Toxicity assessment of polycyclic aromatic hydrocarbons using an air-tight algal toxicity test

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Abstract The existing toxicity data on the effects of polycyclic aromatic hydrocarbons (PAHs) on *Pseudokirchneriella subcapitata* (green alga) are quite insufficient. These data were derived using different test techniques (e.g. conventional batch test, closed-system test, semi-static test). The relative toxicity relationship for various PAHs is thus difficult to interpret. Consequently, the current toxicity database is insufficient and also inadequate for analyses of the effects of PAHs on *P. subcapitata*. This study evaluated the toxicity of eleven PAHs using an air-tight test technique. The relative toxicity relationship was determined on a uniform basis, and was different from the relationship based on current available data. *P. subcapitata.* was found to be more susceptible to PAHs than *Daphnia magna*, fathead minnow, and *Scenedesmus subspicatus*. Quantitative structure-activity relationship (QSAR) was established based on the chemical's hydrophobicity with R^2 equal to 0.88. Photo-induced toxicity for various PAHs was also explored by exposing PAHs under UV-photoactivation. Toxicity of anthracene, benzanthrone, and benzo[a]anthracene was found to increase 3.5 to 25 times after UV exposure. Phototoxicity was observed when the HOMO-LUMO gap varied between 6.8 and 8.0 eV.

Keywords PAHs; photo-induced toxicity; *Pseudokirchneriella subcapitata*; QSAR

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Introduction

Algal toxicity tests have been widely used in bioassay test batteries to assess the relative toxicity of various toxic chemicals and waste discharges. The traditional batch tests, considering their open test environment and the vigorous mixing provided during the test, have been criticized for their applicability for testing volatile organic toxicants (ECETC, 1996). Sealed test vessels with or without headspace were proposed for testing volatile organic compounds (Galassi and Vighi, 1981; Herman *et al.*, 1990; Brack and Rottler, 1994; Halling-Sørensen *et al.*, 1996; Mayer *et al.*, 2000). In these test systems, concentrated CO_2 gas or enriched bicarbonate buffer was employed to provide extra carbon source and maintain stable pH. Large headspace may cause a significant portion of the volatile compound to partition from the aqueous phase and, thus, the exposure concentration might be altered significantly (Mayer *et al.*, 2000). On the other hand, enriched carbonate buffer may also result in increased ionic strength and lower test sensitivity (Brack and Rottler, 1994; Lin *et al.*, 2005). Overall, the aforementioned closed-system test methods are relatively more complex in experimental design and also more tedious compared to the conventional algal batch tests. Although the Organization for Economic Co-Operation and Development has suggested that a sealed exposure system should be used for testing volatile compounds (OECD, 2000), the closed-system test technique has not yet been standardized.

Polycyclic aromatic hydrocarbons (PAHs) are a prevalent group of toxic and mutagenic contaminants. PAHs are highly hydrophobic and are usually sequestered to sediment and particulate phases in aquatic environments (Karickhoff *et al.*, 1979; Banwart *et al.*, 1982; McElroy *et al.*, 1989). In addition, PAHs absorb solar ultraviolet (UV)

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radiation strongly, resulting in photolysis of the carbon skeleton. This makes the chemicals much more soluble, and increases their bioavailability to aquatic organisms (Katz *et al.*, 1979; Huang *et al.*, 1993; McConkey *et al.*, 1997). Recent work has shown that photolysis of PAHs results in complex mixtures of photoproducts that are more toxic than the parent compounds (Huang *et al.*, 1993; Ren *et al.*, 1994; McConkey *et al.*, 1997). Many studies (Newsted and Giesy, 1987; Mekenyan *et al.*, 1994) have developed a quantitative structure activity relationship (QSAR) to predict both the occurrence and potency of photo-induced toxicity for individual PAHs. The pathway for PAH has been elucidated mainly for vertebrates and invertebrates (Lemaire *et al.*, 1992; Narbonne *et al.*, 1992; Djomo *et al.*, 1996), but very little information is available about *Pseudokirchneriella subcapitata*. This organism is frequently found in all freshwaters and is an important primary producer in aquatic food chains. The few available data for the toxicity of PAHs on *P. subcapitata* were derived using different test methods, e.g. conventional batch test, closed-system test, and semi-static test (US EPA, 1978; Gala and Giesy, 1992; Halling-Sørensen *et al.*, 1996). The relative toxicity relationship for different PAHs is thus difficult to interpret.

The author's recent 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 experimental design is quite simple and the test revealed satisfactory sensitivities to both metallic and organic toxicants. The objective of this study was to evaluate the toxicity of various PAHs on *P. subcapitata*, using the air-tight algal toxicity test method developed previously (Chen *et al.*, 2005; Lin *et al.*, 2005), and furthermore, to establish the QSAR relationship for PAHs based on *Pseudokirchneriella subcapitata*.

Methods

The alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*, UTEX 1648) was grown in a 4-litre 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. 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 (1996) bottle medium with no EDTA content was used for toxicity testing. The dilution water was stripped by nitrogen gas to reduce the dissolved oxygen level. In addition, the N_2 gas contained 0.5% carbon dioxide as an extra carbon source. Two response endpoints were used to evaluate the toxicity of toxicants: Δ cell density and algal growth rate based on cell density. The median effective concentration (EC50) was defined as the toxicant concentration that reduced Δ cell density 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 hrs. 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).

Eleven PAHs including naphthalene (NAP), phenathrene (PHE), anthracene (ANT), fluoranthene (FLU), benzo[a]anthracene (BAA), benzo[b]fluorine (BBF), dibenzo[b,i]anthracene (DBIA), perylene (PER), benzo[b]chrysene (BBC), benzanthrone (BEN), acridine (ACR) 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 PAHs were dissolved in DMSO solvent and stored in foil-wrapped glass containers. Before commencing the experiment,

the concentration of test compounds was analyzed using a HPLC analyzer. Solvent controls were conducted and the results were checked using a *t*-test at $p = 0.05$.

To explore the photo-induced-toxicity phenomenon, PAHs were treated under various UV exposure conditions as specified in previous studies (Newsted and Giesy, 1987; Mekenyan *et al.*, 1994; Veith *et al.*, 1995; Ribeiro and Ferreira, 2005). The light intensities were 1,060 $\mu W/cm^2$ and 2,000 $\mu W/cm^2$ for UVA and UVB, respectively. Exposure duration varied from 2 to 5 days depending on the structural characteristics of the chemical (e.g. 2-ring PAHs: 2 days; 5-ring PAHs: 5 days). The photomodified PAH compounds were then transferred to test vessels for toxicity testing. Results from our HPLC analyses indicated that these exposure periods were sufficient for photolysis.

Results and discussion

Table 1 lists the EC50 values for various PAHs with (+ UV) or without UV-light modification. As expected, EC50 values based on Δ cell density are smaller than those based on algal growth rate, indicating that Δ cell density is a more sensitive parameter. The relative toxicity relationships from the two test endpoints are somewhat different, as shown below. However, BBC and BAA are clearly the most toxic compounds among all PAHs.

$$\Delta \text{ cell density: BBC} > \text{BAA} > \text{FLU} > \text{ANT} > \text{BBF} > \text{BEN} > \text{PER} > \text{PHE} > \text{DBIA} > \text{ACR} > \text{NAP}$$

$$\text{Growth rate: BBC} > \text{BAA} > \text{FLU} > \text{PHE} > \text{BEN} > \text{PER} > \text{BBF} > \text{DBIA} > \text{ACR} > \text{ANT} > \text{NAP}$$

With UV-photoactivation, anthracene (ANT), benzanthrone (BEN), benzo[a]anthracene (BAA), and acridine (ACR) displayed apparent induced toxicity with their toxic effects

Table 1 EC50 values for PAHs with or without UV exposure

Chemical	Δ cell density		Growth Rate		Δ cell density	Growth Rate
	EC50 (mg/l)	95% CL*	EC50 (mg/l)	95% CL	EC50/ EC50 + UV	EC50/ EC50 + UV
BEN	0.048	0.039–0.059	0.211	0.177–0.259	9.6	10.0
BEN (+ UV)	0.005	0.004–0.006	0.021	0.017–0.026		
PHE	0.124	0.117–0.131	0.193	0.177–0.214	1.15	0.92
PHE (+ UV)	0.108	0.098–0.118	0.210	0.184–0.263		
FLU	0.033	0.028–0.039	0.074	0.069–0.079	2.06	1.45
FLU (+ UV)	0.016	0.013–0.019	0.051	0.043–0.061		
ANT	0.402	0.371–0.433	0.913	0.845–0.996	3.44	5.93
ANT (+ UV)	0.117	0.072–0.352	0.154	0.122–0.195		
BAA	0.008	0.001–0.0174	0.050	0.0101–0.219	8.0	25.0
BAA (+ UV)	0.001	0.0005–0.0008	0.002	0.0017–0.0029		
ACR	0.524	0.506–0.542	0.850	0.812–0.893	13.4	5.15
ACR (+ UV)	0.039	0.025–0.058	0.165	0.119–0.239		
BBF	0.043	0.031–0.055	0.395	0.286–0.619	0.8	1.04
BBF (+ UV)	0.054	0.046–0.062	0.379	0.296–0.505		
DBIA	0.194	0.137–0.258	0.767	1.606–1.057	1.11	1.28
DBIA (+ UV)	0.175	0.145–0.209	0.597	0.469–0.809		
PER	0.053	0.039–0.067	0.348	0.285–0.435	1.10	2.32
PER (+ UV)	0.048	0.033–0.063	0.150	0.119–0.195		
BBC	0.005	0.004–0.008	0.012	0.009–0.015	1.25	1.09
BBC (+ UV)	0.004	0.003–0.006	0.011	0.009–0.0134		
NAP	2.50	1.953–3.006	4.793	3.919–5.808	1.23	0.86
NAP (+ UV)	2.040	1.362–2.619	5.527	4.538–7.011		

*CL: confidence limit

increased 3.5 to 25 times. Fluoranthene (FLU) and perylene (PER) also revealed moderate increase of toxicity; on the other hand, no significant increase was observed in toxicity for PHE, BBF, DBIA, BBC, and NAP.

Table 2 lists the literature EC50 or LC50 values for various aquatic organisms. Previous data based on *Pseudokirchneriella subcapitata* appeared to be less sensitive than our data based on Δ cell density, except for the case of ANT (Gala and Giesy, 1992). In Gala and Giesy's study, the semi-static test was employed and ANT was added to the test vessels every 8 hrs. Results from the traditional batch tests were almost 10 times greater than our values. Data derived using closed-system tests (Halling-Sørensen *et al.*, 1996; Dijkman *et al.*, 1997), are closer to our test results. The relative toxicity relationship for PAHs, according to literature data, is ANT > PHE > ACR > FLU > NAP. The above toxicity order for PAHs is different from our results as shown previously. Such a relationship is mainly related to the influences of diverse test methods applied. Data for *Scenedesmus subspicatus* were also derived from closed-system technique (Dijkman *et al.*, 1997). The alga, *Scenedesmus subspicatus*, appears to be more resistant than *P. subcapitata*. Furthermore, *Daphnia magna* is generally less sensitive to PAHs than *P. subcapitata*, except for ANT, DBIA, and PER. Overall, the relative sensitivity for organisms listed in Table 2 can be determined as *P. subcapitata* > *Daphnia magna* > *Scenedesmus subspicatus* > fathead minnow.

Data in Table 2 also revealed that BEN, BAA, and BBC were particularly hazardous to *P. subcapitata*, as compared to other aquatic organisms. Variations in species sensitivity may be the major reason causing the above observations.

In order to establish capability for predicting the toxic potency of other PAHs, quantitative structure–activity relationships (QSARs) were derived using various physical descriptors. Table 3 lists the parameters for QSAR study. For PAHs with no UV activation, good linear relationships were found between the observed toxicity and the 1-octanol:water partition coefficient (log K_{ow}). Equations (1) and (2) describe the QSARs based on either Δ cell density or growth rate. Hence, the toxicity of PAHs is directly related to the chemical's hydrophobicity. Two outliers, i.e. PER and DBIA, were

Table 2 Comparison of EC50 values for various aquatic organisms

Test species	BOD bottle test *P subcapitata*	Algae *P subcapitata*	Algae[a] *Scenedesmus subspicatus*	Water flea[g] *Daphnia Magna*	Fish[i] Fathead minnow
Toxicant	Δ cell density EC50 (mg/l)	Biomass EC50 (mg/l)	Biomass EC50 (mg/l)	EC50 (mg/l)	LC50 (mg/l)
BEN	0.048	–	0.439	–	0.463
PHE	0.124	0.18[b] (closed)	0.737	0.23	0.817
FLU	0.033	4.14[c] (batch)	0.256	0.196[h]	0.095
ANT	0.402	0.112[d] (semi-static)	0.737	0.211*	0.535
BAA	0.0078	–	0.087	0.027	0.083
ACR	0.524	0.78[e](closed)	0.84	1.024[h]	2.3
BBF	0.043	–	–	0.215	–
DBIA	0.194	–	0.01*	0.012*	–
PER	0.053	–	0.029*	0.036*	0.089[j]
BBC	0.0052	–	–	0.012	0.03[j]
NAP	2.50	25[f] (batch, 14day)	5.866	2.5	6.14

[a]Djomoa *et al.*, 2004; [b]Halling-Sørensen *et al.*, 1996; [c]U.S. EPA, 1978; [d]Gala and Giesy, 1992; [e]Dijkman *et al.*, 1997; [f]Gaur, 1988; [g]Abernethy *et al.*, 1986; [h]Munoz and Tarazona, 1993; [i]Foster and Tullis, 1985; [j]Broderius *et al.*, 1995

*More sensitive than *P. subcapitata* (EC50 based on Δ cell density)

Table 3 Parameters for QSAR analyses

Toxicant	log(1/EC50)					
	Δ cell density (mmole/l)	Growth rate (mmole/l)	Log Kow*	E_{LUMO}† ev	E_{HOMO}† ev	Δ GAP ev
BEN	3.68	3.04	4.81	−1.27	−8.70	7.43
PHE	3.16	2.96	4.35	−0.41	−8.62	8.21
FLU	3.79	3.44	4.93	−0.93	−8.63	7.70
ANT	2.65	2.29	4.45	−0.84	−8.12	7.28
BAA	4.47	3.66	5.53	−0.81	−8.21	7.40
ACR	2.53	2.32	3.32	−1.04	−8.58	7.53
BBF	3.70	2.74	5.19	−0.49	−8.48	7.99
DBIA	3.16	2.56	6.32	−1.52	−6.90	5.39
PER	3.68	2.86	6.11	−1.15	−7.86	6.71
BBC	4.73	4.36	6.54	−0.99	−8.05	6.61
NAP	1.71	1.43	3.32	−0.27	−10.88	10.16

*Estimated by the fragment method (http://www.epa.gov/opptintr/exposure/docs/episuite.htm)
†Estimated by Gaussian 98 program package (Frisch *et al.*, 1999)

removed from the regression analyses.

$$\Delta\,\text{cell density}: \ \text{Log}(1/\text{EC50}) = 0.884 \log K_{ow} - 0.792 \quad R^2 = 0.88, n = 9 \tag{1}$$

$$\text{Growth rate}: \ \text{Log}(1/\text{EC50}) = 0.758 \log K_{ow} - 0.656 \quad R^2 = 0.81, n = 9 \tag{2}$$

The photo-induced toxicity for PAHs can also be described by the HOMO–LUMO energy gap. Figure 1 displays the phototoxicity window based on the endpoint of Δ cell density. The window can be illustrated by Eq. (3) and (4), as given below:

$$\text{Log}(\text{EC50}/\text{EC50} + \text{UV}) = 1.096\Delta\,\text{GAP} - 7.23 \quad R^2 = 0.86, \ n = 4 \tag{3}$$

$$\text{Log}(\text{EC50}/\text{EC50} + \text{UV}) = -1.534\Delta\,\text{GAP} + 12.4 \quad R^2 = 0.78, \ n = 5 \tag{4}$$

From Figure 1, one may find that phototoxicity was induced when the HOMO–LUMO energy gap was in the range of 6.8 to 8.0 eV. This range for energy gap is slightly wider than that defined by Mekenyan *et al.* (1994).

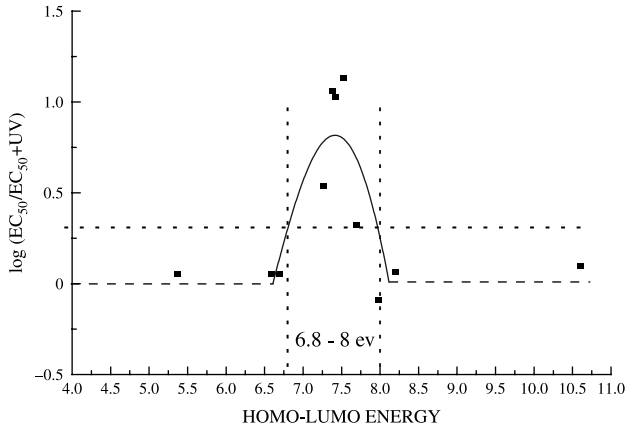

Figure 1 Phototoxicity window for PAHs on *P. subcapitata* (Δ cell density)

Conclusions

The existing toxicity data on the effects of polycyclic aromatic hydrocarbons (PAHs) on *Pseudokirchneriella subcapitata* (green alga) are quite insufficient. These data were derived using different test techniques (e.g. conventional batch test, closed-system test, semi-static test). The relative toxicity relationship for various PAHs is thus difficult to interpret. Consequently, the current toxicity database is insufficient and also inadequate for analyses of the effects of PAHs on *P. subcapitata*. This study evaluated the toxicity of eleven PAHs using an air-tight test technique. The relative toxicity relationship was determined on a uniform basis, and was different from the relationship based on current available data. *P. subcapitata*. was found to be more susceptible to PAHs than *Daphnia magna*, fathead minnow, and *Scenedesmus subspicatus*. A quantitative structure–activity relationship (QSAR) was established based on the chemical's hydrophobicity with R^2 equal to 0.88. Photo-induced toxicity for various PAHs was also explored by exposing PAHs under UV-photoactivation. Toxicity of anthracene, benzanthrone, and benzo[a]anthracene was found to increase 3.5 to 25 times after UV exposure. Phototoxicity was observed when the HOMO–LUMO gap varied between 6.8 and 8.0 eV.

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