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Estimating low-toxic-effect concentrations in closed-system algal toxicity tests

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ABSTRACT

The no-observed-effect concentrations (NOEC) and EC_{10} values for 108 organic compounds were estimated, using multiple endpoints (i.e., biopopulation, growth rate, and dissolved oxygen production), from previous data obtained by a closed-system algal toxicity test (test alga: *Pseudokirchneriella subcapitata*). These low-toxic-effect concentrations are valuable to risk assessment of chemicals and protection of the aquatic environment as such information is quite scarce in existing toxicological databases. Furthermore, based on limited amount of available data, we found that the risk of organic toxicants to phytoplankton may be severely underestimated by existing databases, which are primarily derived by the conventional batch technique. Good correlation relationships between NOEC (or EC_{10}) and EC_{50} values were established. For polar and nonpolar narcotics, quantitative structure–activity relationships (QSARs) based on hydrophobicity, and/or the lowest unoccupied molecular orbital energy (Elumo) were developed with satisfactory predictive powers. The above statistical relationships can be applied to derive a preliminary estimation for the low-toxic-effect levels for other (or new) organic compounds that has no toxicological data available.

1. Introduction

Ecological risk assessments of chemicals are aimed at estimating low, or no toxic effect levels, which may then be used as input for risk assessments, or the development of environmental quality criteria and guidelines for risk management purposes. The no-observed-effect concentration (NOEC) is a traditional parameter adopted by risk assessment procedures. NOEC is derived by hypothesis testing in which treatment responses are compared with a control response to test the null hypothesis that they are the same. The determination of NOEC is highly dependent on the test design, e.g., the selection of test concentrations and the number of replicates (Kooijman et al., 1996). In the past decade, the relevance and utility of the NOEC has been seriously criticized (Chapman et al., 1996, 1998; Chapman and Chapman, 1997; Moore and Caux, 1997). Previous studies pointed out that, NOEC was highly variable and concluded that EC_{50}, and other point estimates (EC_x), are more consistent parameters (Chapman et al., 1996; Chapman and Chapman, 1997). Moore and Caux (1997), based on the analyses of 198 toxicity data sets, found that most NOECs represent 10–30% reductions from control responses, and suggested that the regression-based approach is a better tool than hypothesis testing for estimating low-toxic effects. The Organization for Economic Cooperation and Development Workshop has therefore recommended replacing the NOEC with a regression-based estimation procedure (Chapman, 1997). However, the use of point estimator also suffers from several shortcomings, such as: estimates of low-toxic effects were often model dependent when an extrapolation beyond the toxicity data was required and, confidence intervals can be quite large, at 5% effect and lower (Moore and Caux, 1997). Isnard et al. (2001) showed that EC_5, and the lowest bound of the confidence interval of the EC_{10} were close to the NOEC and concluded that the EC_x approach would lead to no major changes in the risk assessment procedure. Therefore, they questioned the necessity for replacing the traditional hypothesis testing method by the point estimating approach.

Toxicity testing with microalgae has been used extensively in ecotoxicological studies. The traditional batch tests have been applied by most algal toxicity test protocols (OECD, 1984, 2000; ISO, 1987; US EPA, 1996). These tests have been challenged in regard to their applicability for testing volatile organic toxicants (European Centre for Ecotoxicology and Toxicology of Chemicals. 1996), considering their open test environment and the vigorous mixing provided during testing. Several closed-system tests have been proposed by previous researchers (Herman et al., 1990; Galassi and Vighi, 1981; Halling-Sørensen et al., 1996; Mayer et al., 2000). Most of these closed-system tests are considerably more complicated in experimental design, compared to the conventional batch technique. Furthermore, the enriched bicarbonate buffer, as applied by some of the above researchers, may also

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result in increased ionic strength and lower test sensitivity (Lin et al., 2005). Therefore, algal toxicity data derived from closed-system tests are still quite scarce. The author's recent work has proposed a closed-system algal toxicity test technique, with no headspace and with low bicarbonate buffer content (Lin et al., 2005). The experimental design is simple and the test revealed satisfactory sensitivities to both metallic and organic toxicants. The test technique has been successfully applied to assess the toxicity of aldehydes, chlorophenols, anilines, benzenes, alkanes, alcohols, ketones, and nitriles (Tsai and Chen, 2007). In addition, our results showed that, based on EC_{50} values, conventional algal batch tests tend to underestimate the toxicity of organic compounds. Toxicity observed from the closed-system test is approximately 2- to 380-fold higher than that estimated by conventional batch tests (Tsai and Chen, 2007).

In existing toxicity databases, algal toxicity data for low-toxic-effect levels are still not abundant as compared to those based on the median effective concentration. In addition, most of the above data were derived primarily by conventional batch-type tests (open test systems). The objective of this study is to present low-toxic-effect concentrations (in terms of NOEC and EC_{10}) for 108 organic toxicants on *Pseudokirchneriella subcapitata* (green alga), as obtained from our closed-system tests. Furthermore, correlation relationships were established with respect to EC_{50} values, the 1-octanol:water partition coefficient (K_{ow}), and the lowest unoccupied molecular orbital energies (Elumo), to enhance the predictive capability of low-toxic-effect concentrations for other organic toxicants.

2. Materials and methods

In the present study, 108 sets of raw data including aldehydes, nitriles, anilines, chlorophenols, benzenes, alkanes, alcohols, polycyclic aromatic hydrocarbons, and pesticides from the author's previous works (Chen et al., 2006; Yeh and Chen, 2006; Tsai and Chen, 2007) were analyzed for low-toxic-effect concentrations. These toxicants were divided into three categories, i.e., nonpolar narcosis, polar narcosis, and reactive, according to previous studies (Verhaar et al., 1992; Russom et al., 1997; Akers et al., 1999). The test alga is *P. subcapitata*. All chemicals used were of reagent grade. The toxicant concentrations presented in this work are in the form of nominal concentrations. The differences between the nominal concentration and the actual measured concentration were less than 6% (Tsai and Chen, 2007). All tests were conducted in triplicate with test duration of 48 h. Three different endpoints were used to analyze the toxic effects of various organic compounds: dissolved oxygen (DO) production, algal growth rate (GR), and the net production of algal cell density (final cell density−initial cell density, biopopulation). Toxicity tests were conducted using the 300-ml biochemical oxygen demand (BOD) test bottles, with no headspace left. A water seal was provided to ensure a closed-test environment. More detailed description regarding the test technique can be found in the author's previous work (Lin et al., 2005).

One-tail Dunnett's procedure was applied for the estimation of NOEC and LOEC values at 5% level of significance. NOEC was defined as the toxicant concentration which caused no significant difference compared to the test controls, with respect to all test endpoints (i.e., DO production, growth rate, and biopopulation). The studentized range (*SI*) can be calculated as follows:

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

where *Xc* and *Xi* are mean observations from controls and treatments, respectively. *Sw* is the square root of the within-group-variance and, *nc* and *ni* are the numbers of replicates for the control and treatment. A specific treatment is considered to be significantly different from the controls if the corresponding *SI* value is greater than the critical value (*T*) specified by the Dunnett's *T* tables.

The EC_{10} value was determined using the best-fit-model approach as described below: Experimental data were fitted into three different dose–response models, i.e., probit, logit, and Weibull. The best-fit model was determined based on the minimum χ^2 values, which calculate the sum of squares of differences between the observation and the model prediction. EC_{10} values were then estimated using the best-fit model. Experimental data were also analyzed by *G* test in order to test the null hypothesis that the fit of the model was adequate (Moore and Caux, 1997). The observed responses were considered as not significantly different from the model estimates if $p > 0.05$. The equation for computing *G* is given by

$$G = 2\sum_{i=1}^{a} f_i \ln\left(\frac{f_i}{\hat{f}_i}\right) \quad (2)$$

where *a* is the number of replicates summed over all treatments, f_i is the observed response for treatment *i*, and $\hat{f}_i$ is the corresponding model estimate. The value of *G* is then compared with the critical value of χ^2 for *a*-*p*-1 degrees of freedom at $\alpha = 0.05$, where *p* is the number of parameters in the model equation.

Regression analyses were performed by using MINITAB (Ver 14.2, MINITAB, State College, PA, USA) to establish prediction models for NOEC and EC_{10}. Leave-one-out cross-validation was carried out to test the significance of each prediction model. The statistical quality was judged by the square of correlation coefficient (r^2), the Fisher criterion (*F*), the root mean square error (*S*), and the cross-validated correlation coefficient (Q^2).

3. Results

Table 1 presents the NOEC, LOEC, and EC_{10} values for 108 organic toxicants. In addition, EC_{50} values, the 1-octanol:water partition coefficient (K_{ow}), the lowest unoccupied molecular orbital energies (Elumo), and literature NOEC values (*P. subcapitata*, *Daphnia magna*, and fathead minnow) were also listed for discussion. The 108 toxicants were divided into three categories, i.e., nonpolar narcotic (NP), polar narcotic (P), and reactive (R), according to each chemical's modes of toxic action. As indicated in Table 1, 36% of the compounds have yielded identical NOEC values for all three test endpoints (i.e., biopopulation, growth rate, and DO production). Also, 58% (63 sets) of data showed that biopopulation and growth rate were equally sensitive in NOEC determination. Overall, biopopulation was found to be the most sensitive endpoint for approximately 80% of the test compounds. The rest of the compounds (ID numbers: 13, 14, 15, 17, 20, 23, 45, 57, 59, 62, 64, 70, 73, 74, 82, 83, 88, 93, 96, 97, and 105), on the other hand, displayed the most severe toxic effects on dissolved oxygen production. Furthermore, regression analyses showed that satisfactory correlation relationships can only be obtained when all data were derived by a single endpoint. Therefore, all NOEC values in Table 1 are based on biopopulation. However, for toxicants exerted stronger toxic effects on DO production, true NOEC values are specified in brackets. Similarly, all EC_{10} values were calculated based on the biopopulation endpoint, using the best-fit model. The percentages of best model fits for the three different models are: Probit 47.2%, Weibull 13.0%, and Logit 39.8%. However, no obvious model preference was found among the above three models.

In Table 1, only 50% of the cases were tested with low-enough concentrations in order to obtain the actual NOECs. The main reason was that these tests were designed to explore the entire concentration–response relationship. Furthermore, our initial focuses were on the response on growth rate (GR) and DO production, instead of biopopulation. In many cases, a NOEC can be determined based on GR, but not for the endpoint of biopopulation.

Table 2 summarizes the ratios between EC_{50}, NOEC, and EC_{10} values. On average, EC_{10} is 1.65 times higher than the NOEC value. Furthermore, the average acute/chronic ratios (ACR) are 5.80 and 4.20, respectively, with respect to NOEC or EC_{10} values. A small fraction of the compounds were excluded from the regression because their ACR^1 (EC_{50}/NOEC) or ACR^2 (EC_{50}/EC_{10}) values are extremely large, as compared to the majority of data. The modes of action and ACR values for these outliers are listed at the bottom of Table 2. Previous studies showed that, with respect to nonpolar narcotic chemicals and algae, ACR is within the range of 3.5–4.5 (Gray and Sova, 1956; McGrath et al., 2004). Furthermore, Roex et al. (2000) concluded that ACRs for polar narcotic compounds and reactive toxicants are approximately 9.8 and