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Toxicity and quantitative structure–activity relationships of benzoic acids to *Pseudokirchneriella subcapitata*

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ABSTRACT

The present study presents the toxicity data of benzoic acid and its derivatives on *Pseudokirchneriella subcapitata*, in terms of EC50 and NOEC values. Median effective concentrations (EC50) range from 0.55 to 270.7 mg/L (based on final yield) and 1.93 to 726.3 mg/L (based on algal growth rate). No-observed-effect concentration (NOEC) is within the range of <0.0057–179.9 mg/L. From both the NOEC and EC50 values, it was found that, 2,4,6-trihydroxybenzoic acid, 4-chlorobenzoic acid, 3-bromobenzoic acid, 4-bromobenzoic acid, 2,6-dihydroxybenzoic acid, and 2,3,4-trihydroxybenzoic acid possess much higher risks to the aquatic organisms as compared to the other benzoic acids. These data are useful for risk assessment and protection of the aquatic environments, because such information is not available in the existing toxicological databases. The toxicity of halogenated benzoic acids was found to be directly related to the compound's hydrophobicity (the logarithm of the 1-octanol/water partition coefficient, log*Kow*). On the other hand, the number of hydroxyl groups (N_{OH}) had a determinant influence to the toxicity of hydroxybenzoic acids. Quantitative structure–activity relationships were established to correlate the observed toxicity with log*Kow* and N_{OH} values. These statistical correlations are highly significant with the predictive power Q^2 ranging from 0.896 to 0.955. Furthermore, in terms of the species sensitivity, the luminescent bacteria (Microtox) and the alga *P. subcapitata* appeared to be more susceptible to benzoic acids than the water flea and ciliate.

1. Introduction

Benzoic acid is a common additive for preserving foods, fats, fruit juices, alkaloid solutions, and curing tobacco. A major source of benzoic acid and its derivatives released into the aquatic environments are from the effluents of coal refining, paper and pulp mills, and in agricultural runoff [1]. In addition, benzoic acids are the major key metabolites for biodergration of alkyl benzenes or PAH via aerobic, anaerobic, or oxidation pathways [2,3]. Moreover, several benzoic acid derivatives with hydroxyl or halogen substitutes are known to be a group of secondary plant metabolites as well as aerobic microbial degradation products of lignin, an important plant cell wall polymer [4].

The effects of benzoic acids on bacteria, ciliate, daphnids, and fish have been reported by previous researchers [5–8]. Muccini et al. [7] and Zhao et al. [5,6] demonstrated that ionization is an important factor governing the toxicity of benzoic acids and the unionized form of these weak acids is thought to be generally more toxic than the ionized analogue. For the different isomers of benzoic acids, Muccini et al. [7] concluded that benzoic acids with halogens at the meta- and para-positions were more toxic than those with ortho-substitutions. They explained the difference by the general concept that the ortho-halogenated benzoic acids having lower pKa values are more ionized, and therefore less toxic than meta- and/or para-substituted ones. Kamaya et al. [8] indicated that ortho-hydroxylated benzoic acids displayed higher toxicity than the meta- and/or para-hydroxylated ones, pointing out that the hydroxybenzoate derivatives behaved differently from the halobenzoates. On the other hand, toxicity data describing the effects of benzoic acids on algae are very rare. Kamaya et al. [9] studied the effects of some hydoxybenzoates to the freshwater green alga *Pseudokirchneriella subcapitata* and concluded that 2-hydroxybenzoic acid (2-HBA) is relatively more toxic than 4-HBA and 3-HBA. In addition, 4-HBA may stimulate the algal growth at lower concentrations ranging from 0.1 to 1.0 mmol/L.

Quantitative structure–activity relationships (QSARs) attempt to statistically relate the toxicity of a group of compounds to their physio-chemical structure. Kamaya et al. [8] found that the toxicity of benzoic acids can be described by the logarithm of n-octanol/water partition coefficient (log*Kow*) and the number of hydroxyl groups (N_{OH}) of benzoic acid replaced with hydroxyl. Zhao et al. [6] also used pKa, E_{LUMO}, and log*Kow* to construct QSARs for

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prediction of the toxicity of halobenzoic acids to *Daphnia magna*, *V. fischeri*, and fish.

The alga *P. subcapitata* (formerly known as *Selenastrum capricornutum*) is a common biological indicator studied most extensively by ecotoxicologists. However, the effects of benzoic acids on phytoplankton have rarely been studied. In addition, the low toxic effects of benzoic acids on algae are seldom found in literature. The objective of the present study was to estimate the toxicity (in terms of EC50 and NOEC) of benzoic acids on *P. subcapitata* based on two response endpoints, i.e., final yield and algal growth rate. Furthermore, quantitative structure–activity relationships were derived for the prediction of toxicity of various benzoic acids.

2. Materials and methods

The test technique applied in the present study is a closed-system algal toxicity test, which was originally designed for testing both volatile organic compounds and metallic toxicants [10]. However, the author's previous work also indicated that the test technique works well for both high and low volatile organic compounds [11]. Detailed information regarding the test method and the concept of experimental design can be found in the author's previous work [10]. A brief description for toxicity testing and data analyses is given below.

Algal inoculum was withdrawn from the chemostat operated under a steady state, and transferred into 300 mL BOD bottles, together with dilution water (with growth medium) and toxicants. The BOD bottles were filled completely, leaving no headspace. A 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 at 24 ± 1 °C and 65 μEm-2s-1 (±10%), respectively. US EPA [12] bottle medium, with no EDTA content, was used for toxicity testing. Two response endpoints were used to evaluate the toxicity of the toxicants; the final yield and algal growth rate based on cell density counts. The median effective concentration (EC50) was defined as the toxicant concentration, which reduced the response to half of that obtained by the control. The initial inoculated cell density was 15,000 cells/mL and the duration of the test was 48 h. The population density of the algae was determined using an electronic particle counter (Culter Electronics, Luton, UK). The initial pH for toxicity testing was set at 6.5. All chemicals used were of reagent grade and were tested at least twice, i.e., range finding test and definitive test. For the definitive test, one control and 6 (or 7) different treatments were performed in triplicate. Stock solution was freshly prepared, and its concentration was analyzed using a HPLC analyzer before commencing the experiment.

Probit analysis was applied to determine the concentration–response relationship and the median effective concentration (EC50). One-tail Dunnett's procedure was applied for the estimation of NOEC and LOEC values at 5% level of significance. The studentized range (*SI*) can be calculated according to Eq. (1) as shown below.

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

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*). Obviously, *T* serves as a cut-off point for the Dunnett's test. We may hence calculate the cut-off value (in term of % reduction) by transforming Eq. (1) into the following expression:

$$\%\text{Reduction} = \frac{Xc - Xi}{Xc} \times 100 = \frac{T}{Xc} \times Sw\sqrt{\frac{1}{nc} + \frac{1}{ni}} \times 100 \quad (2)$$

Correlation analyses were performed using MINITAB (Ver 14.2; MINITAB, State College, PA, USA) to establish QSARs. Leave-one-out cross-validation was carried out to test the significance of each QSAR. The statistical quality was judged by the square of the correlation coefficient (r^2), the Fisher criterion (*F*), the root mean square error (*S*), and the cross-validated correlation coefficient (Q^2).

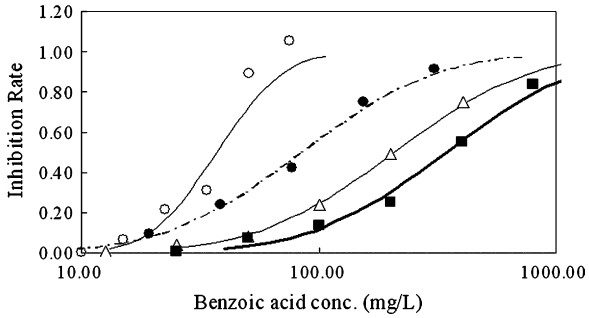

Fig. 1. Effects of benzoic acid at four different initial pH conditions on algal growth rate (○ non-neutralized; ● pH = 6.5; Δ pH = 7; ■ pH = 7.5).

3. Results and discussion

Fig. 1 displays the concentration–response relationships for benzoic acid tested under four different initial pH conditions (6.5, 7.0, 7.5, and non-neutralized), based on the endpoint of the algal growth rate. EC50 values for the aforementioned test conditions were 83.3, 207.5, 342.8, and 37.1 mg/L, respectively. It is obvious that the most severe condition occurred when the test was conducted without pH adjustment. However, for the non-neutralized test, the initial pH level was significantly depressed to below 6.0 when the benzoic acid concentration exceeded 22.5 mg/L. The responses observed under such a condition are partially due to acidity, as the algal growth will be considerably inhibited when pH is below 6.0. Considering that pH level in most aquatic environments is between 6.0 and 9.0, the initial condition of pH equal to 6.5 was chosen for testing the remaining benzoic acids. This means that the assessment is based on a conservative consideration, as in some instances, the pH value in the natural aquatic environment may be higher than 6.5. The reason for increasing toxic effect at decreasing pH is the degree of ionization for a weak acid [7,13]; Although both the ionized and unionized forms of benzoic acids contribute to the toxicity, the unionized fraction contributes significantly to the toxicity

Table 1
Algal responses to benzoic acid.

Conc mg/L	Final cells cells/ml	Growth rate μ	Inihibit rate Final yield	Inihibit rate Growth rate
Control	2.811 E+05	1.470*	0	0
307.84	1.927 E+04*	0.130*	0.980	0.910
153.92	3.112 E+04*	0.370*	0.940	0.750
76.96	8.197 E+04*	0.840*	0.750	0.420
38.48	1.401 E+05*	1.120*	0.530	0.240
19.24	2.159 E+05*	1.330*	0.250	0.090
9.62	2.458 E+05*	1.400	0.130	0.050
4.81	2.779 E+05	1.460	0.010	0.004
EC50			36.39	83.29

* Significantly different from the controls at $p = 0.05$ using the one-tail Dunnett's test.

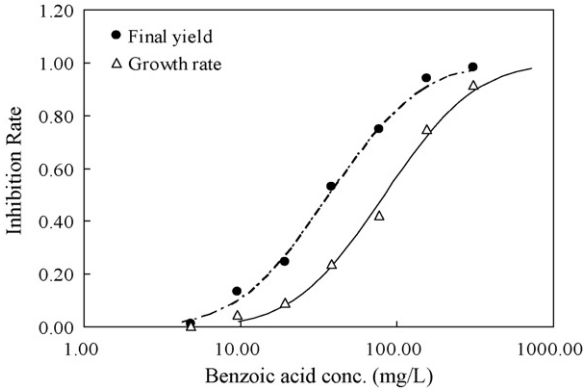

Fig. 2. Dose–response curves of *P. subcapitata* to benzoic acid.

because the uptake of the unionized form is generally faster and easier by biological membranes.

Table 1 displays a typical set of algal data with respect to the toxicity of benzoic acid. For the test control, the cell density was increased from an initial value of 15,000 cells/mL to a final yield of 281,100 cells/mL. Generally speaking, at a specific benzoic acid concentration, the inhibition rate based on final yield is greater than that based on the growth rate. Concentration response curves for the endpoints of final yield and growth rate are shown in Fig. 2. These curves were obtained through linear regression assuming a log-normal distribution (probit model) of tolerances. EC50 values were calculated using the probit analysis and were equal to 36.39 mg/L (final yield) and 83.29 mg/L (growth rate), respectively. The no-observed-effect concentration (NOEC) was determined using the one-tail Dunnett's test at $p = 0.05$ level of significance. Individual treatments that were statistically different from the control were marked with an asterisk. Therefore, NOEC is equal to 4.81 mg/L (final yield) and 9.62 mg/L (growth rate), respectively.

Table 2 lists the NOEC, EC50, and the cut-off values for twenty benzoic acids tested in the present study. NOECs are within the range of <0.0571–179 mg/L. Among all the benzoic acids tested, 2,4,6-trihydroxybenzoic acid is the most toxic compound, with a NOEC as low as 0.057 mg/L. There are several other benzoic acids (i.e., 4-chlorobenzoic acid, 3-bromobenzoic acid, 4-bromobenzoic acid, 2,6-dihydroxybenzoic acid, and 2,3,4-trihydroxybenzoic acid), which should receive more attention for their possible adverse impact on aquatic environments, considering their relatively small NOECs. For most compounds in Table 2, the cut-off values are less than 10% with the mean values equal to 7.89% (final yield) and 6.01% (growth rate), respectively. For a specific treatment, the required degree of inhibition, for producing a significant difference as compared to the controls, is approximately 8% (final yield) or 6% (growth rate), respectively. Referring to Eq. (2), the small cut-off values observed from our algal toxicity test indicate that the within-group variances (or the variations among replicates) for the Dunnett's test are also very small. The application of NOEC has long been criticized as not being able to provide sufficient protection to aquatic organisms than the point estimator EC10 [14–16]. However, on an average basis, the NOECs derived from our algal test technique may provide better protection than EC10 values.

Algal biomass and growth rate are two traditional response endpoints applied most commonly in studies of phytoplankton. The median effective concentrations based on biomass were generally lower and could differ by a factor of two compared with EC50s based on growth rate [17]. However, growth rate endpoint is considered to be more stable, comparable, and ecologically relevant [18]. In the present study, six compounds including benzoic acid, 4-bromobenzoic acid, 2,4-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, 2,3,4-trihydroxybenzoic acid, 2,4,6-trihydroxybenzoic acid, and 3,4,5-trihydroxybenzoic acid, have resulted in NOEC values based on final yield (a biomass endpoint) to be smaller than that on the growth rate endpoint. It was therefore decided that toxicity data of both endpoints should be presented in order to reveal the actual no-observed-effect levels of various benzoic acids.

The EC50 ranged from 0.55 to 270.7 mg/L with respect to the endpoint of final yield. As expected, EC50 values for the growth rate endpoint are considerably larger than those based on the final yield. For both endpoints, 2,4,6-trihydroxybenzoic acid is the most toxic compound of all. Furthermore, based on the final

Table 2
Median effective concentration values (EC50s) and NOECs for benzoic acids.

Toxicant	CAS NO	N_{OH}	log*Kow*	Testing range (mg/L)	NOEC	Final yield Cut-off value (%)	EC50	NOEC	Growth rate Cut-off value (%)	EC50
Benzoic acid	65-85-0	0	1.87	4.8–308	4.81*	7.64	36.39	9.62	6.63	83.29
2-Chlorobenzoic acid	118-91-2	0	2.05	1.5–227	1.58	2.55	21.12	1.58	1.96	81.33
3-Chlorobenzoic acid	535-80-8	0	2.68	0.4–36.3	1.45	15.39	7.03	1.45	10.33	16.15
4-Chlorobenzoic acid	74-11-3	0	2.65	0.5–123	0.57	5.71	6.42	0.57	3.28	17.29
3-Bromobenzoic acid	585-76-2	0	2.87	0.2–32	0.22	5.24	3.87	0.22	4.16	14.39
4-Bromobenzoic acid	586-76-5	0	2.86	0.6–20	0.63*	11.79	4.57	1.28	7.31	13.48
4-Fluorobenzoic acid	456-22-4	0	2.13	1.19–215	2.82	7.66	16.91	2.82	7.10	49.30
4-Aminobenzoic acid	150-13-0	0	0.98	8.4–1213	8.42	6.56	199.9	8.42	5.31	724.4
2-Hydroxybenzoic acid	69-72-7	1	2.26	1.79–115	1.79	4.62	12.32	1.79	5.57	25.46
3-Hydroxybenzoic acid	99-06-9	1	1.50	20–1275	19.91	6.19	143.1	19.91	5.44	342.0
4-Hydroxybenzoic acid	99-96-7	1	1.58	180–968	179.9	8.06	270.7	179.9	9.15	355.0
2,3-Dihydroxybenzoic acid	303-38-8	2	1.20	2.07–300	2.07	4.49	30.10	2.07	3.03	195.80
2,4-Dihydroxybenzoic acid	89-86-1	2	1.63	3.15–184	3.15*	7.31	36.21	5.74	5.51	80.14
2,5-Dihydroxybenzoic acid	490-79-9	2	1.74	1.24–75	2.49	6.29	16.0	2.49	3.14	47.44
2,6-Dihydroxybenzoic acid	303-07-1	2	2.20	0.25–54	0.25	5.46	7.25	0.25	3.60	32.57
3,4-Dihydroxybenzoic acid	99-50-3	2	1.15	34.9–1116	34.87	12.00	267.1	34.87	5.62	726.3
3,5-Dihydroxybenzoic acid	99-10-5	2	0.86	35.5–2218	35.49*	5.90	245.7	70.98	5.34	658.7
2,3,4-Trihydroxybenzoic acid	610-02-6	3	1.05	0.78–25.6	0.78	10.84	3.96	0.78	7.22	8.04
2,4,6-Trihydroxybenzoic acid	83-30-7	3	1.62	0.06–8.20	<0.057*	4.66	0.546	0.114	6.93	1.93
3,4,5-Trihydroxybenzoic acid	149-91-7	3	0.70	0.40–38.4	1.60*	19.40	6.99	3.20	13.66	15.90
Mean						7.888			6.014	

Unit for NOEC and EC50: mg/L.
* NOEC (final yield) < NOEC (growth rate).

yield endpoint (EC50 = 0.546 mg/L), 2,4,6-trihydroxybenzoic acid can be classified as a "R50" compound (very toxic to aquatic organisms, EC50/LC50 <1 mg/L), following the European Union's practice [19]. In addition, seven benzoic acids including 3-chlorobenzoic acid, 4-chlorobenzoic acid, 3-bromobenzoic acid, 4-bromobenzoic acid, 2,6-dihydroxybenzoic acid, 2,3,4-trihydroxybenzoic acid, and 3,4,5-trihydroxybenzoic acid are considered as toxic to aquatic organisms (R51 class, EC50/LC50 between 1 and 10 mg/L), based on the European Union's criteria.

For halobenzoic acids, the brominated homologs were consistently more toxic than the chloro-, fluorine-containing ones (e.g., 4-bromobenzoic acid > 4-chlorobenzoic acid > 4-fluorobenzoic acid; 3-bromobenzoic acid > 3-chlorobenzoic acid). For the different isomers of benzoic acids, halogens at the meta- and para-positions were more toxic than benzoic acids with halogens at the ortho-position (4-chlorobenzoic acid ≒ 3-chlorobenzoic acid > 2-chlorobenzoic acid). Muccini et al. [7] observed the same phenomenon on ciliate *Tetrahymena pyriformis*, and suggested that the ortho-halogenated benzoic acids, which have lower pKa values, are more ionized, and therefore less toxic than meta- and/or para-substituted acids.

For hydroxybenzoic acids, their toxicity was found to correlate with log*Kow* and the number of hydroxyl on benzene. As shown in Fig. 3, the toxicity of trihydroxybenzoic acids (triHBA), dihydroxybenzoic acids (diHBA), and monohydroxybenzoic acids (HBA) were all increased with increasing log*Kow* values. It is also obvious that the number of hydroxyl groups has a determinant influence on toxicity (i.e., triHBAs > diHBAs > monoHBAs, in toxicity order). Furthermore, the toxic effects of monohydroxybenzoic acids were in descending order as 2- >3 >4-HBAs, indicating that toxicity was enhanced by ortho-hydroxyl substitution. Similar observations can be made on di- and triHBAs; 2,6- > 2,5- > 2,4- ≒ 2,3- > 3,4- ≒ 3,5-diHBAs and 2,4,6- > 2,3,4- > 3,4,5-triHBAs. Kamaya et al. [8], based on *Daphnia magna test*, also concluded that hydroxybenzoate derivatives behaved differently from the halobenzoates and polyhydroxybenzoates may have higher affinity to daphnids.

Various descriptors including log*Kow*, the acid dissociation constant (pKa), E_{LUMO}, and E_{HOMO}, were used to model the toxicity of benzoic acids. The results of QSAR analyses show that none of these descriptors alone is capable of describing the toxicity of all the benzoic acids investigated by the present study. However, for halogenated benzoic acids, satisfactory QSARs can be established based on the 1-Octanol-water partition coefficient (*Kow*), as shown by Eqs. (3) and (4). These statistical correlations are highly significant and have very good predictive power, as evidenced by the r^2 and Q^2 values.

$$\mathrm{Log}\left(\frac{1}{\mathrm{EC50}}\right)_{\text{Final yield}} = 0.973\ \log Kow - 1.169 \quad (3)$$

$$n = 8,\ r^2 = 0.986,\ S = 0.080,\ F = 428.54,\ Q^2 = 0.943$$

$$\mathrm{Log}\left(\frac{1}{\mathrm{EC50}}\right)_{\mathrm{GR}} = 1.005\ \log Kow - 1.717 \quad (4)$$

$$n = 8,\ r^2 = 0.998,\ S = 0.030,\ F = 3249.57,\ Q^2 = 0.997$$

Furthermore, the combination of log*Kow* and N_{OH} (the number of hydroxyl substituent) provides excellent descriptions to all the benzoic acids with no outlier. Eqs. (5) and (6) indicate that, for both the final yield and growth rate endpoints, toxicity increases with increasing hydrophobicity and N_{OH} with r^2 = 0.921-0.965. The cross-validation coefficient Q^2 is equal to 0.896 and 0.955, suggesting the correlation relationships are highly significant.

$$\mathrm{Log}\left(\frac{1}{\mathrm{EC50}}\right)_{\text{Final yield}} = 1.124 \log Kow + 0.0266(\mathrm{N_{OH}})^4 - 1.569 \quad (5)$$

$$n = 20,\ r^2 = 0.921,\ S = 0.230,\ F = 98.51,\ Q^2 = 0.896$$

$$\mathrm{Log}\left(\frac{1}{\mathrm{EC50}}\right)_{\mathrm{GR}} = 1.111\ \log Kow + 0.0267(\mathrm{N_{OH}})^4 - 1.997 \quad (6)$$

$$n = 20,\ r^2 = 0.965,\ S = 0.147,\ F = 238.22,\ Q^2 = 0.955$$

Table 3 presents the toxicity data of various benzoic acids, as derived from different tests using water flea (*D. magna*), the luminescent bacteria (Microtox), ciliate (*Tetrahymena pyriformis*), and alga (the present study) as the test organisms. These tests were conducted with different initial pH levels, therefore, the initial pH for each set of data is specified. All data were expressed as (1/EC50) and EC50s are in the form of mmol/L. A graphical representation of these data is shown in Fig. 4. At first glance of Fig. 4, it appears that the relative sensitivity relationship for various aquatic organisms to benzoic acids is Microtox > *P. subcapitata* > *D. magna* > *T. pyriformis*. However, one should also bear in mind that the influence of pH should be taken into consideration. For halogenated benzoic acids (including the benzoic acid), it is apparent that *P. subcapitata* (with an initial pH = 6.5) is more sensitive than *D. magna*, even though the water flea was tested under a more acidic level (pH 6.0). On the other hand, most halogenated benzoic acids should be more toxic under the Microtox test environment (pH = 5.7) than under the algal testing condition. However, the observed inhibitory effects are not significantly different from algal responses. Similarly, for 2-, 3-,

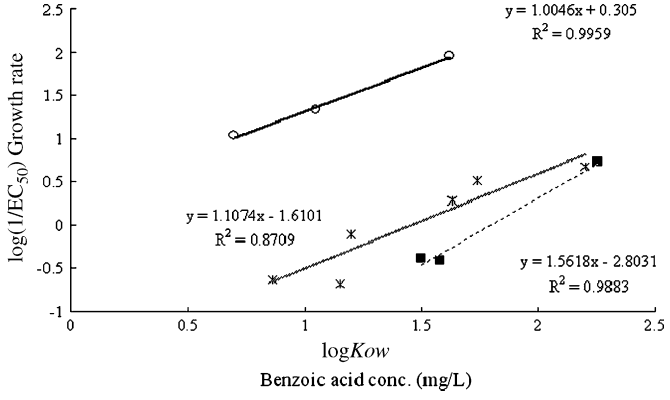

Fig. 3. Correlations between EC50 values and log*Kow* for hydroxybenzoic acids (■ monohydroxybenzoic acid; * dihydroxybenzoic acid; ○ trihydroxybenzoic acid).

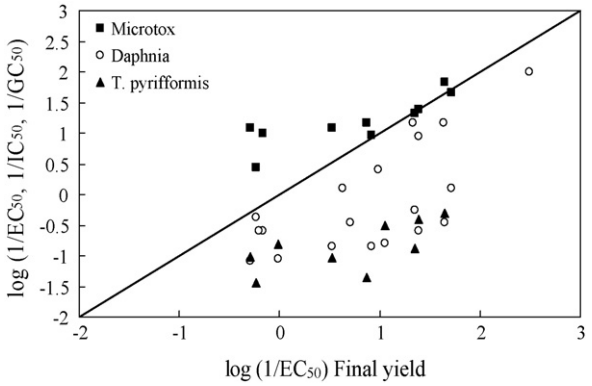

Fig. 4. Comparison of species sensitivity among different aquatic organisms.

Table 3
Comparison of algal toxicity test results with other species of organisms.

Chemicals	Alga (final yield) 48 h Log(1/EC50)	*P. subcapitata* 72 h Log(1/EC50)	*D. magna* 48 h Log(1/EC50)	Microtox 15 min Log(1/EC50)	*T. pyriformis* 40 h Log(1/IGC50)
Benzoic acid	0.5257[6.5]	−0.107[a] [7.45]	−0.260[b][6.0]	1.089[b] [5.7]	−1.030[c] [7.5]
2-Chlorobenzoic acid	0.8700[6.5]		–	1.169[b] [5.7]	−1.360[c] [7.5]
3-Chlorobenzoic acid	1.348[6.5]		0.108[b][6.0]	1.320[b][5.7]	−0.880[c] [7.5]
4-Chlorobenzoic acid	1.387[6.5]		0.018[b][6.0]	1.389[b][5.7]	−0.410[c] [7.5]
3-Bromobenzoic acid	1.716[6.5]		0.290[b][6.0]	1.659[b][5.7]	
4-Bromobenzoic acid	1.644[6.5]		0.410[b][6.0]	1.830[b][5.7]	−0.300[c] [7.5]
4-Fluorobenzoic acid	0.9184[6.5]		−0.390[b][6.0]	0.9580[b][5.7]	–
4-Aminobenzoic acid	−0.1638[6.5]		0.010[b][6.0]	1.000[b][5.7]	–
2-Hydroxybenzoic acid	1.050[6.5]	0.764[a] [7.45]	−0.799[d][7.5]	–	−0.510[c] [7.5]
3-Hydroxybenzoic acid	−0.0154[6.5]		−1.050[d][7.5]	–	−0.820[c] [7.5]
4-Hydroxybenzoic acid	−0.2922[6.5]	−1.00[a] [7.45]	−1.087[d][7.5]	1.036[e][–]	−1.020[c] [7.5]
2,3-Dihydroxybenzoic acid	0.7093[6.5]		−0.4620[d][7.5]	–	–
2,4-Dihydroxybenzoic acid	0.6291[6.5]		0.1080[d][7.5]	–	–
2,5-Dihydroxybenzoic acid	0.9838[6.5]		0.413[d][7.5]	–	–
2,6-Dihydroxybenzoic acid	1.328[6.5]		1.162[d][7.5]	–	–
3,4-Dihydroxybenzoic acid	−0.2388[6.5]		−0.3800[d][7.5]	0.4365[e][–]	−1.442[f] [–]
3,5-Dihydroxybenzoic acid	−0.2026[6.5]		−0.601[d][7.5]	–	–
2,3,4-Trihydroxybenzoic acid	1.633[6.5]		1.162[d][7.5]	–	–
2,4,6-Trihydroxybenzoic acid	2.494[6.5]		2.000[d][7.5]	–	–
3,4,5-Trihydroxybenzoic acid	1.386[6.5]		0.949[d][7.5]	–	–

[]: Initial pH value. Unit: mmol/L.
[a] Data from Kamaya et al. [9].
[b] Data from Zhao et al. [5,6].
[c] Data from Muccini et al. [7].
[d] Data from Kamaya et al. [8].
[e] Data from Fiorentino et al. [20] (pH: non-neutralized).
[f] Data from US EPA, ECOTOX.

and 4-hydroxybenzoic acids, both *D. magna* and *T. pyriformis* were tested at pH 7.5. Again, no significant difference can be observed from the responses of the two test organisms. From the above comparisons, one may conclude that the luminescent bacteria and *P. subcapitata* are apparently more sensitive to benzoic acids than water flea and ciliate. Furthermore, literature data for *P. subcapitata* derived by Kamaya et al. [9] are also listed in Table 3 and compared with results from the present study. The log(1/EC50) values from the present study are consistently greater than previous data [9], indicating that more severe toxic effects were observed from our tests. Such a phenomenon is within our expectation because our tests were conducted at pH 6.5, while Kamaya's tests were conducted at pH 7.5.

4. Conclusions

The present study presents the toxicity data of benzoic acid and its derivatives on *P. subcapitata*, in terms of EC50 and NOEC values. Quantitative structure–activity relationships were established to correlate the observed toxicity with log*Kow* and N_{OH} values. These statistical correlations are highly significant with the predictive power Q^2 ranging from 0.896 to 0.955. In addition, the luminescent bacteria (Microtox) and the alga *P. subcapitata* appeared to be more susceptible to benzoic acids than the water flea and ciliate. The above toxicity data and QSARs are useful for risk assessment and protection of the aquatic environments, because such information is not available in the existing toxicological databases.

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References

[1] M. Sittig (Ed.), Handbook of Toxic and Hazardous Chemicals Carcinogens, 2nd ed., Noyes Publications, Park Ridge, NJ, USA, 1985.
[2] M.R. Smith, Biodegradation of aromatic hydrocarbon by bacteria, Biodegradation 1 (1990) 191–206.
[3] H. Habe, T. Omori, Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria, Biosci. Biotechnol. Biochem. 67 (2003) 225–243.
[4] C.L. Chen, H.M. Chang, Chemistry of lignin biodegradation, in: T. Higuchi (Ed.), Biosynthesis and Biodegradation of Wood Components, Academic Press, Florida, 1985, pp. 535–556.
[5] Y.H. Zhao, X. Yuan, L.H. Yang, L.S. Wang, Quantitative structure–activity relationships of organic acids and bases, Bul. Environ. Contam. Toxicol. 57 (1996) 242–249.
[6] Y.H. Zhao, G.D. Ji, M.T.D. Cronin, J.C. Dearden, QSAR study of the toxicity of benzoic acids to Vibriofischeri, *Daphnia magna* and carp, Sci. Total Environ. 216 (1998) 205–215.
[7] M. Muccini, A.C. Layton, G.S. Sayler, T.W. Schultz, Aquatic toxicities of halogenated benzoic acids to *Tetrahymena pyriformis*, Bull. Environ. Contam. Toxicol. 62 (1999) 616–622.
[8] Y. Kamaya, Y. Fukaya, K. Suzuki, Acute toxicity of benzoic acids to the crustacean *Daphnia magna*, Chemosphere 59 (2005) 255–261.
[9] Y. Kamaya, S. Tsuboi, T. Takada, K. Suzuki, Growth stimulation and inhibition effects of 4-hydroxybenzoic acid and some related compounds on the freshwater green alga *Pseudokirchneriella subcapitata*, Arch. Environ. Contam. Toxicol. 51 (2006) 537–541.
[10] J.H. Lin, W.C. Kao, K.P. Tsai, C.Y. Chen, A novel algal toxicity testing technique for assessing the toxicity of both metallic and organic toxicants, Water Res. 39 (2005) 1869–1877.
[11] K.P. Tsai, C.Y. Chen, An algal toxicity database of organic toxicants derived by a closed system technique, Environ. Toxicol. Chem. 26 (2007) 1931–1939.
[12] US Environmental Protection Agency (US EPA), Ecological Effect Test Guidelines, OPPTS 850.5400, Algal Toxicity, Tiers I and II, 1996.
[13] D.J.W. Blum, R.E. Speece, Quantitative structure-activity relationships for chemical toxicity to environmental bacteria, Ecotoxicol. Environ. Saf. 22 (1991) 198–224.
[14] P.M. Chapman, R.S. Caldwell, P.F. Chapman, A warning: NOECs are inappropiate for regulatory use, Environ. Toxicol. Chem. 15 (1996) 77–79.
[15] P.M. Chapman, OECD workshop recommends phasing out NOECs for aquatic toxicity test, Mar. Pollut. Bull. 34 (1997) 145.
[16] D.R.J. Moore, P.Y. Caux, Estimating low toxic effects, Environ. Toxicol. Chem. 16 (1997) 794–801.
[17] N. Nyholm, Expression of results from growth inhibition toxicity tests with algae, Arch. Environ. Contam. Toxicol. 19 (1990) 518–522.

[18] A. Weyers, G. Vollmer, Algal growth inhibition: effect of the choice of growth rate or biomass as end point on the classification and labeling of new substances notified in the EU, Chemosphere 41 (2000) 1007–1010.
[19] European Commission, Council Directive 92/32/EEC of 5 June 1992 amending for the seventh time Council Directive 67/548/EEC, *Official Journal of European Communities* L152, Brussels, Belgium, 1992.
[20] A. Fiorentino, A. Gentili, M. Ishidori, P. Monaco, A. Nardelli, A. Parrella, F. Temussi, et al., Environmental effects caused by olive mill wastewaters: toxicity comparison of low-molecular-weight phenol components, J. Agric. Food Chem. 51 (2003) 1005–1009.