Bull Environ Contam Toxicol (2011) 86:576–582
DOI 10.1007/s00128-011-0274-3

Estimating Toxic Effect of Copper on Population of Microalgae Through a Three-dimensional Toxic Effect Growth Model

Changyou Wang · Hongli Li · Xiulin Wang · Yong Zhang

Received: 24 March 2010 / Accepted: 12 April 2011 / Published online: 26 April 2011

Abstract A newly proposed three-dimensional model for the effects of heavy metals on the growth of batch cultures of algae that allows the estimation of the no detected toxic effect concentration (NDEC) is presented. Two batch assays with exposure to copper were investigated in situ (ship-based enclosure). As an endpoint in these studies, the carrying capacity B_f, a parameter of the logistic growth model, possesses higher sensitivity and reliability than routine ecotoxicological endpoints. Using B_f as the endpoint, the NDEC from the proposed model is compared to the no observed effect concentration (NOEC), Lowest Observed Effect Concentration (LOEC), the 5% effect concentration (EC_{05}) and no effect concentration (NEC) also calculated from field-derived data. The results show the confidence interval for the NDEC was wholly contained within the corresponding interval between the NOEC and LOEC, as well as within the corresponding much wider confidence interval for EC_{05}. Though the width of the confidence interval for NEC was basically equivalent to the corresponding width for NDEC, the NEC was somewhat higher than the corresponding NDEC. The results indicate that the NDEC is a promising possible alternative parameter to the NOEC.

Keywords Carrying capacity · Endpoint · Copper · Algae

C. Wang (✉) · H. Li
Key Laboratory of Meteorological Disaster of Ministry of Education, NUIST, Nanjing 210044, People's Republic of China
e-mail: chy.w@hotmail.com

C. Wang · H. Li
College of Atmospheric Sciences, Nanjing University of Information Science & Technology, Nanjing 210044, People's Republic of China

X. Wang
College of Chemistry & Chemical Engineering, Ocean University of China, Qingdao 266003, People's Republic of China

Y. Zhang
Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Beijing, People's Republic of China

The effects of chemicals on aquatic biological systems are tested routinely with a set of simple toxicity tests, where sets of organisms, usually originating from a laboratory culture, are exposed to one of several treatments during some standardized period, each treatment being a certain dose level of the test chemical. Current assessments of toxic effects are based on convenient endpoints derived from laboratory data, either by hypothesis tests that determine a 'no observed effect concentration' (NOEC) or regression methods that estimate an 'effect concentration' (EC_x). The NOEC is the highest concentration in a test with a mean response that is not statistically significantly different from the mean response of the control as determined by a post-ANOVA multiple comparison test, such as Dunnett's test. The EC_x is specified by way of a regression model (usually probit or logit) as that concentration affecting a percentage x (usually $x = 50\%$) of a test cohort (Grist et al. 2003).

In the environmental risk assessment of chemicals, the prediction of environmental no-effect concentrations relies heavily on laboratory-derived NOEC values. The perceived advantage of the NOEC is that it is easy to calculate and easy to understand, but its fundamental inadequacy has been shown in a series of papers (Isnard et al. 2001; Kooijman et al. 1996; Hoeven 2004). Apart from the obvious objection that not proving an effect does not prove the absence of an effect, this method has as its most serious

drawback that the NOEC increases if the experiment is performed in a sloppy manner, or with fewer replicates (Hoeven 1998). In addition, because the NOEC can only take the values of a tested concentration, there is no way of producing confidence limits for it. These shortcomings have led many ecotoxicologists to advocate gradual replacement of the NOEC with a regression approach. The current practice is to derive predicted environmental no effect concentrations (PNECs) from EC_{50} data using a fixed application factor scheme. Because the factors are usually selected arbitrarily, some scientists have proposed EC_{05}, EC_{10}, EC_{25} or other 'small' effect values. Such an approach is controversial to apply to risk assessment, because of a lack of consensus about the precise definition of 'small'. Furthermore, the smaller the effect size in descriptive models, the larger the confidence interval and the more the estimate of EC_x becomes dependent on the specific model that has been used to describe the results (Isnard et al. 2001; Kooijman et al. 1996). The method of estimating an EC_x value does not completely overcome the problems associated with hypothesis testing.

In addition, extrapolation from the laboratory to the field is often made in the environmental risk assessment of chemicals. In the natural environment, states and survivorships of organisms are usually different from those in the laboratory, and chemicals are often only partly bioavailable. The translation from the observations in laboratory experiments to environmental standards is fraught with variability and uncertainty. In these circumstances, it is recognized that, although they do not have strong scientific validity, empirically derived assessment factors must be used. In order to avoid the problems when extrapolating the results of laboratory experiments to the field, it is a promising practice to perform in situ experiments, especially algal growth inhibition tests, to study the effects of chemicals on ecosystems (Girling et al. 2000).

In this paper we present and apply a statistical analysis of in situ incubation testing of algae to demonstrate a three-dimensional model (the variables include time, concentration and biomass) approach that can be applied to derive the no detected effect concentration (NDEC) with a percentile confidence interval. The NDEC is a precise parameter that can be used as an alternative to the NOEC. The bootstrap resampling regression is used to generate a percentile confidence interval for quantifying the uncertainty associated with such an estimate. The computer intensive method exploits empirical variation present in the sample data, on the basis that the sample distribution is representative of the (usually unknown) distribution from where it came.

After exposure of chemicals, the "effect" is measured, most typically the exponential growth rate or biomass inhibition at 72 h. However, the exponential growth rate or 72-h biomass inhibition only represents the toxic effect at a certain time, and cannot reflect the ecotoxicological effect over the entire growth period. Therefore, neither is ideal as an endpoint in the natural environment. As already observed, the toxic effects on the tested organisms vary usually in their different growth stages. The toxic effects of individuals in a population are also not always consistent with each other in their growth stages. Based on insights from the logistic growth model, the parameter of the logistic growth model, the carrying capacity (B_f), is used as a time-independent endpoint in ecotoxicological studies, which can integrate responses over time and overcome the limitations of an endpoint selected at a single point in time (Grist et al. 2006).

Materials and Methods

In situ incubation tests were carried out during the winter of 2005 in Jiaozhou Bay, a shallow embayment off the Yellow Sea, China, and during the spring of 2006 in the East China Sea. Inocula for the experiments were collected from surface seawater in the field (36°9′ N, 120°15′ E (Jiaozhou Bay), 29°31′ N, 122°37′ E (East China Sea)). The applied chemicals ($CuCl_2$, NaH_2PO_4, KNO_3 and HNO_3) were guaranteed reagents and were purchased from BASF Electronic Materials Co., Ltd. (Shanghai, China). Transparent cylindrical 5-liter polyethylene bottles were used as culture containers, and were pretreated with ethanol before incubation. A ship-based enclosure, made of white waterproof canvas and sustained by metal frame, was used to keep the culture fluid at the same temperature as the natural seawater.

Surface seawater was collected using polyethylene buckets, prescreened through a 200-μm mesh net to remove major zooplankton, pooled in a 120-liter polyethylene tank and manipulated by the addition of nitrate and phosphate corresponding to levels of 32 and 2 μmol/L, respectively, and then poured into six 15-liter polyethylene buckets (pretreated with ethanol). A copper chloride solution was added to six 15-liter polyethylene buckets at nominal levels of 1, 2, 4, 8, 12 and 16 μg/L during the winter of 2005 in Jiaozhou Bay, and to five 15-liter buckets at levels of 2, 4, 8, 12 and 16 μg/L during the spring of 2006 in the East China Sea. The Cu concentration in each culture was measured analytically. Control cultures without the addition of copper were also included. All water samples to be used for Cu analyses were filtered through Nuclepore ME membrane filters (0.45 μm, constant weight) and stored with a procedure described in another paper (Wang and Wang 2007). After the addition of Cu, the resulting solution in each 15-liter polyethylene bucket was distributed evenly into three 5-liter polyethylene bottles used as

replicates. Subsequently these culture bottles were put in an enclosure and rocked manually two times in the morning and two times in the evening in order to provide sufficient dissolved gas and to prevent algae from congregating. Seawater was poured into the enclosure continuously in order to keep the temperature of the culture fluid close to that of natural seawater. Natural light was provided for algal growth. During a two-week period, 25-mL waters samples were taken every day from the culture bottles, poured into 30-mL polyethylene flasks and fixed for storage in the dark by the addition of Lugol's solution. At the end of the experiment, water samples to be used for Cu analyses were collected from each culture bottle. Algae species composition and cell number were determined by light microscopy (XLE-2, 3DFAMILY Technology Co., Ltd., Nanjing, China) with measurements being repeated 3 times per replicate.

The Cu concentration in each culture was determined using a graphite furnace atomic absorption spectrometer (GF-AAS, Solaar M6, Thermo Elemental, USA). Dissolved Cu was enriched by an extraction procedure involving the adjustment of the seawater pH, addition of the chelating reagent and organic solvent, drainage and evaporation steps (Grasshoff et al. 1999). A recovery of ≥98% was observed with two-fold extraction from spiking experiments. The coefficient of variation of Cu concentrations was less than 10%. The detection limits have been estimated as <0.05 nmol/L Cu. The concentration measurement of Cu in the reference material (Estuarine Water Reference Material for Trace Metals (SLEW-3) of the National Research Council of Canada) was also performed to check the accuracy of the sample analysis. Analytical replication was used for every analysis; the same reference material was used after every tenth analysis and during the whole period. The Cu concentrations measured for water samples were shown in Table 1.

Table 1 Cu concentrations measured for water samples collected at the beginning and the end of the experiment (μg/L)

Jiaozhou Bay			East China Sea		
Nominal	Beginning	End	Nominal	Beginning	End
0	1.8 ± 0.1	1.0 ± 0.1	0	1.6 ± 0.1	0.8 ± 0.1
1	2.7 ± 0.2	2.1 ± 0.2	1[a]		
2	3.9 ± 0.3	2.9 ± 0.2	2	3.7 ± 0.3	2.9 ± 0.2
4	5.9 ± 0.4	5.1 ± 0.4	4	5.7 ± 0.4	4.8 ± 0.4
8	9.3 ± 0.7	8.5 ± 0.8	8	9.8 ± 0.6	8.2 ± 0.7
12	13.6 ± 1.3	12.8 ± 1.1	12	14.1 ± 0.9	14.8 ± 1.2
16	17.1 ± 1.2	17.2 ± 1.5	16	16.5 ± 1.5	16.1 ± 1.3

The test values are mean ± SE (n = 12 for each treatment). Nominal level 0 is the control

[a] The 1 μg/L treatment was deletede in order to reduce workload in the second study in the East China Sea because neither the 1 μg/L nor 2 μg/L treatments inhibited the growth of algae in the Jiaozhou Bay

Heavy metal interactions with aquatic organisms generally involve the following steps: (1) advection or diffusion of the heavy metal in the bulk solution to the cell membrane surface; (2) sorption/surface complexation of the heavy metal at binding sites on the cell membrane surface; and (3) uptake (transport) of the heavy metal through the cell membrane into the organism. Therefore, a heavy metal must first interact with, or traverse, the cell membrane surface to elicit a biological response. The cell membrane is the primary receptor site for metal interactions with aquatic organisms. The interaction of a free surface receptor site on the cell membrane (i.e., the biotic ligand) with the heavy metal can be described as a surface complexation reaction (Brown and Markich 2000).

As can be assumed, minute algal cells with abundant surface receptor sites (i.e., the biotic ligands), bind with the heavy metal to form the surface complexes, and are almost instantaneously in equilibrium with the environmental concentration of heavy metal (Morel 1983; Gin et al. 2002). Consider n heavy metal ions (M) reacting with a biotic ligand (L) (charges omitted for brevity) (Christensen and Nyholm 1984) (Fig. 1):

$$nM + L = L - M_n \quad (1)$$

Equilibrium equation for the binding of M to L sites can be written as (conditional) stability constant expressions of the form (Schamphelaere and Janssen 2002):

$$K = \frac{[L - M_n]}{[M]^n[L]} \quad (2)$$

where K is the stability constant for M binding to L sites, [M] the M concentration, [L] the concentration of unoccupied L sites, $[L - M_n]$ the concentration of M bound to the L. K will be incorporated in a model parameter which is determined by model regression, so ignoring corrections

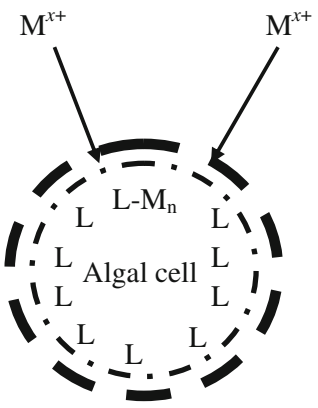

Fig. 1 Schematic diagram of the heavy metal (M^{x+}) reactivity with the biotic ligands (L). The *thick dashed line* is cell wall; the *thin dashed line* cell membrane; the *dots* biotic ligands

for K have no influence on the model outcome. For simplicity, ionic strength corrections of K that describe M binding to L are not carried out. Incorporation of both the complexation reaction and the stability constants of the equilibrium equation lead to the establishment of the following toxic effect model.

The main phenomenon of algal growth inhibition is that cell division is blocked; namely, the number of daughter cells decreases. In ecology, the change in population over time can be quantified as the change in the number of individuals in a population using "per unit number and per unit time" for measurement, which is termed population relative growth rate, of which the maximum is named for the more specific demographic term population intrinsic growth rate R (Li 1991). As for algal growth, the meaning of R is essentially the number of cell divisions completed per cell and per time unit and represents the capacity for cell division. Thus, a change in R can denote the toxic effect of heavy metal on algal growth.

In in situ algal growth inhibition tests, with an increase in heavy metal concentration from the background to c_s (defined below), $[L - M_n]$ increases from the background level to $[L - M_n]_s$. The population intrinsic growth rate at this environmental concentration of heavy metal (R_s) does not show a significant difference from that at the background concentration (R_c). The no detected toxic concentration (c_s) is defined as the highest concentration that does not result in a statistically significant deviation of the population from its natural state with the background, which indicates the two populations being compared are not different in a statistical test.

With an increase in heavy metal concentration from c_s to $c_s + dc$, $[L - M_n]$ increases from $[L - M_n]_s$ to $[L - M_n]_s + d[L - M_n]$, and the algal population intrinsic growth rate decreases from R to R–dR. The probability that cell division will be blocked because of an increase in toxicant concentration from c to $c_s + dc$ can, therefore, be written as –dR/R. The basic assumption is now that this probability is proportional to $[L - M_n]^{\alpha}$ (the concentration of blocked receptors $[L - M_n]$ to the power of α) and the increase in the concentration of blocked receptors $d[L - M_n]$:

$$-dR/R = k[L - M_n]^{\alpha} d[L - M_n] \quad (3)$$

where k is a constant. After transformation, Eq. 2 can be expressed in the form:

$$[L - M_n] = K[M]^{n}[L] \quad (4)$$

Combining Eqs.3 and 4, after a simple rearrangement and integration, yields

$$-dR/R = k(K[L])^{\alpha+1}(\alpha + 1)^{-1} d[M]^{n(\alpha+1)} \quad (5)$$

The total number of biotic ligands is the sum of the metal-bound ligands and the unbound ones. For a large number of biotic ligands on the algal cell surface, the number of the metal-bound ligands is usually very small in comparison with that of the unbound, so that the total number of biotic ligands is approximately equal to the metal-unbound ones ([L]). Therefore, [L] may be considered constant. Making $a = k(K[L])^{\alpha+1}/(\alpha + 1)$, $b = n(\alpha + 1)$, $c = [M]$, Eq. 5 can be written as:

$$-dR/R = adc^{b} \quad (6)$$

Integration of Eq. 5 yields the "no detected effect concentration" (i.e., NDEC) model:

$$R = R_c e^{-a\left(c^b - c_s^b\right)} \quad (7)$$

This parameter R can be readily computed by numerical algal growth models. Then in tandem with a suitable regression model, R serves as an endpoint to chart the effect of a set of treatments on the natural increase of a population and to estimate the parameter c_s by a non-linear simulation procedure (e.g., this can be made by using the simulating tool in the software of OriginPro 7.5). The uncertainty associated with an estimate for R under a specified treatment may be calculated by using a resampling method to generate a bootstrap confidence interval. But the 'best fit' curve obtained by regression to a set of such point R estimates (derived for different treatments) is subject to the combined uncertainty of all these estimates. Such a curve will be one out of many "best fit" curves that might have been obtained, depending on what (uncertain) values are assigned to each of the treatment estimates in the regression. This leaves an open question as to the accuracy of a toxicity estimate because only those "best fit" R values are used to estimate the parameter c_s.

In order to avoid the open question from 'two-step' simulations, the NDEC model is inserted into the algal growth model to estimate c_s directly by a three-dimensional non-linear simulated method (e.g., Matlab 7).

Because of better fitting and parameters with specific biological significance, the logistic growth model has been widely applied to estimate population growth of bacteria, algae, animals and even human beings as long as the growth curve assumes a sigmoidal shape, especially suitable for describing slow growth in early phase and limited resources (Schanz and Zahler 1981; Wang et al. 2002). The algal incubation density and nutrient concentrations in in situ tests are low, resulting in slow growth of algal populations. Therefore, the logistic growth model is adequate and selected to describe in situ algal growth. The expression of the logistic growth model is

$$B_t = \frac{B_f}{(1 + \frac{B_f - B_0}{B_0} e^{-R \cdot t})} \quad (8)$$

where B_f is the carrying capacity of algae, B_0 the initial algal biomass, t the culture time and B_t the biomass at

time t. Because $R/B_f = D$ (a constant) (Li 1991), we have the following expression of the NDEC model:

$$B_f = B_f^c e^{-a\left(c^b - c_s^b\right)} \quad (9)$$

where B_f^c is the algal carrying capacity under natural conditions.

Inserting expressions 6 and 8 into 7, we then obtain the algal growth model under heavy metal contamination, which is designated as the toxic effect growth model:

$$B_t = \frac{B_f^c e^{-a(c^b - c_s^b)}}{\left(1 + \frac{B_f^c e^{-a(c^b - c_s^b)} - B_0}{B_0} e^{R_c e^{-a(c^b - c_s^b)} \cdot t}\right)} \quad (10)$$

By this model, we can estimate c_s directly with the measured data (c, t, B_t) in the experiment. All the parameters of the model are fitted by non-linear regression using Marquardt's algorithm.

Confidence intervals of c_s are estimated using a bootstrap resampling technique. The procedure is based on random resampling of the original data sets (c, t, B_t) using replicates of each treatment. This is done by placing each original data set (c, t, B_t) of p replicates into a pool from which p sets are randomly selected with replacement. As a consequence, any effect could have been chosen more than once, or not at all in the bootstrap replicate. For each combination of the original data, the bootstrap sample value of c_s is calculated. The above procedure is done n times and yields n c_s values. The average of c_s is calculated to be the NDEC and its confidence interval from the resulting frequency distribution of pseudo-values (the 2.5th and 97.5th percentile values) is estimated. In this procedure, when the bootstrap sample value of c_s is estimated by regression using our model, the corresponding B_f is also worked out, and then the Dunnett's test is performed to check if the B_f under the c_s is significantly different from those under the control. If that is the case, the c_s will not be used to calculate the average. This bootstrap confidence interval, compared with asymptotic one, is anti-conservative, but it is representative of the experimental data (Isnard et al. 2001).

Results and Discussion

The algae species in the initial culture fluid from Jiaozhou Bay included *Skeletonema costatum*, *Nitzschia pungens*, *Coscinodiscus asteromp halus*, *Guinardia flaccida*, *Chaetoceros debilis*, and *Leptocylindrus danicus* as well as other species, and those from the East China Sea included *Prorocentrum donghaiense* Lu, *Karenia mikimotoi* and *Eutreptiella gymastica* as well as others. The algal density from Jiaozhou Bay was 0.5×10^6 cell/L, and that from the East China Sea was 1.95×10^6 cell/L. In Jiaozhou Bay, the dominant algal taxa were *Skeletonema costatum*, and in the East China Sea it was *Prorocentrum donghaiense* Lu; their percentages were as much as 96% at the beginning and always exceeded 95% in the course of culturing. So only the dominant species was used to estimate NDEC values.

As shown in Table 2, the NDEC value at each site (for our data set) was somewhat higher than the corresponding NOEC. In particular, the confidence interval for the NDEC was wholly contained within the corresponding interval between the NOEC and LOEC (Lowest Observed Effect Concentration). This result implies that our experimental design was appropriate regarding concentration gradient and the spacing around the given NDEC to be estimated, and that it was sufficient for dose–response experiments. The NDEC is clearly different from the corresponding EC_{05}; the EC_{05} is not included in the confidence interval of

Table 2 Statistics of toxic effect of Cu for various end points (μg/L)

Algal Species	Endpoints	NDEC (c_s)			NOEC		LOEC		EC_{05}			NEC		
		Mean	CI_{95}	R^2	Value	p	Value	p	Value	CI_{95}	R^2	Mean	CI_{95}	R^2
Skeletonema costatum	B_f	4.2	3.9–4.5	0.97	3.9	0.71	5.9	0.71	4.8	3.8–5.8	0.99			
	$B_{72\ h}$				5.9	0.14	9.3	0.14	4.4	3.0–5.7	0.96			
	μ				5.9	0.1	9.3	0.1	7.3	6.7–7.9	0.99	9.6	9.0–9.9	–
Prorocentrum donghaiense Lu	B_f	4.1	3.7–4.4	0.97	3.7	0.66	5.7	0.66	3.2	1.8–4.6	0.99			
	$B_{72\ h}$				3.7	0.38	5.7	0.38	2.5	−0.5–5.4	0.95			
	μ				5.7	0.19	9.8	0.19	3.9	1.0–6.8	0.97	5.5	5.0–5.7	–

The NDEC was determined by nonlinear regression using the toxic growth effect model (Eq. 10) presented in this paper. A 95% confidence interval (CI_{95}) for NDEC was computed by bootstrapping. A hypothesis test approach based on Dunnett's test was used in determining the NOEC and LOEC. The EC_{05} with an asymptotic CI_{95} was determined by nonlinear regression using a log-logistic model (Pascal et al. 2000). The NEC with CI_{95} was calculated using the method chosen in the DEBtox model (experiment: population growth; model: growth) through the software tool DEBtox (downloaded freely from: http://www.bio.vu.nl/thb/deb/deblab/). B_f is the carrying capacity of algae, $B_{72\ h}$ the 72-h biomass of algae and μ the exponential growth rate of algae

the NDEC. However, the confidence interval for the NDEC is wholly contained within the corresponding much wider confidence interval for EC_{05}. An interesting phenomenon may be observed: the ratio of EC_{05} to NDEC for *Skeletonema costatum* is much higher than that for *Prorocentrum donghaiense* Lu. In other words, the EC_{05} is over the corresponding upper boundary of the NDEC for *Skeletonema costatum* and below the lower one for *Prorocentrum donghaiense* Lu. The NEC developed by Kooijman (Kooijman et al. 1996), another alternative parameters to the NOEC or EC_x, was somewhat higher than the corresponding NDEC (for our data set). However, the width of confidence interval for NEC was basically equivalent to the corresponding width for NDEC. In addition, NOECs from different endpoints were found to be different and more conservative with the B_f as an endpoint. It is the same with the EC_{05}s.

The method proposed in this paper shows the presented model can be used successfully to describe the result of algae inhibition growth tests. They do not have more parameters than the routine analysis associated with the logistic growth model or the log-logistic dose–response model, which relate the population intrinsic growth rate to the heavy metal concentration.

This method avoids the complexities inherent to the NOEC and EC_x. As already observed, NOEC depends on the choice of test concentration, and there is no way of producing confidence limits for it. A series of papers have also been published showing the inadequacy of the EC_{05}, and scientists have noted that the smaller the effect size, the larger the confidence interval (Isnard et al. 2001; Kooijman et al. 1996; Hoeven 2004). Moreover, the estimates of low effects (e.g., EC_{05}) are found to be model dependent (Moore and Caux 1997). The statistical summary parameter NDEC is a real parameter with a confidence interval, not just one of the tested concentrations, and does not suffer from the statistical problems of the NOEC. It is the accurate highest concentration that does not significantly differ from the control, and does not suffer from the problems in defining the 'x' for the EC_x, or the large confidence interval issue for a small value of x. Other alternative parameters to the NOEC or EC_x have been suggested, such as NEC, developed by Kooijman, based on the Dynamic Energy Budget (DEB) model. However, for our data set, the NEC was somewhat higher than the corresponding NDEC. This probably resulted from the application of different algal growth models, the exponential growth model for NED (Kooijman et al. 1996) and logistic growth model for NDEC. The logistic growth model is better for in situ algal growth due to slow growth in early phase and limited resources (Schanz and Zahler 1981; Wang et al. 2002). The DEB model, which provides a mechanistic basis, directly gives a no-effect concentration, but it seems too complex to be used routinely in many ecotoxicological laboratories, and probably still needs extensive validation studies (Isnard et al. 2001; Kooijman and Bedaux 1996). The derivation of the NDEC does not require the assumption of the specific biotic ligand for different heavy metals (Niyogi and Wood 2004) or a specific process-based model such as the DEB model (Kooijman et al. 1996). Further, the NDEC model does not require the identification of the specific mode of action between the heavy metals and ligands, which is practical because routine toxicity tests are not very suitable for this purpose.

An additional advantage of this method is the application of the parameter of the logistic growth model—the carrying capacity (B_f)—as the endpoint in the studies of ecotoxicology. As already observed, the tested organisms are usually of different adaptability in their different growth stages. The sensitivity and reliability of biological endpoints are enhanced with the increase of their represented growth time (Zhu et al. 2006). Microalgae growth is characterized by a sigmoid or logistic function (Cid et al. 1995), usually including three stages: the initial, exponential and stable growth stage. The 72-h biomass (B_{72h}), a routine algal endpoint, is not related to the sequential growth stages and varies with initial density. The exponential growth rate (μ), another routine algal endpoint, only represents the exponential growth stage. However, B_f models the entire algal growth period, and represents the ultimate biomass limit in the environment. It is determined jointly by the environmental resources and limiting factors. Therefore, B_f is a parameter that reflects the ecotoxicological effect over the entire growth process. Furthermore, B_f is not related to the initial state according to the analysis of the logistic growth model. Results from another experiment also showed that B_f is basically uniform in the same culture conditions, and is not affected by the algal incubation density or physiological state (Zhang et al. 2005). Based on the analysis above, B_f possessed higher sensitivity and reliability than the routine ecotoxicological endpoints. As can be seen from Table 2, using the B_f as an endpoint instead of B_{72h} or μ, the NOEC values were more conservative and the test power p values were higher, and the EC_{05} was more protective and its confidence interval estimate was narrower. Such an endpoint is ecologically significant for evaluation of the consequences of contaminants/effluents in the environment. It is important to be compatible between the model for risk assessment and for scientific purposes.

In principle, the model parameter endpoint can be extended to the other population growth model as long as the parameter is of specific ecological significance and importance. Similar approaches to those described in this paper may be adopted for other chemicals only if the

biological elicited responses are caused mainly by an interaction between the chemicals and receptor sites on the cell membrane. However, there are undoubtedly some practical constraints. These include difficulties in distinguishing hormesis resulting from exposure to low concentrations of some chemicals from raw data variance for which the developed model is inappropriate, and in obtaining adequate growth model parameters with specific ecological significance from which meaningful toxicological endpoints may be chosen. Nevertheless the benefits seem sufficiently important to justify efforts to address these deficiencies.

A new three-dimensional model approach was proposed and applied to estimate a no detected effect concentration (NDEC) with a percentile confidence interval, which proved to be a precise alternative parameter to the NOEC. The carrying capacity, a parameter of the logistic growth model, was used as an endpoint in the in situ studies of ecotoxicology, and proved to possess high sensitivity and reliability. The NDEC of Cu calculated for *Skeletonema costatum* was 4.2 μg/L, and that for *Prorocentrum donghaiense* Lu was 4.1 μg/L.

Acknowledgments This study was supported by the Sino-UK International Cooperation Project of Science and Technology (Project No. 2004DFA03600), by the Science Research Foundation of Nanjing University of Information Science & Technology, China, and by the National Natural Science Foundation of China (Project No. 41006040).

References

Brown PL, Markich SJ (2000) Evaluation of the free ion activity model of metal-organism interaction: extension of the conceptual model. Aquat Toxicol 51:177–194

Christensen ER, Nyholm N (1984) Ecotoxicological assays with algae: Weibull dose-response curves. Environ Sci Technol 18:713–718

Cid A, Herrero C, Torres E, Abalde J (1995) Copper toxicity on the marine microalga *Phaeodactylum tricornutum*: effects on photosynthesis and related parameters. Aquat Toxicol 31:165–174

Gin KYH, Tang YZ, Aziz MA (2002) Derivation and application of a new model for heavy metal biosorption by algae. Water Res 36:1313–1323

Girling AE, Pascoe D, Janssen CR, Peither A, Wenzel A, Schafer H, Neumeier BA, Mitchell GC, Taylor EJ, Maund SJ, Lay JP, Juttner AI, Crossland NO, Stephenson RR, Persoone G (2000) Development of methods for evaluating toxicity to freshwater ecosystems. Ecotox Environ Safe 45:148–176

Grasshoff K, Ehrhardt M, Kremling K (1999) Methods of seawater analysis, 3rd edn. Weinheim Press, Berlin

Grist EPM, Crane M, Jones C, Whitehouse P (2003) Estimation of demographic toxicity through the double bootstrap. Water Res 37:618–626

Grist EPM, O'Hagan A, Crane M, Sorokin N, Sims I, Whitehouse P (2006) Bayesian and time-independent species sensitivity distributions for risk assessment of chemicals. Environ Sci Technol 40:395–401

Hoeven NVD (1998) Power analysis for the NOEC: what is the probability of detecting small toxic effects on three different species using the appropriate standardized test protocols? Ecotoxicology 7:355–361

Hoeven NVD (2004) Current issues in statistics and models for ecotoxicological risk assessment. Acta Biotheor 53:201–217

Isnard P, Flammarion P, Roman G, Babut M, Bastien P, Bintein S, Essermeant L, Ferard FJ, Gallotti SS, Saouter E, Saroli M, Thiebaud H, Tomassone R, Vindimian E (2001) Statistical analysis of regulatory ecotoxicity tests. Chemosphere 45:659–669

Kooijman SALM, Bedaux JJM (1996) Analysis of toxicity tests on Daphnia survival and reproduction. Water Res 30:1711–1723

Kooijman SALM, Hanstveit A, Nyholm N (1996) No-effect concentration of algal growth inhabitation tests. Water Res 30:1625–1632

Li SX (1991) Mathematical methods commonly applied in biology. Ocean University of China Press, Qingdao

Moore DRJ, Caux PY (1997) Estimating low toxic effects. Environ Toxicol Chem 16:794–801

Morel F (1983) Principles of Aquatic Chemistry. Wiley, New York

Niyogi S, Wood CM (2004) Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. Environ Sci Technol 38:6177–6192

Schamphelaere KACD, Janssen C (2002) A Biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effects of calcium, magnesium, sodium, potassium, and pH. Environ Sci Technol 36:48–54

Schanz F, Zahler U (1981) Prediction of algal growth in batch cultures. Aquatic Sci—Res Across Boundaries 43:103–113

Wang CY, Wang XL (2007) Spatial distribution of dissolved Pb, Hg, Cd, Cu and As in the Bohai sea. J Environ Sci 19:1061–1066

Wang XL, Zhang L, Han XR, Zhu CJ, Ge M (2002) Effect of nutrient on marine phytoplankton growth—study on mathematic model. Adv Mar Sci 20:96–101

Zhang HQ, Jin JL, Liu B, Gao PJ (2005) Difficulties in estimating microbial growth parameters by using logistic models. Chin J Appl Environ Biol 11:595–599

Zhu YF, Lin X, Xu TC (2006) Analyses of ecotoxicological index in rotifers. Fisheries Sci 25:146–149