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# Toxicity and quantitative structure–activity relationships of nitriles based on *Pseudokirchneriella subcapitata*

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**Abstract**

This study presents the toxicity data of various nitriles to *Pseudokirchneriella subcapitata* using a closed algal toxicity testing technique with no headspace. Two different response endpoints, i.e., dissolved oxygen (DO) production and algal growth rate, were used to evaluate the toxicity of nitriles. In general, the DO endpoint revealed higher inhibitory effects than that from algal growth rate. Furthermore, halogen-substituted nitriles were found to be extremely toxic to *P. subcapitata*. With increasing numbers of the halogen atoms, stronger toxicity was observed. The bromine substitutent also seems to be more toxic than chlorine substitutent. Quantitative structure–activity relationships (QSARs) were established based on the chemicals' Elumo values and hydrophobicity ($\log K_{ow}$). Such relationships may thus be useful in predicting the toxicity of other compounds of the same mode of toxic action. Furthermore, for various aquatic organisms, the relative sensitivity relationship is: *Pimephales promelas* $\geqslant$ *P. subcapitata* > *Tetrahymena Pyriformis* > *Daphnia magna* > luminescent bacteria (Microtox). The alga, *P. subcapitata*, was found to be quite sensitive to nitriles compared to other organisms.


*Keywords:* Toxicity; Nitriles; *Pseudokirchneriella subcapitata*; QSAR; Algae

## 1. Introduction

The development of the narcosis quantitative structure–activity relationships (QSARs) has led to a general classification of organic chemicals. Toxicity of organic compounds can be compiled into two general categories: reversible non-specific toxicity (i.e. narcosis) and irreversible specific toxicity (Schultz, 1997). Approximately 70% of all industrial organic chemicals are thought to exhibit a narcosis mode of toxic action (Bradbury and Lipnick, 1990). Narcotic effects are estimated by the ability of a compound to interact with cellular membranes as quantitated by the 1-octanol:water partition coefficient ($K_{ow}$). Narcotic effects are thought to result in non-covalent interactions including the disruption of van der Waals interactions between lipid and/or protein components within the membrane (Franks and Lieb, 1990). Specific toxicity is related to the ability of a toxicant to elicit a covalent interaction with a biological system. Toxicants exhibit specific toxicity are also called the reactive toxicants. They have been further divided into four different categories, i.e., electrophilic, proelectrophilic, cyanogenic, and multiple, according to their mechanisms of toxicity (Lipnick, 1991).

According to Lipnick's classification, nitriles are belonged to the category of reactive toxicants (cyanogenic). The toxicity of nitriles is primarily due to the release of cyanide ions through hydrolysis (Lipnick, 1991). The impact of nitriles to aquatic organisms has rarely been investigated by ecotoxicologists: Bringmann and Kuhn (1980) compared the toxicity of various chemicals to *Pseudomonas putida* (bacteria), *Entosiphon sulcatum* (protozoa), and *Scendesmus quadicauda* (alga). They concluded that phytoplankton was not sensitive to nitriles. Protic and Sabljic (1989), based on effects on fish, found that nitriles with larger molecular size usually resulted in higher toxicity. Akers et al. (1999), by testing on *Terahymena pyriformis*, showed that hydrophobicity ($\log K_{ow}$) did not correlate well with the toxicity of nitriles. Instead, the

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energy of the lowest unoccupied molecular orbital (Elumo) provided better correlation ($r^2 = 0.59$) with toxicity. Elumo represents the quantum activation state of molecular frontier orbitals, and therefore serves as a generalized descriptor of soft electrophilicity. Furthermore, a combination of $\log K_{ow}$ and Elumo resulted in even better description of the toxicity ($r^2 = 0.915$). On the other hand Russom et al. (1997), based on observations from fathead minnow, found that the toxicity of nitriles and halogen-substituted nitriles to be narcotic.

The traditional batch-test approach has been adopted by most standard algal test protocols to assess the relative toxicity of various toxic chemicals and waste discharges (ASTM, 1994; IOS, 1987; US EPA, 1996; OECD, 1984). Previous studies indicated that algal toxicity tests were relatively insensitive to organic toxicants compared to tests using fish, water flea and luminescent bacteria (Toussaint et al., 1995; Pederson and Peterson, 1996). The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETC, 1996) also concluded that current algal toxicity test protocols are unsuitable for assessing the effects of volatile compounds. In addition, the Organization for Economic Co-Operation and Development has suggested that sealed exposure system should be used for testing volatile compounds (OECD, 2000).

Several studies tried to solve the above problems using closed test system and providing large headspace for additional carbon supply (Herman et al., 1990; Brack and Rottlern, 1994; Galassi and Vighi, 1981; Halling-Sørensen et al., 1996). Large headspace may cause a significant portion of the volatile compound to partition from the aqueous phase into the headspace until equilibrium is reached. The exposure concentrations were thus altered significantly (Mayer et al., 2000). To determine actual exposure concentration requires taking samples from sealed vessels and is not realistic because it may shift the gas–liquid equilibrium for the volatile compound and is too tedious for routine analyses. Sealed test vessels with no headspace were also used to test volatile substances (Mayer et al., 2000). For such an approach, an enriched bicarbonate buffer was added as a carbon source. However, enriched carbonate buffer may also result in increased ionic strength and lower test sensitivity (Brack and Rottlern, 1994; Lin et al., 2005). The author's previous works have proposed a closed-system algal toxicity test technique with no headspace and with a low bicarbonate-buffer content (Chen et al., 2005; Lin et al., 2005). The test revealed much better sensitivities to organic toxicants as compared to the conventional batch tests.

*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) is a common biological indicator that was studied most extensively by ecotoxicologists. However, toxicity data for nitriles on *P. subcapitata* are hardly found from literatures. The objective of this study was to evaluate the toxicity of nitriles and their derivatives using the air-tight algal toxicity test technique. Two response endpoints, i.e., algal growth rate and DO production, were used to evaluate the toxic effects of various toxicants.

## 2. Materials and methods

The alga *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 BOD bottles, 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 operated at 100 rpm. Temperature and light intensity were kept at $24 \pm 1$ °C and 65 μEm-2s-1 ($\pm 10\%$), respectively. US EPA (1996) bottle medium with no EDTA content was used for toxicity testing. The dilution water was stripped by nitrogen gas to reduce the DO level. In addition, the $N_2$ gas contained 0.5% carbon dioxide as an extra carbon source. The DO level at the beginning of the test was approximately 1–3 mg/L. Two response endpoints were used to evaluate the toxicity of toxicants: DO production (ΔDO) and algal growth rate based on cell density. The median effective concentration (EC50) was defined as the toxicant concentration that reduced the final DO or algal growth rate to half of that obtained by the control. The initial inoculated cell density was 15 000 cells/mL and the duration of the test was 48 h. A detail description of the test method and the concept of experimental design can be found from the author's previous work (Lin et al., 2005).

Twelve compounds including acetonitrile, chloroacetonitrile, dichloroacetonitrile, trichloroacetonitrile, bromoacetoitrile, propionitrile, 3-chloropropionitrile, butyronitrile, isobutyronitrile, 4-chlorobutyronitrile, benzonitrile, and malononitrile 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. Toxicants with low solubility were dissolved in acetone solution. Solvent controls were conducted and the results were checked using *t*-test at $P = 0.05$. Before commencing the experiment, stock solution was freshly prepared and its concentration was analyzed using a total organic carbon (TOC) analyzer.

Probit analysis was applied to determine the concentration-response relationship and the median effective concentration (EC50). QSARs were generated using log(1/EC50) in millimolar as the dependent variable and $\log K_{ow}$ and the energy of the lowest unoccupied molecular orbital (Elumo) as the independent variables. The lowest unoccupied molecular orbital energies were calculated using the Gaussian 98 program package. Specific chemicals were first analysed at the density functional theory level (B3LYP/6–31G) and the results were submitted to subsequent ab initio calculations (Frisch et al., 1999). To test the stability of QSAR models, leave-one-out cross validation is carried out using the MINITAB program (version 14.2, MINITAB Inc., 2005). Model quality was characterized by the number of observations ($n$), the square of correlation coefficient ($R^2$), the Fisher criterion ($F$), and the cross-validated correlation coefficient ($Q^2$).

## 3. Results and discussions

Table 1 displays a typical set of algal responses with respect to the toxicity of propionitrile. For the test control, the DO concentration increased from 1.48 mg/L at the beginning to a final DO concentration of 8.54 mg/L. The cell density increased from an initial value of 15,000 cells/mL to a final yield of 275,750 cells/mL. For most treatments, the inhibition rate based on DO production is greater than that based on algal growth rate. Concentration response curves for the aforementioned response endpoints are shown in Fig. 1. These curves were obtained

Table 1
Dose-response relationships for propionitrile

| Conc. (mg/L) | Initial DO (mg/L) | Final DO (mg/L) | Delta DO (mg/L) | Inhibition rate (DO) | Final cells (cells/mL) | $\mu$[a] ($Day^{-1}$) | Inhibition rate (growth rate) |
|---|---|---|---|---|---|---|---|
| Control | 1.48 | 8.54 | 7.06 | 0 | 275750 | 1.46 | 0 |
| 716.72 | 1.82 | 3.03 | 1.21 | 0.829 | 41600 | 0.51 | 0.649 |
| 358.36 | 1.63 | 4.16 | 2.53 | 0.641 | 83945 | 0.861 | 0.410 |
| 238.91 | 1.60 | 4.84 | 3.24 | 0.541 | 114000 | 1.014 | 0.305 |
| 119.45 | 1.44 | 6.42 | 4.98 | 0.295 | 165380 | 1.20 | 0.179 |
| 59.73 | 1.41 | 7.63 | 6.22 | 0.119 | 199500 | 1.294 | 0.114 |
| 14.93 | 1.59 | 8.35 | 6.76 | 0.042 | 228210 | 1.361 | 0.068 |
| | | | EC50 = | 223.09 | | | 480.26 |

[a] $\mu$: specific growth rate.

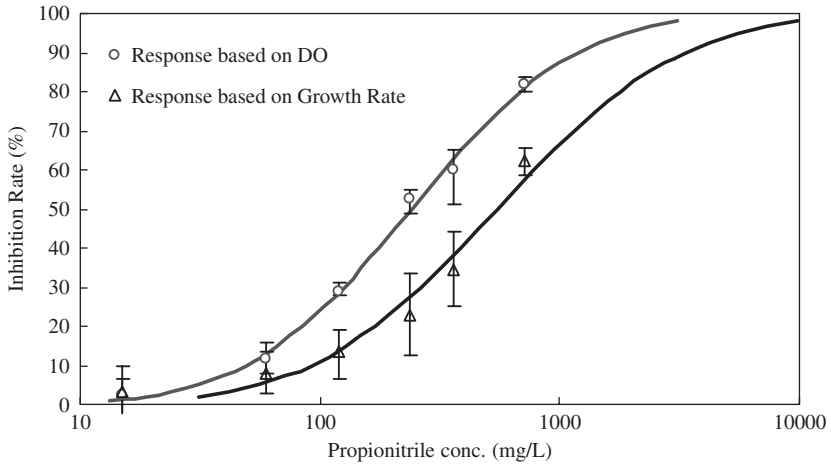


Fig. 1. Dose–response curves for propionitrile.

Table 2
EC50 values and their 95% confidence intervals for nitriles

| Chemical | Response based on DO | | Response based on growth rate | |
|---|---|---|---|---|
| | $EC_{50}$ (mg/L) | 95% Confidence limit | $EC_{50}$ (mg/L) | 95% Confidence limit |
| Acetonitrile | 5842 | 5433–6378 | 8065 | 7558–8843 |
| Chloroacetonitrile | 10.91 | 6.45–22.35 | 19.34 | 8.91–49.90 |
| Dichloroacetonitrile | 2.62 | 2.29–2.96 | 3.45 | 0.86–6.26 |
| Trichloroacetonitrile | 0.051 | 0.046–0.056 | 0.063 | 0.0148–0.126 |
| Bromoacetoitrile | 0.113 | 0.090–0.143 | 0.106 | 0.080–0.147 |
| Propionitrile | 223.09 | 196.47–253.21 | 480.26 | 339.13–826.16 |
| 3-Chloropropionitrile | 199.39 | 189.62–210.27 | 193.39 | 186.35–200.77 |
| Butyronitrile | 756.39 | 542.51–1293.1 | 1037.8 | 843.40–1689.6 |
| Isobutyronitrile | 742.62 | 687.25–798.03 | 1149 | 1101–1199 |
| 4-Chlorobutyronitrile | 616.87 | 284.97–800.37 | 655.50 | 333.43–783.50 |
| Benzonitrile | 29.175 | 22.70–39.8 | 33.251 | 21.73–49.72 |
| Malononitrile | 20.423 | 14.97–26.03 | 40.119 | 28.63–55.16 |

through linear regression assuming a log-normal distribution (probit model) of the tolerances. Based on the probit analyses, EC50 values were found to be equal to 223.09 mg/L ($\Delta$DO) and 480.26 mg/L (growth rate based on cell density), respectively. According to the dose–response curves in Fig. 1, we may also conclude that endpoint based on DO production is more sensitive than growth rate.

Table 2 lists the EC50 values and their 95% confidence intervals for various nitriles tested in this study. The

Table 3
Parameters for QSAR models

| Chemicals | Molecular weight | log $K_{ow}$ | Elumo (hartree) | log(1/$EC_{50}$) | | log Te[a] | |
|---|---|---|---|---|---|---|---|
| | | | | DO | GR | DO | GR |
| Acetonitrile | 41.05 | −0.39 | −0.00957 | −2.123 | −2.293 | −1.110[b] | −1.020[b] |
| Chloroacetonitrile | 75.5 | 0.45 | −0.05739 | 0.806 | 0.577 | −0.679 | −0.567 |
| Dichloroacetonitrile | 109.94 | 0.93 | −0.07284 | 1.594 | 1.426 | −0.699 | −0.538 |
| Trichloroacetonitrile | 144.39 | 2.09 | −0.08528 | 3.425 | 3.427 | 0.477 | 0.654 |
| Bromoacetoitrile | 119.95 | 0.36 | −0.07412 | 3.022 | 3.053 | 0.665 | 1.136[b] |
| Propionitrile | 55.08 | 0.14 | −0.01085 | −0.607 | −0.940 | 0.336 | 0.171 |
| 3-chloropropionitrile | 89.53 | 0.34 | −0.02648 | −0.366 | −0.378 | −0.239 | −0.042 |
| Butyronitrile | 69.11 | 0.66 | −0.00505 | −1.028 | −1.153 | 0.215 | 0.133 |
| Isobutyronitrile | 69.11 | 0.44 | −0.01006 | −1.034 | −1.364 | −0.051 | −0.272 |
| 4-chlorobutyronitrile | 103.55 | 0.56 | −0.01807 | −0.758 | −0.760 | −0.193 | −0.0693 |
| Benzonitrile | 103.12 | 1.56 | −0.0658 | 0.540 | 0.457 | −1.390[b] | −1.295[b] |
| Malononitrile | 66.06 | −1.20 | −0.03392 | 0.453 | 0.219 | 0.201 | 0.495 |

[a]log Te = log(1/EC50)−(predicted value from Eq. (5) or (6)).
[b]Outliers for QSARs.

median effective concentrations (EC50) range from 0.051 to 5842 mg/L (based on DO production). Among the 12 nitriles tested, ten of them displayed stronger inhibitory effects on DO production than that on algal growth rate. In particular, the toxic effect of malononitrile and propionitrile on DO production is twice as large as that revealed by the growth-rate endpoint. The apparent difference in sensitivity between the two test endpoints is a consequence of comparing an arithmetric increase in DO (DO production) with a logarithmic increase in cell density (algal growth rate). Furthermore, halogen-substituted nitriles were found to be extremely toxic to *P. subcapitata*. Chloroacetonitrile's toxicity was enlarged for 400–600 times as compared to acetonitrile. For both DO and growth rate endpoints, trichloroacetonitrile was found to be the most toxic compound among all nitriles tested. Furthermore, for chloroacetonitriles, toxicity is directly related to the number of chlorine atoms contained by the compound. This phenomenon is in consistency with our previous observations from chlorophenols (Chen and Lin, 2006). Also, the bromine substitutent also seems to be more toxic than chlorine substitutent.

Table 3 lists the log $K_{ow}$, Elumo, and log (1/EC50) values for establishing the QSARs. The unit for EC50 values is in terms of mmol/L. Log $K_{ow}$ was found to have no linear relationship with EC50 values for both DO and growth rate (GR) endpoints. On the other hand, good linear relationships ($R^2 = 0.85$) were identified between toxicity and the chemicals' Elumo values. The above relationships based on the descriptor Elumo, which reflects hydrogen bonding donor capacity, indicate that the toxicity mechanisms for nitriles are reactive in nature (specific toxicity). The negative values of the slopes for the QSAR models suggest that toxicity increases with an increase in the hydrogen bonding donor capacity. Eqs. (1) and (2) describe the QSARs based on Elumo. Results from leave-one-out cross-validation, with $Q^2$ equal to 0.78 and 0.77, indicate that these QSARs are quite significant. Fig. 2 depicts the

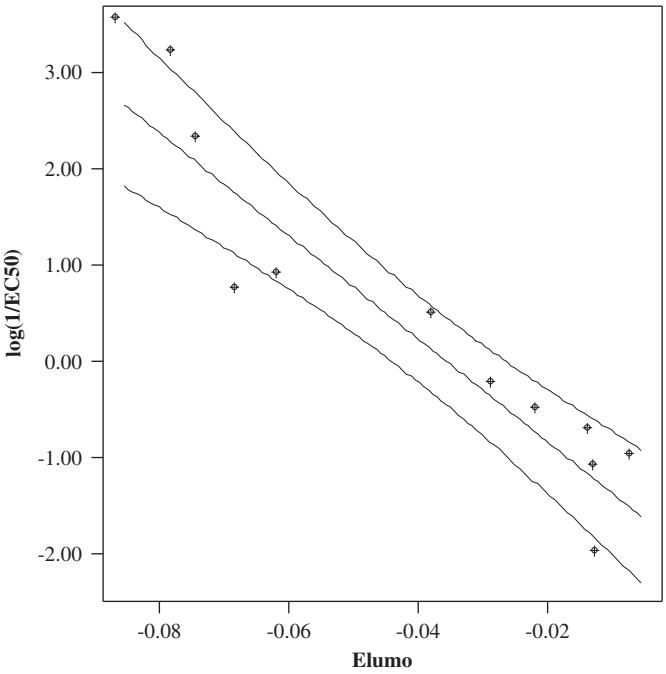


Fig. 2. The relationship between Elumo and EC50 (GR) values.

QSAR between EC50 (DO) and Elumo values.

$$\log(1/\mathrm{EC50}) = -51.8\ \mathrm{Elumo} - 1.70$$
$$R^2 = 0.85,\ Q^2 = 0.78,\ F = 57.85,\ n = 12 \quad \text{(DO)}, \quad (1)$$

$$\log(1/\mathrm{EC50}) = -53.7\ \mathrm{Elumo} - 1.91$$
$$R^2 = 0.85,\ Q^2 = 0.77,\ F = 57.78,\ n = 12 \quad \text{(GR)}. \quad (2)$$

Correlation analyses were further conducted to establish QSARs based on the *n*-octanol/water partition coefficient (log $K_{ow}$) and Elumo. Results from surface–response analyses (Eqs. (3) and (4)) showed no significant improvement on regression when both log $K_{ow}$ and Elumo were included in the QSAR model. The cross-validated

correlation coefficients ($Q^2$) are both equal to 0.71 and are less significant compared to that in Eqs. (1) and (2). Furthermore, the negative coefficients for $\log K_{ow}$ indicate that toxicity increases with a decrease of chemical's hydrophobicity. Such a relationship does not agree with the general consensus that toxicity increases with increasing hydrophobicity.

$$\log(1/EC50) = -1.71 - 0.075 \log K_{ow} - 52.96 \text{ Elumo}$$
$$R^2 = 0.85,\ Q^2 = 0.71,\ F = 26.23,\ n = 12 \quad \text{(DO)}, \quad (3)$$

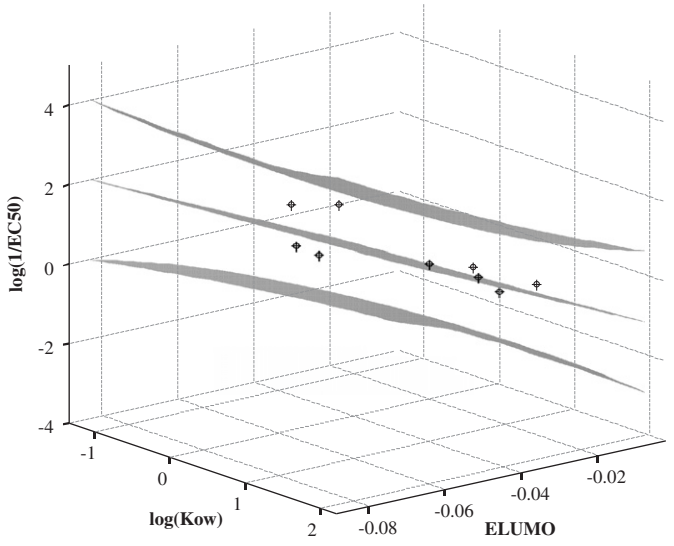


Fig. 3. The correlation between EC50 (GR), $\log K_{ow}$, and Elumo values.

$$\log(1/EC50) = -1.92 - 0.034 \log K_{ow} - 54.23 \text{ Elumo}$$
$$R^2 = 0.85,\ Q^2 = 0.71,\ F = 26.04,\ n = 12 \quad \text{(GR)}. \quad (4)$$

The above two-parameter QSAR models can be improved by excluding statistical outliers from the regression. As Blum (1989) pointed out, the achievable accuracy of a QSAR for predicting toxicity to bacteria is approximately one order of magnitude. In Table 3, the log Te (residue) values for both acetonitrile and benzonitrile were greater than one and were thus considered as outliers. As shown in Eq. (5), for the DO endpoint, the $R^2$ value is 0.92 and is apparently better than that obtained by Eq. (3). Furthermore, the goodness of prediction was also improved as $Q^2$ for Eq. (5) equals to 0.81. Similarly, for the growth-rate endpoint, $R^2$ value for Eq. (6) is also significantly improved to 0.92 when three statistical outliers were excluded (i.e., acetonitrile, benzonitrile, and bromoacetoitrile). However, $Q^2$ value for Eq. (6) is only 0.51 and is not sufficiently significant. More data is still necessary to validate the QSAR described by Eq. (6). Fig. 3 depicts the correlation between EC50 values based on growth rate and descriptors (($\log K_{ow}$ and Elumo) as described by Eq. (6). The coefficients for $\log K_{ow}$ are relatively small compared to that for Elumo. However, refer to Table 3, the numerical value for $\log K_{ow}$ is one to three orders in magnitude greater than that for Elumo. Hence, it seems that both parameters exerted significant influences on the observed toxicity.

$$\log(1/EC50) = -1.51 + 0.0056 \log K_{ow} - 52.14 \text{ Elumo}$$
$$R^2 = 0.92,\ Q^2 = 0.81,\ F = 41.12,\ n = 10 \quad \text{(DO)}, \quad (5)$$

Table 4
Relative sensitivities of different aquatic organisms to nitriles

| Test species | BOD bottle test (*P. subcapitata*) | | Fish (*Pimephales Promelas*)[a] | Protozoa (*Tetrahymena Pyriformis*)[b] | Water flea (*Daphnia magna*) | Microtox |
|---|---|---|---|---|---|---|
| Chemicals | DO $\log EC_{50}^{-1}$ | Growth rate $\log EC_{50}^{-1}$ | $\log LC_{50}^{-1}$ | $\log EC_{50}^{-1}$ | $\log EC_{50}^{-1}$ | $\log EC_{50}^{-1}$ |
| Acetonitrile | −2.12 | −2.29 | −1.60* | — | −3.5563[c] | −4.2014[f] |
| Chloroacetonitrile | 0.81 | 0.58 | 1.75* | 0.85 | | |
| Dichloroacetonitrile | 1.59* | 1.43 | — | 0.97 | | |
| Trichloroacetonitrile | 3.43* | 3.43* | — | 1.88 | | |
| Bromoacetoitrile | 3.02 | 3.05* | — | 2.23 | | |
| Propionitrile | −0.607* | −0.94 | −0.88 | — | −2.39794[e] | |
| 3-Chloropropionitrile | −0.37* | −0.38 | — | −1.12 | | |
| Butyronitrile | −1.03* | −1.15 | — | — | <−2.04139[e] | |
| Isobutyronitrile | −1.03* | −1.36 | — | — | <−1.97451[e] | |
| 4-Chlorobutyronitrile | −0.76* | −0.76* | — | −0.93 | | |
| Benzonitrile | 0.54* | 0.46 | −0.01 | — | −2.08636 (24 h)[d] | |
| Malononitrile | 0.45 | 0.22 | 2.07* | 0.22 | | −2.34439[f] |

Unit: mmol/L.
*The most sensitive species.
[a]Data from Russom et al. (1997).
[b]Data from Akers et al. (1999).
[c]Data from Tong et al. (1996).
[d]Data from Bringmann and Kuhn (1982).
[e]Data from Eastman Kodak Company (2003).
[f]Data from Chen and Yeh (1996).

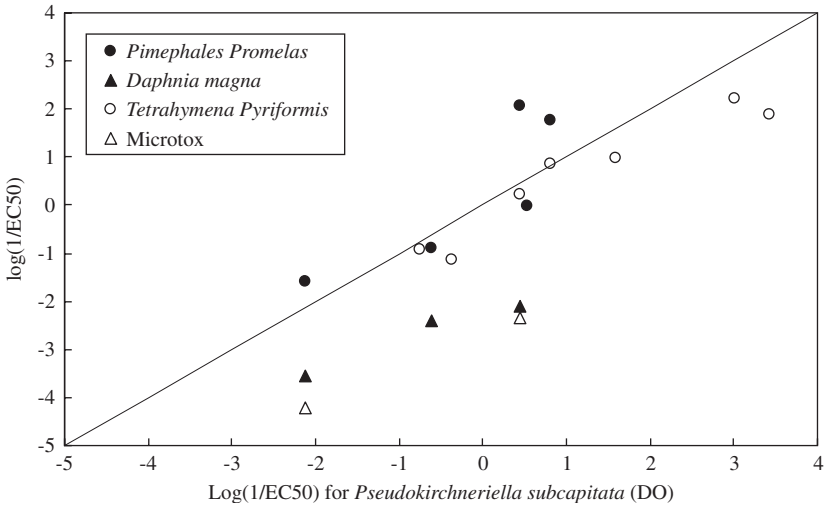


Fig. 4. Comparison of species sensitivity (DO endpoint).

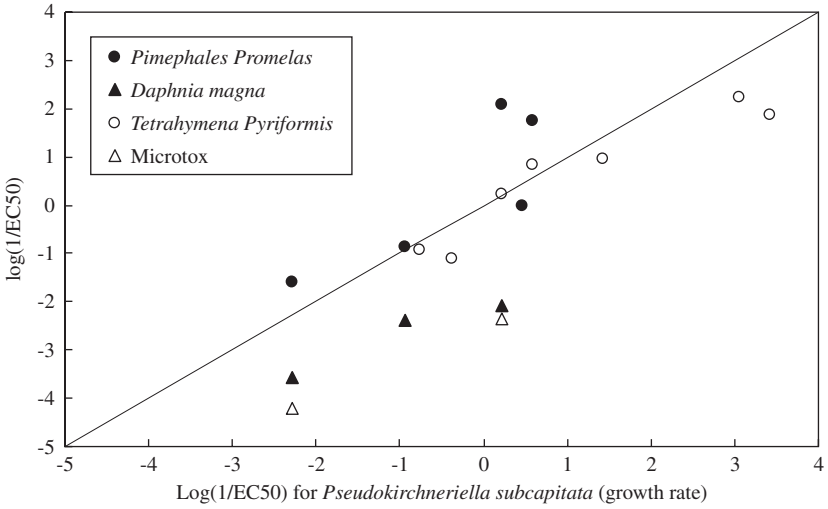


Fig. 5. Comparison of species sensitivity (Growth rate endpoint).

$$\log(1/\mathrm{EC50}) = -1.65 + 0.19\log K_{\mathrm{ow}} - 47.20 \text{ Elumo}$$
$$R^2 = 0.92,\ Q^2 = 0.51,\ F = 36.94,\ n = 9 \quad \text{(GR)}. \qquad (6)$$

Table 4 compares the log (1/EC50) values of different aquatic organisms to nitriles. To a specific compound, the most sensitive organism was marked with an asterisk. Among the 12 compounds listed in Table 4, *P. subcapitata* (DO endpoint) is the most sensitive species for eight chemicals. Fathead minnow (*Pimephales promelas*), on the other hand, reveals the highest sensitivity to the other three nitriles. Fig. 4 and Fig. 5 compare the overall species sensitivity between the five aquatic organisms in Table 4. A data point located below the diagonal line indicates that, the sensitivity of a specific test organism is lower compared to *P. subcapitata*. *Daphnia magna* and the luminescent bacteria (Microtox) appeared to be rather insensitive to nitriles. Based on the available data, the relative sensitivity relationship is: *P. promelas* $\geqslant$ *P. subcapitata* > *Tetrahymena pyriformis* > *D. magna* > luminescent bacteria (Microtox). However, the above relationship is just a comparison based on insufficient data. It is still necessary to produce more data to refine the relative sensitivity relationship. Nevertheless, the above comparison indicates that *P. subcapitata* is a sensitive aquatic species to nitriles.

Literature data (Bringmann and Kuhn, 1978, 1980) were compared with our test results. The lowest-observed-effect concentrations (LOEC) for acetonitrile were found to be 7300 mg/L (*Scenedesmus quadricauda*) and 520 mg/L (*Anacystis aeruginosa*), respectively. On the other hand, for benzonitrile, LOEC values were 75 mg/L (*S. quadricauda*) and 3.4 mg/L (*A. aeruginosa*). Compared to the EC50 values in Table 2, one may find that *P. subcapitata* reveals apparently higher sensitivity than *S. quadricauda* to nitriles. Such a difference could be related to both species sensitivity and the influence of different test techniques that applied. The relative sensitivity relationship between

*P. subcapitata* and *A. aeruginosa*, however, still requires further investigation.

## 4. Conclusions

This study presents the toxicity data of various nitriles to *P. subcapitata* using a closed algal toxicity testing technique with no headspace. For most nitriles, the DO endpoint revealed better sensitivity than algal growth rate. Furthermore, halogen-substituted nitriles were found to be extremely toxic to *P. subcapitata*. With increasing numbers of the halogen atoms, stronger toxicity was observed. The bromine substitutent also seems to be more toxic than chlorine substitutent. Quantitative structure–activity relationships (QSARs) were established based on the chemicals' Elumo values and hydrophobicity ($\log K_{ow}$). Such relationships may thus be useful in predicting the toxicity of other compounds of the same mode of toxic action. Furthermore, for various aquatic organisms, the relative sensitivity relationship is: *P. promelas* $\geqslant$ *P. subcapitata* $>$ *T. pyriformis* $>$ *D. magna* $>$ luminescent bacteria (Microtox). The alga, *P. subcapitata*, was found to be quite sensitive to nitriles compared to other organisms.

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