}) = 2.2886\log K_{ow} - 4.8790E_{LUMO} - 0.742$	6	0.850	2.86	22.67	–	

its actual mechanism is respiratory inhibition, instead of nonpolar narcosis. Therefore, the high excess toxicity for malononitrile is due to its reactive nature of toxic action. As for acetone (ID90), the reason for its lower toxicity is not yet clear; however, the same phenomenon was observed on the fathead minnow (Russom et al., 1997). For outliers that appeared only in the QSAR for NOEC (and vice versa: outliers for EC_{10} QSAR only), it is difficult to speculate the reasons. Therefore, these outliers (ID14, ID80, ID19, etc.) were considered as purely statistical. Finally, since the available data is not abundant ($n = 18$), it is still desirable to further improve the QSAR between NOEC and $\log K_{ow}$ in future studies.

For polar narcotic chemicals, the combination of $\log K_{ow}$ and Elumo was found to provide a satisfactory description for the observed toxicity, with r^2 equal to 0.88 and 0.77, respectively (Table 3). However, the predictive powers for these QSARs ($Q^2 = 0.57$ and 0.60) are less significant as compared to those for nonpolar narcotic compounds. This could be due to the fact that polar narcotics are mechanistically more complicated than nonpolar chemicals. It is thus more difficult to obtain excellent descriptions for this type of chemical, particularly at low-toxic-effect levels. The predictive power for polar narcotic QSARs can be further improved, as more data become available in the future.

In the present work, there are three main types of reactive toxicants, i.e., aldehydes, halogenated nitriles, and pesticides. No proper descriptor was found which can adequately describe all three types of toxicants, and the only valid QSAR is based on the combination of $\log K_{ow}$ and Elumo for halogenated nitriles. However, the predictive powers for these QSARs were not specified due to the relatively small sample sizes.

Literature NOEC values for algae (*P. subcapitata*), water flea, and fish were compared with results from our closed-system technique. Since literature data were derived using different test methods, and/or endpoints, comparison is based on the assumption that these data reflect the general performance, or sensitivity, of various organisms and is subject to change when more data become available. Algal data were based on conventional batch tests (open system) and biomass-type endpoints such as biomass, population, and chlorophyll content. Literature NOEC data are quite scarce because there are only 22 sets of data, and some of them are actually LOEC values. As shown in Table 1, most literature NOECs (72- or 96-h test duration) are considerably greater than those of our closed-system data (48-h test duration), with the exception of 4-chlorophenol. For the 13 sets of data that NOEC values have been specified, comparison was made by calculating the NOEC ratios (open-system test/closed-system test). The NOEC ratio varies from 1.1 to 2000, with the mean equal to 192. Such a large mean value for the NOEC ratio is mainly due to the compound—fluoranthene (NOEC ratio is greater than 2000). A second calculation, made by removing the fluoranthene data, produced a mean value of 11.9, which means that, on average, the conventional batch technique has overestimated the NOEC levels for more than one order of magnitude, as compared

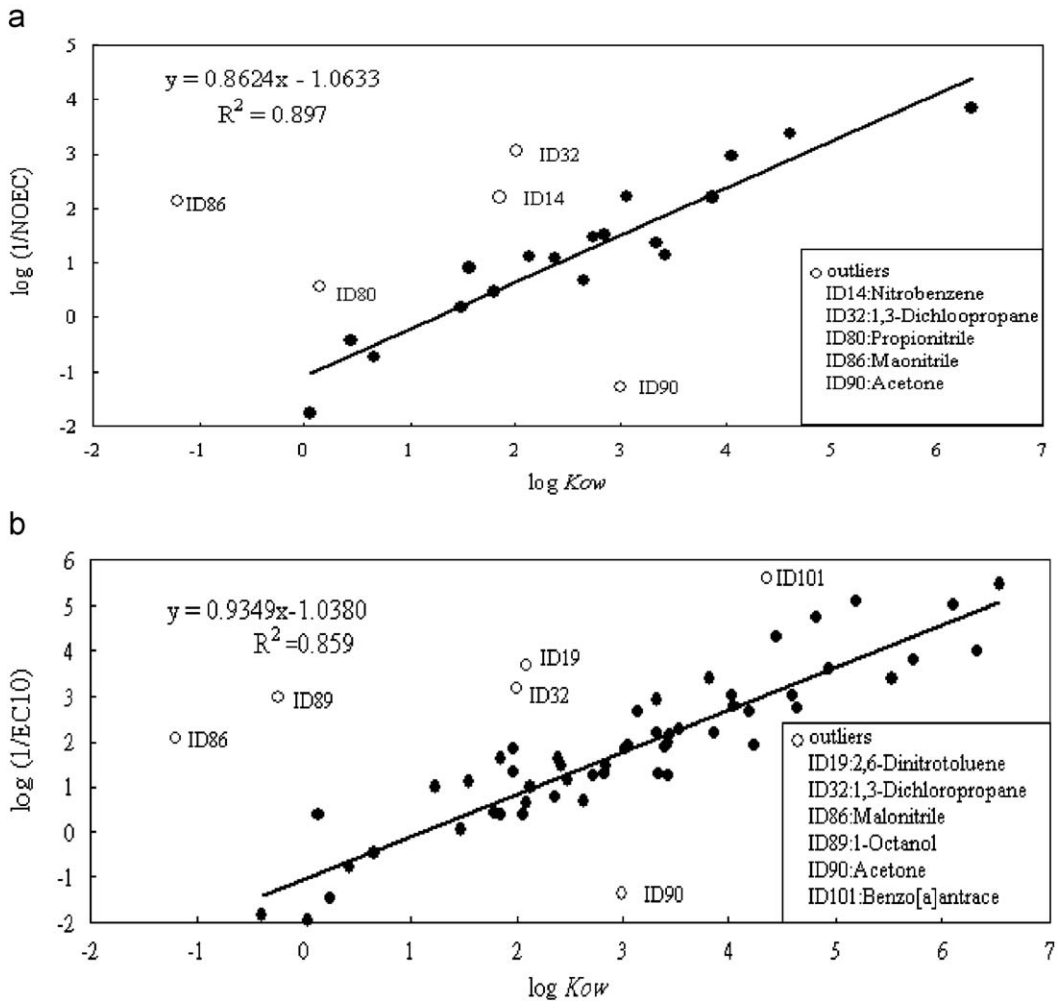

Fig. 2. The relationships between low-toxic-effect levels with $\log K_{ow}$ (the baseline toxicity relationships): (a) NOECs and (b) EC_{10}s.

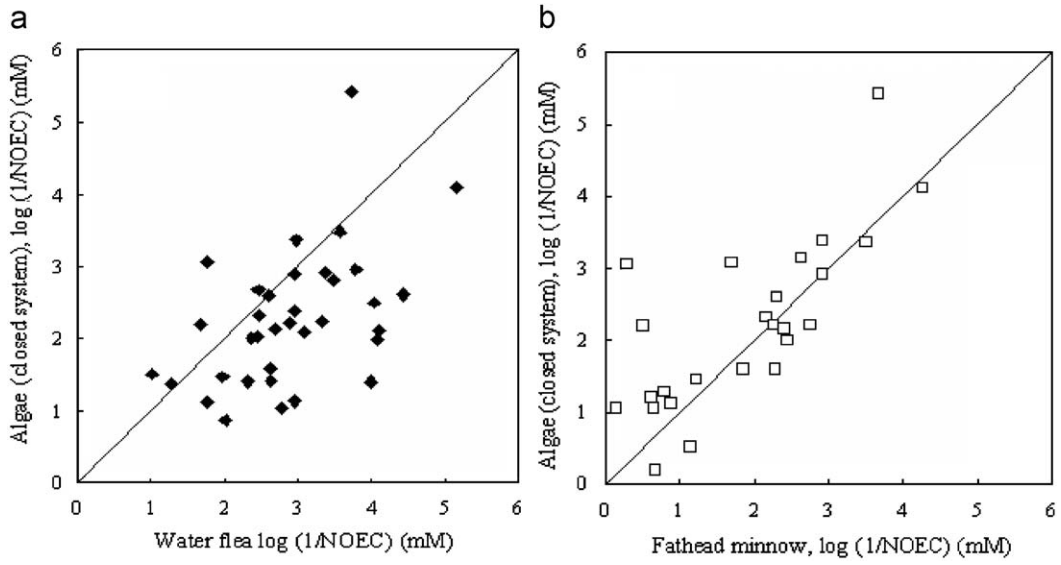

Fig. 3. Comparisons of NOEC values obtained by algal, water flea, and fish tests: (a) algae vs. water flea and (b) algae vs. fathead minnow.

to those by the closed-system test. Normally, one may expect that longer exposure time will result in more sensitive test results. However, our 48-h test results are consistently smaller than data from the traditional batch tests. The losses of organic compounds due to the open test environment applied by the batch tests should be the major reason causing the above differences in NOEC

values. Though the above comparison was based on limited amounts of data, it does reveal a possibility that the risks of organic toxicants to phytoplankton were severely underestimated by existing databases.

Fig. 3(a) compares the species sensitivity between algae and water flea (*D. magna*). Approximately 81% of the data show that *D. magna* is more sensitive than *P. subcapitata*, at NOEC levels. Such a conclusion is in contrast to our previous comparison based on EC_{50} values (Tsai and Chen, 2007). The discrepancy here may be due to the fact that in the case of water flea, different endpoints and/or different durations were used for NOEC and EC_{50} determinations. On the other hand, in algal toxicity test, NOEC and EC_{10} are based on the same test duration and test endpoint. In Fig. 3(b), NOEC values from the fathead minnow test were compared with closed-system test results. Fifty-six percent of the data indicate that algae are more sensitive than fathead minnow. However, the sensitivities for the two aquatic organisms are generally quite similar because only 4 sets of data displayed a difference greater than one order of magnitude. Furthermore, a good linear relationship can be found between algae and the fathead minnow (Eq. (4)), by removing 4 statistical outliers (i.e., nitrobenzene, 1,3-dichloropropane, pentachlorophenol, and 1-octanol). Thus, good correlation relationships existed between the fathead minnow and *P. subcapitata* existed for both NOEC and EC_{50} values (Tsai and Chen, 2007; Hsieh et al., 2006). Hence, the toxicity of organic toxicants to fathead minnow can be reasonably estimated by closed-system algal toxicity data:

$$\mathrm{Log}(1/\mathrm{NOEC})_{\text{fathead minnow}} = 0.9775\,\log(1/\mathrm{NOEC})_{\text{algae}} + 0.0207$$
$$n = 21,\ r^2 = 0.828,\ S = 4.07,\ F = 91.65,\ Q^2 = 0.789 \quad (4)$$

In current practice of chemical release control (European Commission, 2003a, 2003b, 2006), NOEC values from algal toxicity tests have not yet been given an important role in the assessment of chemical safety. The reasons could be related to insufficient algal toxicity data available, the low test sensitivity as revealed by the traditional batch technique, and/or the short exposure duration of the test. As a matter of fact, algal toxicity tests are known to be life-cycle tests because several generations can be produced within a short test period. The test endpoints (biomass, growth rate, or DO production) are sublethal and the responses are quantitative, instead of quantal. Our results also show that the sensitivity of alga (*P. subcapitata*) is better than that of fathead minnow, in terms of NOEC values. The above features indicate that algal toxicity test (the closed-system technique) is a time-saving and ideal assessment tool for deriving the ultimate safety evaluations for various organic compounds. More importantly, algae are known to be the primary producers in the aquatic food-chain. Any change in the algal community will inevitably affect other organisms in the aquatic environment. Therefore, the NOEC database presented in the present work will be useful for risk assessment of chemicals, and, thus, deserves more attention.

5. Conclusions

This paper presents the low-toxic-effect levels (NOEC and EC_{10}) for 108 organic compounds with respect to *P. subcapitata*, as derived by the closed-system algal toxicity test. There is a general consensus that the closed-system technique provides much better control to the exposure concentration and, thus, provides more meaningful concentration–response relationships for various organic toxicants. Since existing data for low-toxic-effect levels on *P. subcapitata* are still quite limited, data presented in the present study can be valuable to risk assessment of chemicals and protection of the aquatic environment. Furthermore, as revealed by the present study, it is likely that the risk of organic toxicants to phytoplankton has been severely underestimated by existing databases, which are primarily derived by the conventional batch technique. For both NOEC and EC_{10}, good correlation relationships between low-toxic-effect concentrations (i.e., NOEC, LOEC, and EC_{10}) and the EC_{50} values were established with the cross-validated correlation coefficients (Q^2) varying from 0.83 to 0.91. In addition, for polar and nonpolar narcotics, QSARs based on descriptors such as hydrophobicity (1-octanol:water partition coefficient, K_{ow}) and/or the lowest unoccupied molecular orbital energy (Elumo) were developed, with respect to NOEC and EC_{10} values. The above statistical relationships will be useful when it is necessary to derive a preliminary estimation for the low-toxic-effect levels for other (or new) organic compounds that have no toxicological data available.

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