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The relationship between logPow and molecular weight of polycyclic aromatic hydrocarbons and EC50 values of marine microalgae

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Abstract : The effect of five polycyclic aromatic hydrocarbons (dibenzothiophene, phenanthrene, naphthalene, fluorene and hydroxybiphenyl) on the growth of eight marine microalgae (Bacillariophyceae; *Skeletonema costatum, Chaetoceros calcitrans*, Prasinophyceae; *Tetraselmis tetrathele*, Haptophyceae; *Isochrysis galbana, Pavlova lutheri,* Dinophyceae; *Prorocentrum minimum,* Euglenophyceae; *Eutreptiella* sp. and Chlorophyceae; *Dunaliella tertiolecta*) was investigated. *D. tertiolecta* to all polycyclic aromatic hydrocarbons (PAHs) was the most tolerant species of the microalgae tested, EC50 values of dibenzothiophene, phenanthrene and naphthalene on *D. tertiolecta* were higher than the highest concentrations tested and therefore could not be determined in this experiment. On the other hand, the most sensitive microalgae varied with the compounds of PAHs. *Eutreptiella* sp. to dibenzothiophene, *P. lutheri* to phenanthrene, fluorene and naphthalene, *P. minimum* to phenanthrene and C. *calcitrans* to hydroxybiphenyl were the most sensitive species. Linear equation between the octanol/water partition coefficient (logPow) of all PAHs tested and EC50 values (log (1/EC50)) of all microalgae tested was $\log(1/EC50) = 0.87 \times \log Pow - 0.76$ ($r^2 = 0.75$). The ranges of the upper and lower 95% confidence limits were more than 1.4, the variation of algal sensitivity was more than twenty-five fold. EC50 values of C. *calcitrans* and *T. tetrathele* tested had a higher correlation to the molecular weight than to the logPow of the PAHs.

Keywords : *Toxicity tests, Marine microalgae, Aromatic hydrocarbons, Octanol/water partition coefficient, Molecular weight*

INTRODUCTION

Toxicity tests of petroleum components have been examined to predict the influence of accidental pollution on aquatic organisms. The toxicity effects on microalgae, primary producers in the aquatic food web, have also been reported (PULICH *et al.,* 1974, WINTERS *et al.,* 1976, HSIAO, 1978, BATE and CROFFORD, 1985, VANDERMEULEN and LEE, 1986,VANDERMEULEN, 1986, MORALES-LOO and GOUTX, 1990, EL-DIB *et al.,* 1997). Several of these studies report that the toxic concentration for microalgae varied with the origin of crude oil (WINTERS *et al.,* 1976, HSIAO, 1978), because the components of aromatic hydrocarbons in crude oil vary with the source of origin. Therefore, toxicity effects of individual aromatic hydrocarbons which along with paraffin and asphalt from the main components in crude oil, on microalgae should be examined. Although the toxic effects on freshwater microalgae (U.S. EPA, 1980, HERMAN *et al.,* 1990, HERMAN *et al.,* 1991, SHEEDY *et al.,* 1991), on marine microalgae (DUNSTAN *et al.,* 1975, KUSK, 1980, 1981a, 1981b) have been investigated, there is little available data on polycyclic aromatic hydrocarbons (PAHs) on marine microalgae.

Recently, predictions of toxicity have led to the examination of relationships between the physicochemical properties of chemicals (water solubility, octanol/water partition coefficient etc.) and the bioconcentration factor, or between the physicochemical properties and toxic effects. Although there are several reports for

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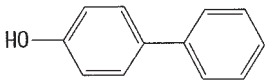
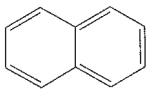
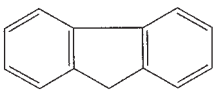
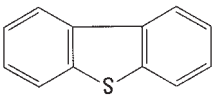
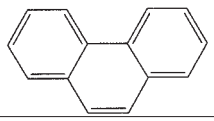
Table 1 Structure, molecular weight and logPOW of aromatic hydrocarbons in this study.

Generic name	Structure	Molecular weight	logPOW	Reference of logPOW
Hydroxybiphenyl		170.21	3.20	HANSCH *et al.* (1995)
Naphthalene		128.18	3.30	HANSCH *et al.* (1995)
Fluorene		166.22	4.18	HANSCH *et al.* (1995)
Dibenzothiophene		184.26	4.38	HANSCH *et al.* (1995)
Phenanthrene		178.24	4.46	HANSCH *et al.* (1995)

freshwater microalgae (GEYER *et al.,* 1981, GEYER *et al.,* 1984, CALAMARI *et al.,* 1983, WONG *et al.,* 1984, SHIGEOKA *et al.,* 1988, HERMAN *et al.,* 1991) and several aquatic organisms (GALASSI *et al.,* 1988, IKEMOTO *et al.,* 1992, FUKUSHIMA, 1983), there are few reports which have investigated the relationship between EC50 (Effective Concentration of 50%) values in marine microalgae and octanol/water partition coefficient (Pow) of PAHs, particularly, reports which investigate multiple species of marine microalgae under the same test conditions are limited.

The OECD guidelines (OECD, 1984), one of the manuals for standard methods of toxicity tests, recommends that test species of microalgae are freshwater microalgae; *Selenastrum capricornutum, Scenedesmus subspicatus* and *Chlorella vulgaris*. However, test species of marine microalgae, that are necessary to toxicity estimation of chemicals in the marine environment were not selected. Therefore, it is important for establish methods to select suitable species for algal sensitivity testing.

The purpose of this study is to determine the EC50 values of PAHs on eight species of marine microalgae. Moreover, we compared the sensitivity among test microalgae, or the toxicity among test chemicals. The relationship between EC50 values of microalgae and physicochemical properties (logPow or logM.W.) of PAHs are investigated.

MATERIALS AND METHODS

Test organisms

Eight species of marine microalgae were used in the experiments as test organisms. Bacillariophyceae; *Skeletonema costatum* (NIES −324) was obtained from The Microbial Culture Collection, National Institute for Environmental Studies (NIES-Collection), Ministry of the Environment Japan. Prasinophyceae; *Tetraselmis tetrathele*, Haptophyceae; *Pavlova lutheri* were obtained from Marine Ecology Research Institute, Japan. Haptophyceae; Isochrysis galbana, Dinophyceae; *Prorocentrum minimum*, and Euglenophyceae; *Eutreptiella* sp. were obtained from JANUS Co., Japan. Chlorophyceae, *Dunaliella tertiolecta* and Bacillariophyceae, *Chaetoceros calcitrans* were obtained from Kitasato University, Japan.

Test chemicals

The PAHs used in the experiments were dibenzothiophene ($C_6H_4C_6H_4S$), phenanthrene

Table 2. Estimated EC50 values with slope, y intercept and r^2 of linear regression.

	estimated EC50	Slope	y intercept	r^2 Squared
Dibenzothiophene				
S. costatum	0.20	128.2	139.0	0.99
C. calcitrans	0.14	63.9	103.6	0.98
T. tetrathele	0.14	48.2	91.1	0.97
I. galbana	0.14	53.3	95.4	0.89
P. lutheri	0.12	60.7	105.4	0.98
P. minimum	0.16	270.6	267.5	1.00
Eutreptiella sp.	0.06	78.1	147.7	0.95
D. tertiolecta	>0.49	—	—	—
Phenanthrene				
S. costatum	0.15	60.6	99.7	0.93
C. calcitrans	0.34	153.7	122.8	1.00
T. tetrathele	0.29	156.3	133.7	1.00
I. galbana	0.14	62.4	103.5	0.97
P. lutheri	0.09	63.1	115.0	0.97
P. minimum	0.09	105.4	157.9	0.99
Eutreptiella sp.	0.13	107.1	144.7	1.00
D. tertiolecta	>0.46	—	—	—
Naphthalene				
S. costatum	1.83	196.8	−1.8	1.00
C. calcitrans	3.73	114.4	−15.4	0.97
T. tetrathele	5.39	274.3	−150.7	0.97
I. galbana	0.84	102.5	57.8	1.00
P. lutheri	0.66	120.4	71.9	0.96
P. minimum	1.63	330.5	−20.2	0.79
Eutreptiella sp.	1.14	73.5	45.9	0.98
D. tertiolecta	>13.8	—	—	—
Fluorene				
S. costatum	0.17	64.5	100.2	0.99
C. calcitrans	0.34	79.7	87.3	0.98
T. tetrathele	0.67	81.5	64.0	1.00
I. galbana	0.11	50.7	99.2	0.95
P. lutheri	0.08	44.8	100.0	0.98
P. minimum	0.24	104.4	114.6	0.96
Eutreptiella sp.	0.23	109.0	119.9	0.99
D. tertiolecta	1.07	40.4	48.8	0.98
Hydroxybiphenyl				
S. costatum	1.11	119.5	44.5	0.99
C. calcitrans	0.59	108.9	74.7	1.00
T. tetrathele	0.85	160.3	61.5	1.00
I. galbana	1.54	87.5	33.6	0.92
P. lutheri	0.73	66.7	59.3	0.57
P. minimum	1.06	79.1	48.1	0.90
Eutreptiella sp.	1.27	61.0	43.6	0.94
D. tertiolecta	3.61	50.8	21.7	0.94

(unit of EC50s: mg/l)

($C_{14}H_{10}$),naphthalene ($C_{10}H_8$),fluorene ($C_6H_4CH_2C_6H_4$) and hydroxybiphenyl ($C_6H_5C_6H_4OH$). All PAHs tested were purchased from Wako Chemicals, Japan. The chemical structure, molecular weight (M.W.) and logPow of the PAHs tested are shown in Table 1. Relationship between logPow and logM.W. of naphthalene, fluorene and phenanthrene showed strong linear relationships ($\log M.W. = 0.12 \times \log Pow + 1.7$, $r^2 = 0.99$), while the correlations of all PAHs contain hydroxybiphenyl and dibenzothiophene ($\log M.W. = 0.0067 \times \log Pow + 2.0$, $r^2 = 0.43$) were weaker than of naphthalene, fluorene and phenanthrene.

Culture conditions

Each alga was cultured in a 300 ml of Erlenmeyer flask containing 200 ml of f/2 medium (GUILLARD and RYTHER, 1962) in stock culture. In toxicity tests, glass test tubes (25×200mm, 64 ml) containing 30 ml of f/2 medium were used to directly measure *in vivo* fluorescence using a fluorescence meter (Turner designs 10–005R). All growth media were autoclaved at 121℃ for 20 minutes. The algae were cultured at a temperature of 20±1℃, under light intensity of 3500 to 4500 lux (38.9 to 50.0 μmol m^{-2} s^{-1}) and a 14:10 light: dark cycle for the stock cultures and toxicity tests.

Toxicity tests

Firstly, the test concentrations of each PAH were adjusted. The maximum concentration of each PAH was prepared by adding a specific volume to the filtered sterile f/2 medium, shaking in a 1000ml beaker for about 24 hours under dark conditions and filtering using sterile glass fiber filters (Whatman GF/F). Each test concentration was prepared by diluting the maximum concentration medium with filtered sterile f/2 medium.

Secondly, test tubes containing 30ml of the growth medium into which the PAHs were mixed at appropriate concentrations, and 0.6ml of the algal stock solution at exponential growth phase were inoculated into the growth media. The experiments were carried out in triplicate. The growth of the algae was monitored daily by *in vivo* fluorescence using a fluorescence meter (Turner designs 10–005R),

which has been shown to have a strong relation to the Chl *a* concentration (LORENZEN, 1966, STRICKLAND, 1968) and cell numbers (YAMAGUCHI, 1994), and has been used as a measurement of microalgal biomass (LEWIS, 1995). The test period was 4 days.

Thirdly, after each chemical in test medium was extracted using *n*-hexane (Ministry of the Environment, 1998), chemical concentrations were measured by using gas chromatography (Shimadzu GC–14B) coupled with flame-ionization detecter (FID) (Standard methods, 1998).

Data analysis

EC50 values were calculated using modified methods of the OECD guidelines (OECD 1984), as outlined below.

The area under the growth curve of individual test vessels was calculated using the following equation.

$$A = (N_1-N_0)/2\times t_1+(N_1+N_2-2N_0)/ 2\times(t_2-t_1)+\ldots+(N_{n-1}+N_n-2N_0)/ 2\times(t_n-t_{n-1})$$

where;

A = area under the growth curve,

N_0 = *in vivo* fluorescence intensity at t_0 (relative units),

N_1 = *in vivo* fluorescence intensity at t_1 (relative units),

Nn = *in vivo* fluorescence at t_n (relative units),

t_1 = time of first measurement after beginning of test,

t_n = time of nth measurement after beginning of test.

Then, the percent inhibition of the growth area of the mean value for individual test concentration to the mean value for controls in each experiments was calculated using the following equation.

$$Ia=(Ac-At)/Ac\times 100.$$

Where;

Ia = percent inhibition of the growth area for an individual test vessel.

At = mean area of each test concentration,

Ac = mean area of the controls in each experiment.

The *Ia* value was plotted against each test concentration on a semi- logarithmic scale. The growth inhibition rates were calculated by linear regression analysis. EC50 is the concentration showing a $Ia=50\%$.

After calculating the EC50 values, the mean rank of the toxicity of PAH and the algal sensitivity and PAHs toxicity were determined by using the statistical method; Friedman test using SPSS 10.0J for Windows, SPSS Inc.. 95% confidence limits of the linear relationship between logPow of PAHs and log(1/EC50) of all microalgae except for *D. tertiolecta* were calculated using methods of KAWABATA (1995) and SNEDECOR (1963).

RESULTS

Algal sensitivity

The EC50 values of PAHs on marine microalgae are shown in Table 2. The most sensitive species of microalgae varied with the compounds of PAHs. *Eutreptiella* sp. was most sensitivite to dibenzothiophene. *P. lutheri* was most sensitive to phenanthrene, naphthalene and fluorene. *P. minimum* and *P. lutheri* were of equally the most sensitive to phenanthrene. *C. calcitrans* was the most sensitive to hydroxybiphenyl. On the other hand, *D. tertiolecta* had the lowest sensitivity to all PAHs of all the microalgae. EC50 values of dibenzothiophene, phenanthrene and naphthalene for *D. tertiolecta* were not measured, because the inhibition values on the maximum concentrations used in the toxicity testing were less than 50%, and were approximately 30%, 10%, and 40%, respectively. The EC50 value of fluorene for *D. tertiolecta* was approximately six times as high as *P. lutheri*. The EC50 value of hydroxybiphenyl for *D. tertiolecta* was approximately thirteen times as high as *C. calcitrans*.

The mean rank calculated using the Friedman nonparametric test was *P. lutheri* < *Eutreptiella sp.* < *I. galbana* < *P. minimum* < *C. calcitrans* < *S. costatum* < *T. tetrathele* (The value of the chi-square statistic is 12.906, with a significant of 0.045).

Toxicity of PAHs

In this study, EC50 values for dibenzothiophene were between 0.06 and >0.49ppm. EC50 values for phenanthrene were between 0.09 and >0.46ppm. Those for naphthalene were between 0.66 and >13.8ppm, and for fluorene between 0.08 and 1.07ppm and for hydroxybiphenyl between 0.59 and 3.6ppm. The mean rank calculated by the Friedman test was dibenzothiophene< fluorene< phenanthrene< hydroxybiphenyl< naphthalene (The value of the chi-square statistic is 22.540, with a significant of 0.000).

Relationship between the EC50 values (mM/l) and the physical properties of PAHs

The square of the regression coefficients between logPow of naphthalene, fluorene and phenanthrene gave a strong linear relationship with the molecular weight, and the log (1/EC50) values of *S. costatum, T. tetrathele, P. lutheri, I. galbana, P. minimum, Eutreptiella* sp. and *C. calcitrans* were 0.97, 0.99, 0.92, 0.91, 0.99, 0.99 and 0.96, respectively. All values for individual microalga except for *D. tertiolecta* , were more than 0.91 (data not shown in figure). The square of the regression coefficient of all microalgae except for *D. tertiolecta* was approximately 0.78 ($\log(1/EC50) = 1.05 \times \log Pow - 1.56$). The square of regression coefficients between log Pow of all PAHs and log (1/EC50) ranged from 0.57 to 0.95 (Fig. 1). In test microalgae, the square of the regression coefficients of *S. costatum, I. galbana, P. minimum, P. lutheri* and *Eutreptiella* sp. were more than 0.91, while for *C. calcitrans* and *T. tetrathele* were less than 0.61. The square of the regression coefficients between the molecular weight of all PAHs and logEC50, except for *D. tertiolecta* ranged from 0.35 to 0.93 (Fig. 2). The square of the regression coefficients of *S. costatum, I. galbana, P. minimum, P. lutheri* and *Eutreptiella* sp. were less than 0.65, while for *C. calcitrans* and *T. tetrathela* approximated 0.93. The linear equation between all PAHs and microalgae tested, except for *D. tertiolecta* , was $\log(1/EC50) = 0.87 \times \log Pow - 0.76$ ($r^2 = 0.75$). The upper and lower ranges of the 95% confidence limits of the linear equation were more than 1.4.

DISCUSSION

Algal sensitivity

In this experiment, *D. tertiolecta* was most tolerant to the PAHs of the microalgae tested and therefore as a test species *D. tertiolecta* should not be use. On the other hand, the most sensitive microalgae varied with the compounds of PAHs, although *P. lutheri* was the most sensitive species to all the PAHs tested (from the results of Friedman test). From these results, it can be conducted that sensitivity testing should be carried out on several species of microalgae. In similar results regarding algal toxicity, *D. tertiolecta* was more tolerant to PCBs and DDT than diatoms (MOSSER *et al.,* 1972, MENZEL *et al.,* 1970), more tolerant to benzene, toluene and xylene than *S. costatum* (DUNSTAN *et al.,* 1975), and was the most tolerant to five organic solvents in nine marine microalgae tested (Y. OKUMURA, personal communication). VANDERMEULEN (1986) reported that the sensitivity of *P. lutheri* to naphthalene was higher than other microalgae. However, algal sensitivities to oils varied with the type of oils (PULICH *et al.,* 1974, WINTERS *et al.,* 1976). Contrary to our results, there is a report that *D. tertiolecta* is the more sensitive to crude oil than *S. costatum* and *P. minimum* (MORALES-LOO and GOUTX, 1990). One factor by which algal sensitivity varies with components of oil is the variation of oil type, or the components of chemicals in the oil, such as the ratio between the aromatic, paraffinic and asphaltic fractions, or the volatile fractions. PULICH *et al.* (1974) reported that the volatile fractions of oils have variable effects on microalgae. BATE *et al.,* (1985) reported that the different oil treatments have different effects on several microalgae. In this study, the slopes of the linear regressions between the EC50 values and the logPow of naphthalene, fluorene and phenanthrene, show strong linear relationships to all microalgae except for *D. tertiolecta* , and ranging from 0.88 to 1.20, with the intercepts of the linear regression ranging from −0.67 to −2.60. The slopes and the intercepts of the linear regressions varied with the algal species. Some of the linear regressions intersected. So, it is possible that the sensitivity of each microalgae

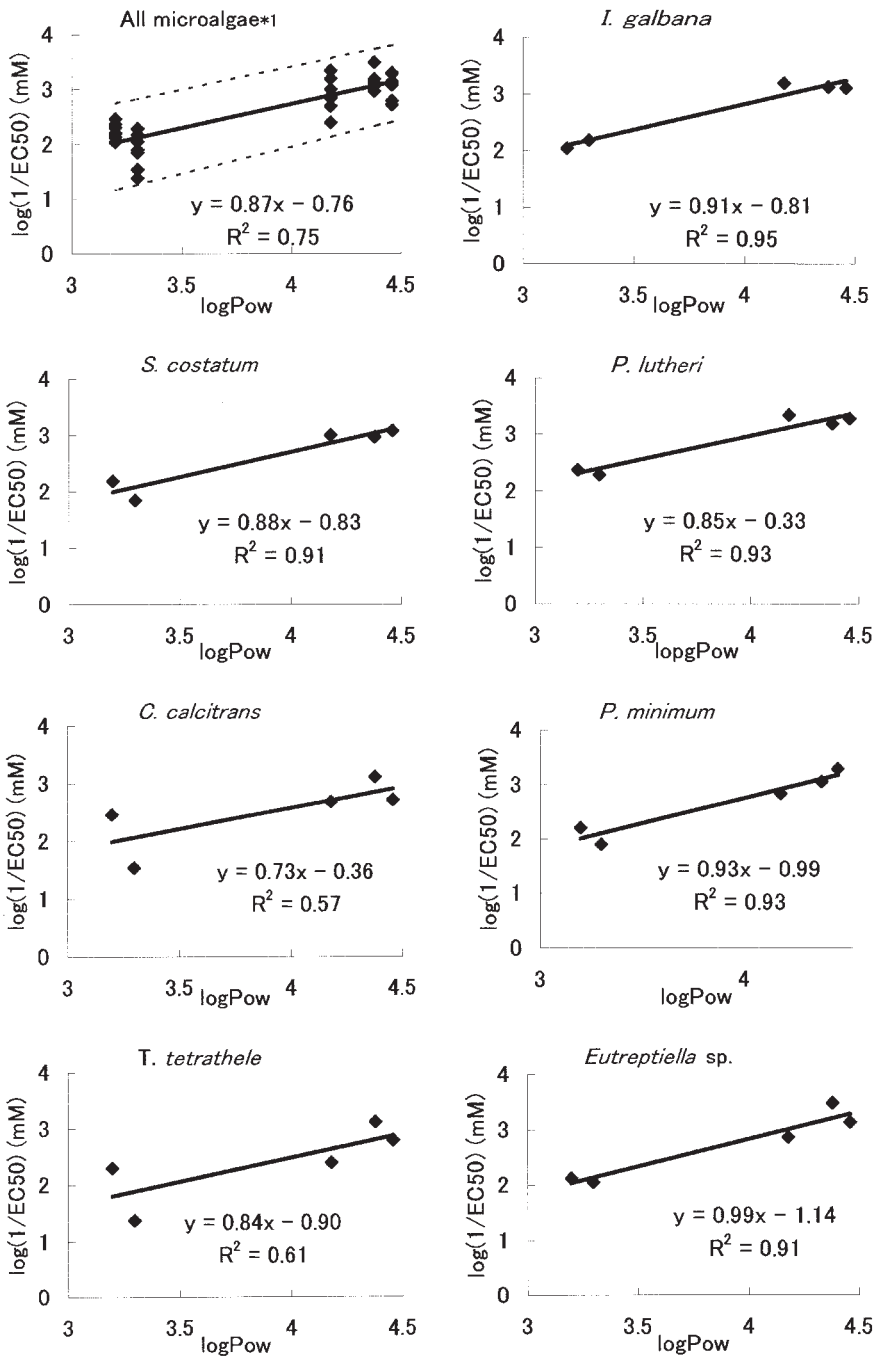


Fig. 1. The linear regression equation relationship between logPow of the five PAHs and log (1/EC50) of the microalgae tested. *1 is the linear equation and upper and lower 95% confidence limits of all microalgae except for *D. tertiolecta* .

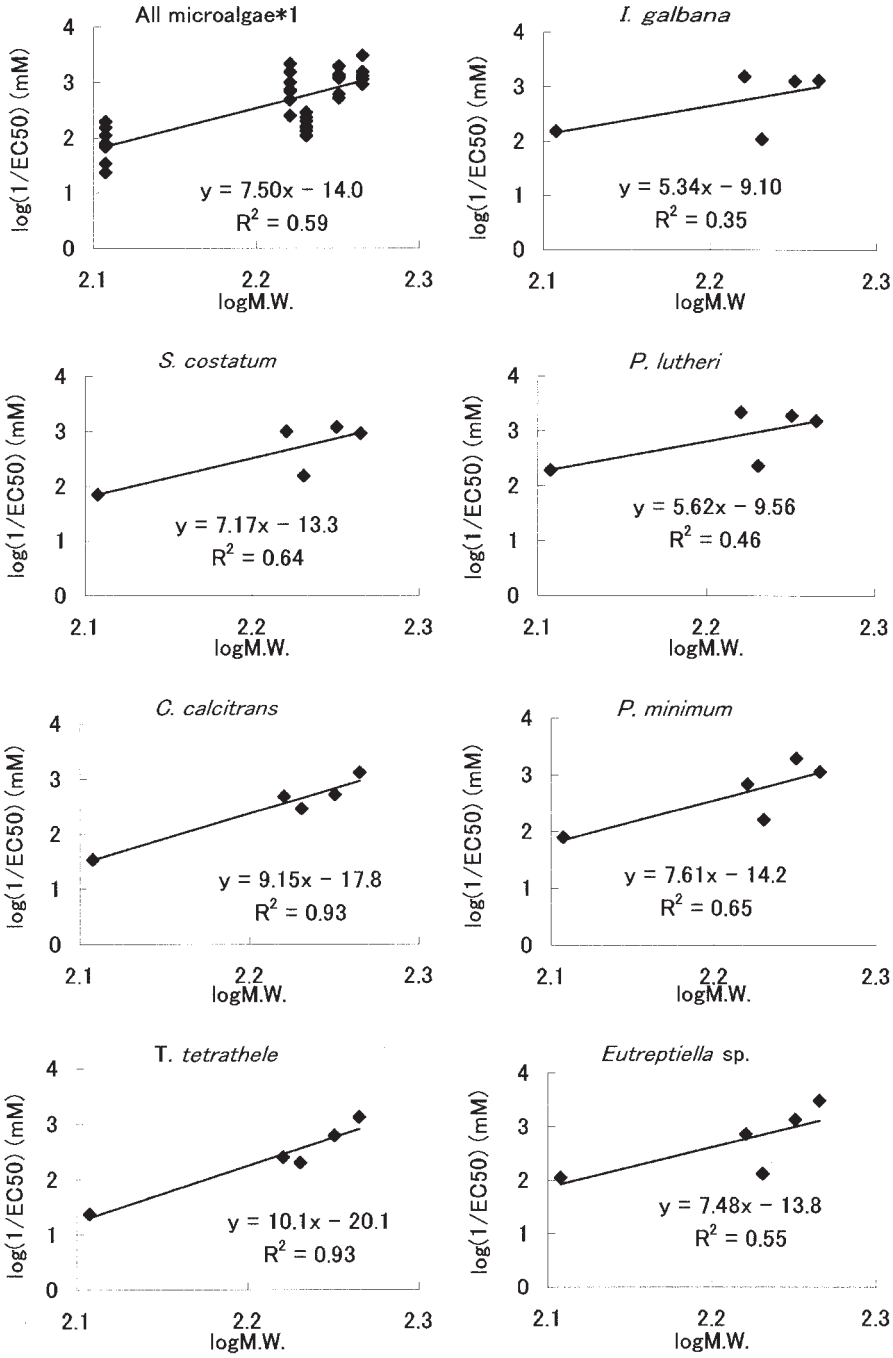


Fig. 2. The linear regression equation relationship between logM.W. of the five PAHs and log (1/EC50) of the microalgae tested. *1 is the linear equation of all microalgae except for *D. tertiolecta*.

Table 3 The relationship between logPow and EC50

chemicals	habitat of test algae	linear regression equation	correlation coefficient r	test periods	unit	reference
polycyclic aromatic hydrocarbons	marine	log (1/EC50) =0.87×logPow−0.76	0.75	96-h	mM/l	This study
clorobenzenes	freshwater	log (1/EC50) =0.92×logPow−1.4	0.97	96-h	mM/l	CALAMARI *et al.* 1983
clorobenzenes	freshwater	log (1/EC50) =0.99×logPow−1.8	0.997	3-h	mM/l	CALAMARI *et al.* 1983
clorobenzenes	freshwater	log (1/EC50) =1.000×logPow−2.676	0.968	4-h	mM/l	WONG *et al.* 1984
chlorophenols	freshwater	log (1/EC50) =0.887×logPow−1.545	0.98	96-h	mM/l	SHIGEOKA *et al.* 1988
chlorophenols	freshwater	log (1/EC50) =0.543×logPow−0.909	0.845	96-h	mM/l	SHIGEOKA *et al.* 1988
volatite aromatic hydrocarbons	freshwater	log (1/EC50) =0.94×logPow−3.59	0.99	12-h	mg/l	HERMAN *et al.* 1991
aromatic hydrocarbons	freshwater	log (1/EC50) =0.91×logPow−4.45	0.9471	72-h	mM/l	GALASSI *et al.* 1988
alcohols, benzenes	freshwater	log (1/EC50) =0.935×logPow−3.341	0.964	120-h	mM/l	IKEMOTO *et al.* 1992

was reversed by logPow of test PAHs. For example, it may be that one microalgae is more sensitive to PAHs of low logPow than the other microalgae, which is less sensitive to PAHs of high logPow than the other microalgae, or that one microalgae is less sensitive to PAHs of low logPow than the other microalgae, is more sensitive to PAHs of high logPow than the other microalgae. Dibenzothiophene and hydroxybiphenyl for which the ratio of logM.W. to logPow was higher than values of naphthalene, fluorene and phenanthrene for some of the microalgae (*C. calcitrans* and *T. tetrathele*) and tended to have a higher toxicity than the other PAHs. The regression coefficients between logM.W. and log (1/EC50) values of these microalgae tended to be higher than of the other microalgae, and the regression coefficients between logPow and log (1/EC50) values of the microalgae. So, it may be that PAHs are the ratio of logM.W. to logPow are higher, varied with algal sensitivity.

Regarding strains of test microalgae, FISHER *et al*. (1973) and MURPHY *et al*. (1980) reported that algal sensitivity, even if within the same species of microalgae, varied with environmental factors. It may be that the strain influences the algal sensitivity.

SHEEDY *et al*. (1991) reported that the 14day-EC50 value of naphthalene on the freshwater algae, *Selenastrum capricornutum* was 25ppm. US-EPA (1980) reported that the 48hour-EC50 value of naphthalene on *Chlorella vulgaris* was 33ppm. In our study, EC50 values of naphthalene were between 0.66 to >13.8ppm. The sensitivity of marine microalgae to naphthalene tended to be higher than the freshwater microalgae.

Comparison of linear regression equations between logPow and EC50

Several reports regarding the linear regression equation between logPow of chemicals and EC50 values of microalgae have been published (Table 3). The slope of the linear equations ranged from 0.54 to 1.00, the intercept ranged from −4.45 to −0.91. In this study, the slope and intercept of all PAHs tested were 0.87 and −0.76, respectively. The slope and intercept of the linear regression equation to naphthalene, fluorene and phenanthrene were 1.05 and −1.56, respectively. Our equations are approximately in agreement with the equations of given in the references in the Table 3. The ranges of the

upper and lower 95% confidence limits of the linear regression equations were more than 1.4, the variation of algal sensitivity was more than twenty-five fold. Although the species of test chemicals and the range of logPow of chemicals were limited, the upper 95% confidence limit of our equation was highest and in the linear regression equations of given in the references in the Table 3, the upper 95% confidence limit of our equation was the most sensitive compared to other values in the literature.

From our equation, for example it is clear that the toxicity of phenanthrene, for which the logPow is 4.46, is higher in toxicity than naphthalene, which has a logPow of 3.3, while the water solubility of phenanthrene is lower than naphthalene. So, we conclude that the PAHs such as hydroxybiphenyl and dibenzothiophene for which the ratio of molecular weight to Pow is high, have a higher toxicity than naphthalene, fluorene and phenanthrene.

References

BATE, G.C. and S.D. CROFFORD (1985): Inhibition of phytoplankton photosynthesis by the WSF of used lubricating oil. Mar. Pollut. Bull., **16**, 401–404.

CALAMARI, D.,S. GALASSI, F. SETTI and M.VIGH (1983): Toxicity of selected chlorobenzenes to aquatic organisms. Chemosphere, **12**, 253–262.

DUNSTAN, W.M., L.P. ATKINSON and J. NATOLI (1975): Stimulation and inhibition of phytoplankton growth by low molecular weight hydrocarbons. Mar, Biol., **31**, 305–310.

EL-DIB, M.A., H.F. Abou-Waly and A.M.H. El-NABY (1997): Impact of fuel oil on the freshwater alga *Selenastrum capricornutum*. Bull. Environ. Contam. Toxicol., **59**, 438–444.

FISHER, N.S., L.B. GRAHAM and E.J. CARPENTER (1973): Geographic differences in phytoplankton sensitivity to PCBs. Nature, **241**, 548–549.

FUKUSHIMA, M. (1983): Evaluation of water pollution by determining chemicals in plankton. Seitai Kagaku, **6**, 4–15, in Japanese.

GEYER, H., R. VISWANATHAN, D. FREITAG and F. KORTE (1981): Relationship between water solubility of organic chemicals and their bioaccumulation by the alga *Chlorella.* Chemosphere, **10**, 1307–1313.

GEYER, H., G. POLITZKI and D. FREITAG (1984): Prediction of ecotoxicological behaviour of chemicals: relationship between n-octanol / water partition coefficient and bioaccumulation of organic chemicals by alga *Chlorella*. Chemosphere, **13**, 269–284.

GALASSI, S., M. MINGAZZINI. L. VIGANO, D. CESAREO and M.L. TOSATO (1988): Approaches to modeling toxic responses of aquatic organisms to aromatic hydrocarbons. Ecotoxicol Environ Safety, **16**, 158–169.

GUILLARD, R.R.L. and J.H. RYTHER (1962): Studies of marine planktonic diatoms. I. Cyclotellanana Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol, **62**, 229–239.

HANSCH, C., A. LEO and D. HOEKMAN (1995): Exploring QSAR -Hydrophobic, Electronic, and Steric Constants. ACS Professional Reference Book, American Chemical Society, Washington, DC, 97–118.

HERMAN, D.C., W. E.INNISS and C.I. MAYFIELD (1990): Impact of volatile aromatic hydrocarbons, alone and in combination, on growth of the freshwater alga *Selenastrum capricornutum*. Aquat. Toxicol., **18**, 87–100.

HERMAN, D.C., W.E. INNISS and C.I. MAYFIELD (1991): Toxicity testing of aromatic hydrocarbons utilizing a measure of their impact on the membrane integrity of the green alga Selenastrum capricornutum. Bull. Environ. Contam. Toxicol., **47**, 874–881.

HERMAN, D.C., C.I. MAYFIELD and W.E. INNISS (1991): The relationship between toxicity and bioconcentration of volatile aromatic hydrocarbons by the alga *Selenastrum capricornutum*. Chemosphere, **22**, 665–676.

HSIAO, S.I.C. (1978): Effects of crude oils on the growth of arctic marine phytoplankton. Environ Pollut., **17**, 93–107.

IKEMOTO, Y. K. MOTOBA T. SUZUKI and M. UCHIDA (1992): Quantitative structure-activity relationships of nonspecific and specific toxicants in several organism species. Environ Toxicol Chem, **11**, 931–939.

KAWABATA, K. (1995): Kaiki to soukan (regression and correlation). *In* Ouyou Toukei Handbook, OKUNO *et al*. (eds) Ouyou Toukei Handbook, Youkendo, Japan, p91–105, in Japanese

KUSK, K.O. (1980): Effects of crude oils and aromatic hydrocarbons on the photosynthesis of three species of *Acrosiphonia* grown in the laboratory. Bot, Mar., **23**, 587–593.

KUSK, K.O. (1981a): Effects of hydrocarbons on respiration, photosynthesis and growth of the diatom *Phaeodactylum tricornutum*. Bot. Mar., **24**, 413–418.

KUSK, K.O. (1981b): Effects of naphthalene on the diatom *Phaeodactylum tricornutum* growth under varied conditions. Bot. Mar., **24**, 485–487.

LEWIS, M.A. (1995): Algae and vascular plant tests. *In* Fundamental of aquatic toxicology. Secnd edition, RAND, G.M. (ed) Fundamental of aquatic

toxicology. Second edition, Taylor and Francis, USA, p144–155

LORENZEN, C.J. (1966): A method for the continuous measurement of *in vivo* chlorophyll concentration. Deep-Sea Res, **13**, 223–227.

MENZEL, D.W. J. ANDERSON and A. RANDTKE (1970): Marine phytoplankton vary in their response to chlorinated hydrocarbons. Science, **167**, 1724–1726.

MINISTRY of the ENVIRONMENT (1998): Benzothiophene, dibenzothiophene. *In* the analytical methods of chemicals. Tokyo, p.176–185.

MORALES-LOO, M.R. and M. GOUTX (1990): Effects of water-soluble fraction of the Mexican crude oil "Isthmus Cactus" on growth, cellular content of chlorophyll *a*, and lipid composition of planktonic microalgae. Mar. Biol., **104**, 503–509.

MOSSER, J.L., N.S. FISHER, T.S. TENG and C.F. WURSTER (1972): Polychlorinated biphenyls: Toxicity to certain phytoplankters. Science, **175**, 191–192.

MURPHY, L.S. and R.A. BELASTOCK (1980): The effect of environmental origin on the response of marine diatoms to chemical stress. Limnol Oceanogr, **25**, 160–165

Organization for Economic Cooperation and Development (1984): Alga growth inhibition test. TG201. *In* OECD Guidelines for Testing of Chemicals. Paris, p. 1–14.

PULICH, W.M., K.WINTERS and C.VAN BAALEN (1974): The effects of a No. 2 fuel oil and two crude oils on the growth and photosynthesis of microalgae. Mar. Biol., **28**, 87–94.

SHEEDY, B.R., J.M. LAZORCHAK, D.J. GRUNWALD Q.H. PICKERING, A. PILLI, D. HALL and R.WEBB (1991): Effects of pollution on freshwater organisms. Res. Jour. WPCF., **63**, 619–696.

SHIGEOKA, T. Y. SATO, Y. TAKEDA, K. YOSHIDA and F. YAMAUCHI (1988): Acute toxicity of chlorophenols to green algae, *Selenastrum capricornutum and Chlorella vulgaris*, and quantitative structure-activity relationships. Environ Toxicol Chem, **7**, 847–854.

SNEDECOR, G.W. (1963): linear regression. *In* Statistical methods. Fifth Edition, HATAMURA, M., T. OKUMO and Y. TSUMURA (translators), Statistical methods. Fifth edition, Iwanami Shoten, Japan, p. 127–131, in Japanese.

Standard methods for the examination of water and wastewater (1998): polynuclear aromatic hydrocarbons. CLESCERI L.S., A.E. GREENBERG and A.D. EATON (eds), p. 6–79–6–84.

STRICKLAND, J.D.H. (1968): Continuous measurement of *in vivo* chlorophyll;a precautionary note. Deep-Sea Res, **15**, 225–227.

U.S. Environmental Protection Agency (1980): Ambient water quality criteria for naphthalene. B–10. EPA440/5–80–059. NTIS/PB81–117707. Washington DC.

VANDERMEULEN, J.H. (1986): Altered grazing patterns in an experimental copepod- alga ecosystem exposed to naphthalene and Kuwait crude oil. Bull. Environ. Contam. Toxicol., **36**, 260–266.

VANDERMEULEN, J.H. and R.W. LEE (1986): Lack of mutagenic activity of crude and refined oils in the unicellular alga *Chlamydomonas reinhardtii*. Bull. Environ. Contam. Toxicol., **36**, 250–253.

WINTERS, K., R. O'DONNELL, J.C. BATTERTON and C. VAN BAALEN (1976): Water-soluble components of four fuel oils: chemical characterization and effects on growth of microalgae. Mar. Biol., **36**, 269–276.

WONG, P.T.S. Y.K. CHAU J.S. RHAMEY and M. DOCKER (1984): Relationship between water solubility of chlorobenzenes and their effects on a freshwater green alga. Chemosphere, **13**, 991–996.

YAMAGUCHI, M. (1994): Physiological ecology of the red tide flagellate *Gymnodinium Nagasakiense* (Dinophyceae) - Mechanism of the red tide occurrence and its prediction -, Bull Nansei Natl Fish Res Inst, **27**, 251–394, in Japanese

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