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TOXICITY OF SUBSTITUTED ANILINES TO *PSEUDOKIRCHNERIELLA SUBCAPITATA* AND QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIP ANALYSIS FOR POLAR NARCOTICS

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Abstract—This study evaluated the toxic effects of substituted anilines on *Pseudokirchneriella subcapitata* with the use of a closed algal toxicity testing technique with no headspace. Two response endpoints (i.e., dissolved oxygen production [DO] and algal growth rate) were used to evaluate the toxicity of anilines. Both DO and growth rate endpoints revealed similar sensitivity to the effects of anilines. However, trichloroanilines showed stronger inhibitory effects on microalgal photosynthetic reactions than that on algal growth. For various aquatic organisms, the relative sensitivity relationship for anilines is *Daphnia magna* > luminescent bacteria (Microtox) ≥ *Pocelia reticulata* ≥ *Pseudokirchneriella subcapitata* ≥ fathead minnow > *Tetrahymena pyriformis*. The susceptibility of *P. subcapitata* to anilines is similar to fish, but *P. subcapitata* is apparently less sensitive than the water flea. The lack of correlation between the toxicity revealed by different aquatic organisms (microalgae, *D. magna*, luminescent bacteria, and *P. reticulata*) suggests that anilines might have different metabolic routes in these organisms. Both hydrogen bonding donor capacity (the lowest unoccupied molecular orbital energy, E_{lumo}) and hydrophobicity (1-octanol:water partition coefficient, K_{OW}) were found to provide satisfactory descriptions for the toxicity of polar narcotics (substituted anilines and chlorophenols). Quantitative structure–activity relationships (QSARs) based on E_{lumo}, log K_{OW}, or both values were established with r^2 values varying from 0.75 to 0.92. The predictive power for the QSAR models were found to be satisfactory through leave-one-out cross-validation. Such relationships could provide useful information for the estimation of toxicity for other polar narcotic compounds.

Keywords—*Pseudokirchneriella subcapitata* Anilines Toxicity Median effective concentration

INTRODUCTION

A significant portion of industrial organic pollutants is thought to exhibit a narcosis mode of toxic action [1]. Narcotic effects are estimated by the ability of a compound to interact with cellular membranes as quantitated by the 1-octanol:water partition coefficient (K_{OW}). For nonpolar narcotics, high correlations exist between log K_{OW} and the effect concentrations, representing the so-called baseline toxicity [2]. The toxicity of nonpolar chemicals is thus considered as primarily hydrophobicity dependent [3,4]. Moreover, polar narcotics were found to display higher toxicity (5–10 times) than predicted from baseline toxicity [5]. The polar nature of these chemicals is believed to be the main reason contributing the observed excess toxicity [4,6]. Reports also indicate that the above-mentioned differences are only due to differences in their partitioning to membrane phospholipids and that both groups (nonpolar and polar narcotic compounds) act by the same mode of toxic action [7,8].

Polar narcotic pollutants consist of phenols, anilines, nitrobenzenes, pyridines, and aliphatic amines [5,9]. Marchini et al. [10] found that the effect concentration of aniline to daphnids is approximately two orders of magnitude lower than to fish larvae. Vaal et al. [11,12] also found that daphnids are highly sensitive to anilines. Urrestarazu Ramos et al. [7,13] reached similar conclusions from their study of the acute toxicity of polar narcotic chemicals toward fish, snails, and water fleas. In addition, the effects of polar narcotic pollutants (phenols, nitrobenzenes, and anilines) on the alga *Chlorella pyrenoidosa* have been reported, and quantitative structure–activity relationship (QSAR) models were established with hydrophobicity and hydrogen bonding capacity as descriptors [14].

Toxicity testing with green microalgae is often required for regulatory purposes to assess the potential environmental hazard of chemicals. The traditional batch technique was found to be relatively insensitive to organic toxicants compared with tests in which fish, water flea, and luminescent bacteria were used [15,16]. The reason for the above phenomenon is mainly due to the inadequate experimental design (open test environment and vigorous mixing) for batch tests. The Organization for Economic Cooperation and Development has thus suggested that sealed exposure system should be used for testing volatile compounds [17]. The lead author's previous works have proposed a closed-system algal toxicity test technique [18,19]. The test revealed satisfactory sensitivities to both metallic and organic toxicants compared with the conventional batch tests [19]. With the use of the above-mentioned technique, it was also demonstrated that good QSARs were found between the toxicity of chlorophenols and descriptors such as the *n*-octanol/water partition coefficient or the acid dissociation constant [20].

Pseudokirchneriella subcapitata (formerly known as *Selenastrum capricornutum*) is a common microalgal indicator that was studied most extensively by ecotoxicologists. However, toxicity data for anilines on *P. subcapitata* are hardly found in the literature. The objective of this study was to evaluate the toxicity of substituted anilines according to the aforementioned closed-system test technique. To derive more general QSARs for polar narcotics, previous data for chlorophenols [20] were also included for regression analyses.

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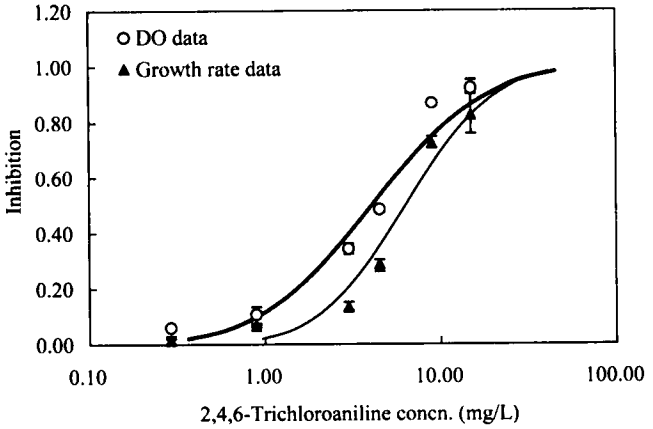


Fig. 1. Concentration–response relationships for 2,4,6-trichloroaniline (DO = dissolved oxygen production).

MATERIALS AND METHODS

The microalga *P. subcapitata* (UTEX 1648) was grown in a 4-L transparent chemostat incubator operated under steady state. Algal inoculum were withdrawn from the chemostat and transferred into 300-ml biochemical oxygen demand (BOD) test bottle, together with dilution water (with growth medium) and toxicants. 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 (Ferstek, Model S103, Medclub, Hsinchu, Taiwan) operated at 100 rpm. Test microalgae were exposed under continuous cool-white lighting (65 $\mu E\ m^{-2}\ s^{-1} \pm 10\%$) and the temperature was kept at 24 ± 1°C. U.S. Environmental Protection Agency bottle medium [21] with no ethylenediaminetetraacetic acid content was used for toxicity testing. The dilution water was stripped by nitrogen gas (containing 0.5% carbon dioxide) to reduce the dissolved oxygen level. The dissolved oxygen production (DO) level at the beginning of the test was approximately 1 to 3 mg/L. Two response endpoints were used to evaluate the toxicity of toxicants, dissolved oxygen production (ΔDO), and algal growth rate on the basis of cell density. The median effective concentration (EC50) was defined as the toxicant concentration that reduced the DO production or algal growth rate to half of that obtained by the control. The initial inoculated cell density was 15,000 cells/ml, and the test duration was 48 h. A detailed description of the test method and the concept of experimental design can be found from the lead author's previous work [19].

Thirteen substituted anilines, including 3-chloroaniline; 4-chloroaniline; 2,4-dichloroaniline; 2,5-dichloroaniline; 2,6-dichloroaniline; 3,4-dichloroaniline; 3,5-dichloroaniline; 2,4,5-trichloroaniline; 2,4,6-trichloroaniline; 3,4,5-trichloroaniline; 2-bromoaniline; 2,3-dimethyaniline; 3,4-dimethyaniline, were tested in this study. The toxicant concentrations presented in this work are in the form of nominal concentration. All chemicals used were of reagent grade, and all 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 by high-performance liquid chromatography (HPLC; 2996 Photodiode Array Detector, Waters, Milford, MA, USA). Concentration controls were conducted as described in our previous work [19] to ensure a good quality for the test data. The lowest unoccupied molecular orbital energies (E_{lumo}) were calculated with the Gaussian 98 program package (CambridgeSoft Corporation, Cambridge, MA, USA). Specific chemicals were first analyzed at the density functional theory level (B3LYP/6-31G) and the results were submitted to subsequent ab initio calculations [22].

Linear regression and surface response analysis were employed to derive QSARs. Leave-one-out cross-validation is carried out with the MINITAB program (Ver. 14.2, MINITAB, State College, PA, USA) to determine the predictive power for a QSAR model. The statistical quality was judged by the number of observations (n), the square of correlation coefficient (r^2), the Fisher criterion (F), the cross-validated correlation coefficient (Q^2).

RESULTS AND DISCUSSION

Figure 1 displays the concentration–response curves for 2,4,6-trichloroaniline on the basis of two different endpoints: inhibition on DO and algal growth rate. These curves were obtained through linear regression assuming a log-normal distribution (probit model) of the tolerances. On the basis of the probit analyses, EC50 values were found to be equal to 4.07 mg/L (ΔDO) and 6.32 mg/L (growth rate), respectively. From Figure 1, one can see that 2,4,6-trichloroaniline exerted stronger inhibitory effects on DO production than on growth rate.

Table 1 lists the EC50 values and their 95% confidence intervals for various anilines tested in this study. The EC50

Table 1. Median effective concentration values (EC50s) and 95% confidence intervals for anilines for *Pseudokirchneriella subcapitata*

	DO production[a]		Growth rate	
Chemical	EC50 (mg/L)	95% Confidence interval	EC50 (mg/L)	95% Confidence interval
3-Chloroaniline	21.35	17.69–26.13	13.52	10.43–16.16
4-Chloroaniline	3.37	2.84–3.96	3.45	2.82–4.33
2,4-Dichloroaniline	9.42	7.51–13.38	6.33	5.17–7.75
2,5-Dichloroaniline	12.12	10.15–14.59	9.87	8.85–10.87
2,6-Dichloroaniline	25.66	23.81–27.77	24.99	23.39–26.74
3,4-Dichloroaniline	3.64	3.42–3.87	3.43	3.21–3.68
3,5-Dichloroaniline	10.62	4.25–35.37	8.24	5.53–10.80
2,4,5-Trichloroaniline	2.27	1.94–2.72	2.76	2.13–3.92
2,4,6-Trichloroaniline	4.07	2.69–6.70	6.32	4.51–9.03
3,4,5-Trichloroaniline	2.05	1.69–2.59	5.44	2.94–8.34
2-Bromoaniline	26.20	23.74–29.19	11.26	5.96–20.36
2,3-Dimethylaniline	71.39	53.69–93.24	88.47	60.94–137.77
3,4-Dimethylaniline	7.12	1.05–13.43	12.18	8.26–15.9

[a] DO = dissolved oxygen.

Table 2. Comparison of toxicity of anilines to various aquatic organisms[a]

	Log(1/EC50)						
	Microalga			Protozoa		Fish	
Chemical	*Pseudokirchneriella subcapitata* DO	*Pseudokirchneriella subcapitata* Growth rate	Bacterium Microtox [25]	*Tetrahymena pyriformis* [26]	Water flea *Daphnia magna* (48 h)	Fathead minnow (96 h)	*Pocelia reticulata* [27]
3-Chloroaniline	0.78	0.97	0.96	0.09	2.56[27][b]	—	0.98[b]
4-Chloroaniline	1.58	1.57	1.40	1.36	2.61[27][b]	0.61[28]	0.69
2,4-Dichloroaniline	1.24	1.41	1.54[b]	0.56	1.78[27][b]	—	1.41[b]
2,5-Dichloroaniline	1.13	1.22	1.63[b]	0.58	—	—	1.99[b]
2,6-Dichloroaniline	0.8	0.81	1.98[b]	0.33	2.06[27][b]	—	—
3,4-Dichloroaniline	1.65	1.67	2.40[b]	1.14	2.57[29][b]	1.33[28]	1.41
3,5-Dichloroaniline	1.18	1.29	1.19	—	—	—	—
2,4,5-Trichloroaniline	1.94	1.85	2.12[b]	1.30	2.01[30]	—	2.00
2,4,6-Trichloroaniline	1.68	1.49	1.63	1.01	1.52[31][b]	1.79[b] (1.29–2.29) [32][d]	—
3,4,5-Trichloroaniline	1.98	1.56	1.77	1.51	—		—
2-Bromoaniline	0.80	1.18	—	—	1.75[27][b]		—

[a] Units for median effective concentration are mmol/L. DO = dissolved oxygen production.
[b] Organism showed higher sensitivity than *P. subcapitata*.
[c] Reference 31, http://www.pesticideinfo.org/Index.html/.
[d] Reference 32, http://md1.csa.com/partners/viewrecord.php?requester=gs&collection=ENV&recid=7212074&q=&uid=789852453&setcookie=yes.

ranged from 2.05 to 71.39 mg/L on the basis of the endpoint of DO. 3,4,5-Trichloroaniline is the most toxic compound of them all. Considering the growth rate endpoint, EC50 is within the range of 2.76 to 88.47 mg/L. The most toxic chemical is 2,4,5-trichloroaniline, instead of 3,4,5-trichloroaniline. 2,3-Dimethyaniline appears to be the least toxic compound among all anilines. Basically, the two response endpoints applied in this study were not distinctly different. However, for mono- and dichloroanilines, the growth rate endpoint appeared to be more sensitive than DO production with the exception of 4-chloroaniline. On the other hand, DO production revealed stronger inhibitory effects than algal growth rate for all trichloroanilines. The above phenomenon indicates that chloroanilines containing more chlorine atoms tend to exhibit stronger toxic effects on microalgal photosynthetic reactions. Similar observations were found from our previous study dealing with toxicity of chlorophenols [20].

Table 2 compares log(1/EC50) values of different aquatic organisms to anilines. For each test compound, an organism displaying higher sensitivity than *P. subcapitata* was marked with an asterisk. 2,3-Dimethylaniline and 3,4-dimethylaniline were not included in Table 2 because of lack of data for other organisms. It is apparent that *Daphnia magna* and the luminescent bacteria (Microtox) appeared to be very sensitive to aniline compared with *P. subcapitata*. Fish (fathead minnow and *Pocelia reticulata*) are similar in sensitivity to *P. subcapitata*. On the other hand, *Tetrahymena pyriformis* (protozoan) was found to be quite resistant to polar narcotics. Figure 2 displays the overall species sensitivity between the six aquatic organisms in Table 2. A data point located below the diagonal line indicates that the sensitivity of a specific test organism is lower than *P. subcapitata*. In Figure 2, the relative sensitivity relationship for anilines is *D. magna* > luminescent bacteria (Microtox) ≥ *P. reticulata* ≥ *P. subcapitata* ≥ fathead minnow > *T. pyriformis*. The above relationship, however, is a rough conclusion based on insufficient data. It is still necessary to produce more data to refine the relative sensitivity relationship. Furthermore, the lead author's previous work [23] showed that the relative sensitivity for aquatic organisms to nonpolar narcotics is *P. subcapitata* > luminescent bacteria > *Nitrosomonas* sp. > fathead minnow > *D. magna* > polytox > activated sludge. For *D. magna*, luminescent bacteria, and *P. subcapitata*, the relative sensitivity relationship is reversed for nonpolar chemicals compared with that for polar narcotics. It shows that polar and nonpolar chemicals have quite different chemical selectivity for aquatic organisms.

The correlation between log(1/EC50) values for various aquatic organisms was analyzed. Good linear relationships between *P. subcapitata* and *T. pyriformis* were found with r^2 equal to 0.91 (DO endpoint) and 0.75 (growth rate endpoint), respectively (Fig. 3a and b). On the other hand, the correlations

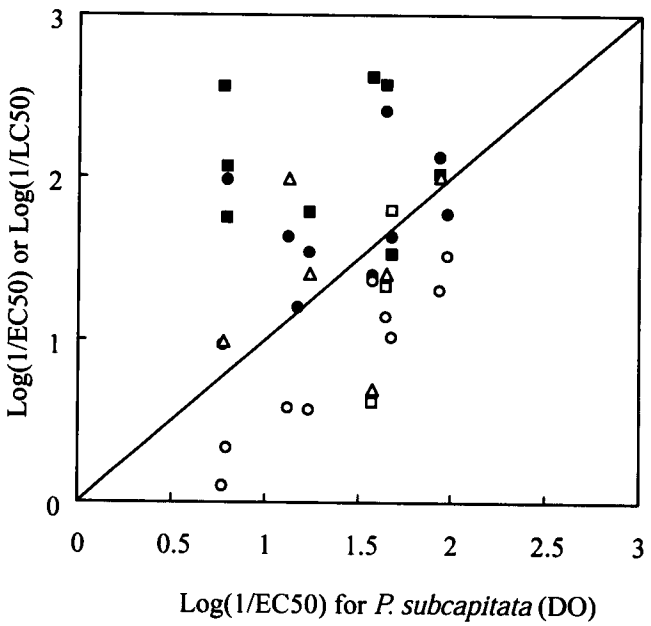
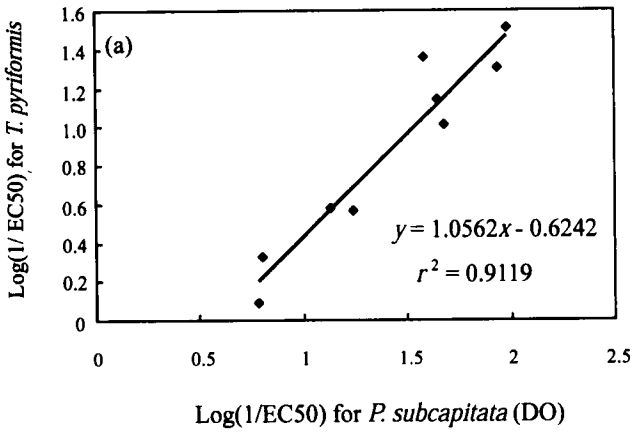


Fig. 2. Comparison of species sensitivity on the basis of dissolved oxygen production (DO). ●, Microtox; ○ *Tetrahymena*; ■, *Daphnia magna*; □, Fathead minnow; △, *Pocelia reticulate*. EC50 = median effective concentration; LC50 = median lethal concentration; *P. subcapitata* = *Pseudokirchneriella subcapitata*.



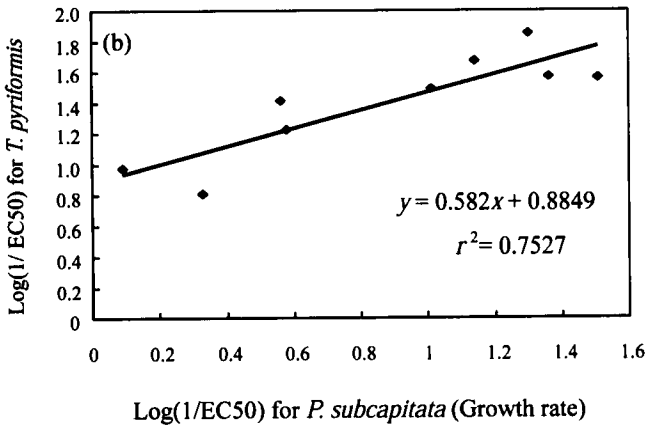
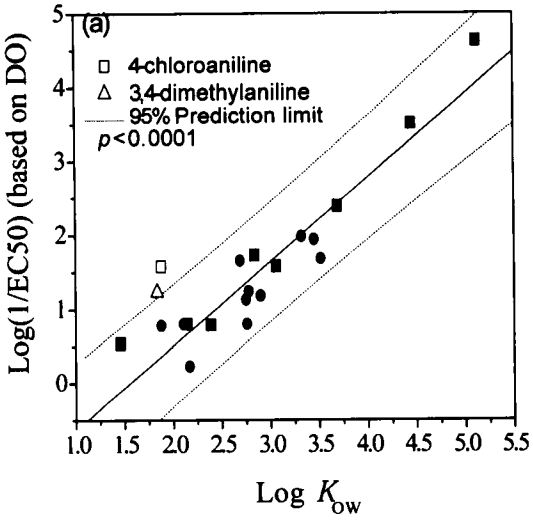


Fig. 3. Correlations between toxicity revealed by *Pseudokirchneriella subcapitata* and *Tetrahymena pyriformis* (**a**) dissolved oxygen production (DO) and (**b**) growth rate (EC50 = median effective concentration).



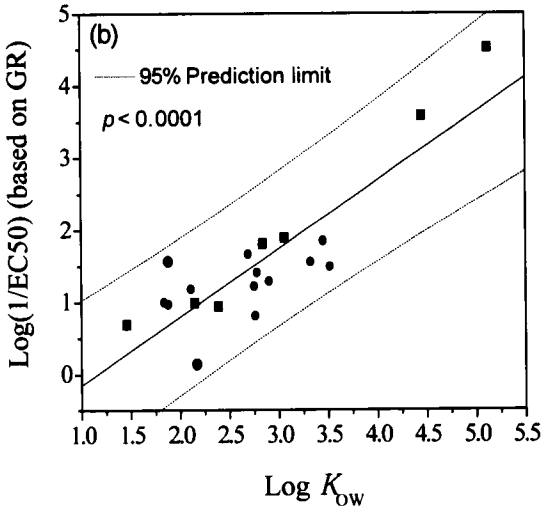


Fig. 4. Quantitative structure–activity relationship between toxicity and log K_{ow} on the basis of (**a**) dissolved oxygen production (DO) and (**b**) growth rate (GR). ●, anilines; ■, phenols; EC50 = median effective concentration, K_{ow} = 1-octanol:water partition coefficient.

between toxicity revealed by microalgae, *D. magna*, luminescent bacteria, and *P. reticulata* were poor, with r^2 values varying from 0.0004 to 0.24. Vaal et al. [11,12] pointed out that the high sensitivity for water flea to anilines could be because of a different mode of action of anilines in daphnids. The lack of correlation between the toxicity from various aquatic organisms (microalgae, *D. magna*, luminescent bacteria, and *P. reticulata*) is another important indication that anilines could have different metabolic routes in these organisms.

Table 3 lists the log K_{ow}, E_{lumo}, and log(1/EC50) values for establishing the quantitative structure–activity relationships. Twenty-one compounds, including anilines and chlorophenols [20], were used to establish QSARs for polar narcotics. Figure 4a and b depict the relationship between log K_{ow} and toxicity on the basis of the DO and growth rate endpoints. For DO production, good linear relationships (r^2 = 0.885) were found, indicating that toxicity increases with increasing hydrophobicity. Two outliers (i.e., 4-chloroaniline and 3,4-dimethylaniline) were identified from the regression because these two data points were located outside the 95% prediction limits. Similarly, toxicity data based on growth rate also depicted satisfactory correlation with log K_{ow} (r^2 = 0.75, no outlier). Results from leave-one-out analyses indicate that Equations 1 and 2 are quite significant because the cross-validated correlation coefficients (Q^2) are 0.829 and 0.715, respectively. The QSAR models are summarized below.

$$\log(1/EC50) = 1.136 \log K_{ow} - 1.771$$

$$r^2 = 0.885, \quad Q^2 = 0.829, \quad F = 130.762,$$

$$S = 0.372, \quad p < 0.0001, \quad n = 19 \quad \text{(DO)} \quad (1)$$

$$\log(1/EC50) = 0.946 \log K_{ow} - 1.100$$

$$r^2 = 0.753, \quad Q^2 = 0.715, \quad F = 54.80,$$

$$S = 0.499, \quad p < 0.0001, \quad n = 20 \quad \text{(GR)} \quad (2)$$

The E_{lumo} descriptor, which reflects hydrogen bonding donor capacity, also provided good estimations for the toxicity of polar narcotics on *P. subcapitata*. Equations 3 and 4 describe the linear relationships for the QSARs on the basis of E_{lumo}. For both endpoints, 4-chloroaniline and 3,4-dimethylaniline

Table 3. Descriptors and toxicity data for quantitative structure-activity relationships[a]

Chemical	Molecular mass	Log K_{ow}	E_{lumo} (eV)	Log(1/EC50)[b] DO	Log(1/EC50)[b] Growth rate
3-Chloroaniline	127.57	1.88	0.38	0.78	0.97
4-Chloroaniline	127.57	1.88	0.47	1.58	1.57
2,4-Dichloroaniline	162.02	2.78	0.12	1.24	1.41
2,5-Dichloroaniline	162.02	2.75	0.03	1.13	1.22
2,6-Dichloroaniline	162.02	2.76	0.07	0.8	0.81
3,4-Dichloroaniline	162.02	2.69	0.13	1.65	1.67
3,5-Dichloroaniline	162.02	2.9	0.06	1.18	1.29
2,4,5-Trichloroaniline	196.46	3.45	−0.20	1.94	1.85
2,4,6-Trichloroaniline	196.46	3.52	−0.18	1.68	1.49
3,4,5-Trichloroaniline	196.46	3.32	−0.13	1.98	1.56
2-Bromoaniline	172.03	2.11	0.32	0.80	1.18
2,3-Dimethylaniline	121.18	2.17	0.59	0.23	0.14
3,4-Dimethylaniline	121.18	1.84	0.61	1.23	1.00
Phenol[c]	98.96	1.46	0.40	0.54	0.69
2-Chlorophenol[c]	128.56	2.15	0.03	0.8	0.99
4-Chlorophenol[c]	128.56	2.39	0.10	0.79	0.94
2,3-Dichlorophenol[c]	163.01	2.84	−0.26	1.73	1.81
2,4-Dichlorophenol[c]	163.01	3.06	−0.24	1.58	1.89
2,4,6-Trichlorophenol[c]	197.45	3.69	−0.50	2.39	—
2,3,4,6-Tetrachlorophenol[c]	231.89	4.45	−0.75	3.51	3.58
Pentachlorophenol[c]	266.34	5.12	−0.98	4.63	4.52

[a] eV = Electron voltage; DO = dissolved oxygen production.
[b] Units for median effective concentration (EC50) is mmol/L.
[c] Data from Chen and Lin [20].

are outliers from the regression analyses. The negative values of the slopes for the QSAR models indicate that toxicity increases with an increase in the hydrogen bonding donor capacity and a decrease in E_{lumo}. The cross-validated correlation coefficients (Q^2) are 0.833 and 0.821, respectively, which indicates that both Equations 3 and 4 provide satisfactory predictive power to the toxicity of polar narcotic chemicals.

$$\log(1/EC50) = -2.572E_{lumo} + 1.410$$
$$r^2 = 0.886, \quad Q^2 = 0.833, \quad F = 132.391,$$
$$S = 0.370, \quad p < 0.0001, \quad n = 19 \quad \text{(DO)} \quad (3)$$

$$\log(1/EC50) = -2.491E_{lumo} + 1.486$$
$$r^2 = 0.879, \quad Q^2 = 0.821, \quad F = 115.813,$$
$$S = 0.368, \quad p < 0.0001, \quad n = 18 \quad \text{(GR)} \quad (4)$$

Regression analyses were further conducted by including both log K_{ow} and E_{lumo}. The correlation between toxicity and the above two descriptors were improved with r^2 values of 0.92 (DO) and 0.88 (growth rate), respectively. Again, 4-chloroaniline and 3,4-dimethylaniline were found to be outliers for the QSARs. Equations 5 and 6 described the relationships between observed toxicity and descriptors (i.e., log K_{ow} and E_{lumo}). Figure 5 depicts the experimental points in relation to log K_{ow} and E_{lumo} according to surface–response analysis. On the basis of Q^2 values, the predictive powers of Equations 5 and 6 are also satisfactory.

$$\log(1/EC50) = -0.24 - 1.331E_{lumo} + 0.588 \log K_{ow}$$
$$r^2 = 0.916, \quad Q^2 = 0.866, \quad F = 87.36,$$
$$S = 0.328, \quad p < 0.0001, \quad n = 19 \quad \text{(DO)} \quad (5)$$

$$\log(1/EC50) = 0.81 - 1.97E_{lumo} + 0.24 \log K_{ow}$$
$$r^2 = 0.88, \quad Q^2 = 0.817, \quad F = 57.365,$$
$$S = 0.371, \quad p < 0.0001, \quad n = 18 \quad \text{(GR)} \quad (6)$$

In the above analyses, 4-chloroaniline and 3,4-dimethylaniline were excluded from most QSAR models. Previous research [24] also indicated that 4-nitroaniline revealed excessively high toxicity and was an outlier for polar QSAR. By examining the log K_{ow} and E_{lumo} values for two groups of isomers (i.e., [3-chloroaniline, 4-chloroaniline] and [2,3-dimethylaniline, 3,4-dimethylaniline]) one might find that the differences in descriptor values are not significant. However, the differences in toxicity for these isomers are almost one

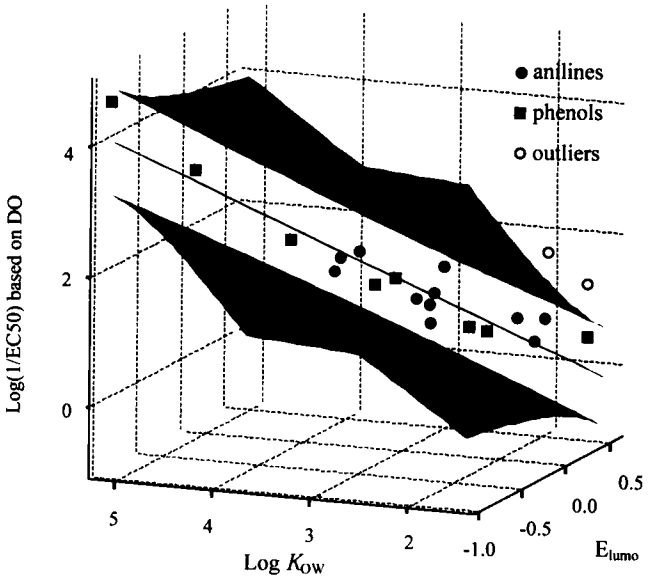


Fig. 5. Quantitative structure–activity relationship on the basis of E_{lumo} and log K_{ow} as descriptors. DO = dissolved oxygen production; EC50 = median effective concentration; K_{ow} = 1-octanol:water partition coefficient; E_{lumo} = the lowest unoccupied molecular orbital energies.

order in magnitude. We suspect that the location of the substituent(s) on the benzene ring could be a key factor causing 4-chloroaniline and 3,4-dimethylaniline to be outliers of polar QSAR.

CONCLUSIONS

Toxicity of substituted anilines on *P. subcapitata* was evaluated with a closed algal toxicity testing technique with no headspace. Both DO and growth rate endpoints revealed similar sensitivity to the effects of anilines. However, trichloroanilines showed stronger inhibitory effects on microalgal photosynthesis reactions than that on microalgal growth. For various aquatic organisms, the relative sensitivity relationship for anilines is *D. magna* > luminescent bacteria (Microtox) ≥ *P. reticulata* ≥ *P. subcapitata* ≥ fathead minnow > *T. pyriformis*. The susceptibility of *P. subcapitata* to anilines is similar to fish but is apparently less sensitive than water flea. The lack of correlation between the toxicity revealed by different aquatic organisms (microalgae, *D. magna*, luminescent bacteria, and *P. reticulata*) suggests that anilines could have different metabolic routes in these organisms. Both hydrogen bonding donor capacity (E_{lumo}) and hydrophobicity (K_{OW}) were found to provide satisfactory descriptions for the toxicity of polar narcotics (substituted anilines and chlorophenols). Quantitative structure–activity relationships on the basis of E_{lumo}, log K_{OW}, or both values were established with r^2 value varying from 0.75 to 0.92. Such relationships might provide useful information for the estimation of toxicity for other polar narcotic compounds.

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