BIOCONCENTRATION OF TETRACHLOROBENZENE IN MARINE ALGAE

WANG Xiu-lin(王修林), MA Yan-jun(马延军), CHENG Gang(程刚)*, 
YU Wei-jun(郁纬军), ZHANG Li-jun(张力军)
(Ocean University of Qingdao, Qingdao 266003)
(Commodity Inspection Administration, Qingdao 266003)
Received July 4, 1996; revision accepted Jan. 10, 1997

Abstract

Bioconcentration of tetrachlorobenzene (TeCB) in Chlorella marine, Nannochloris oculata, Pyramidomonas sp., Platymonas subcordiformis, and Phaeodactylum tricornutum, and toxicity of TeCB to the marine algae were tested. Values of bioconcentration potential parameters, including uptake rate constant $k_1$, elimination rate constant $k_2$ and bioconcentration factor $BCF$, were obtained not only from the time course of TeCB uptake by the marine algae by using a bioconcentration model, but also from the acute toxicity test data for percent inhibition $PI(\%)$ ~ exposure concentration of TeCB-time by using a combined bioconcentration and probability model. The results showed good relationship between $k_{\text{TeCB}}$ and $k_{\text{TeCB-PI}}$, and $k_{\text{TeCB-PI}}$. $BCF_{\text{TeCB}}$ and $BCF_{\text{TeCB-PI}}$, Especially, the values of $BCF_{\text{TeCB-PI}}$ were well consistent with those of $BCF_{\text{TeCB}}$.

Key words: tetrachlorobenzene; phytoplankton; bioconcentration; toxicity

INTRODUCTION

Chlorinated benzenes are widely used in industry and households, and so is of significant environmental concern, because it is often released as waste, contributing to the pollutants in all types of water (Afghan et al., 1979). Although some data on toxicity and bioconcentration of chlorinated benzenes in aquatic organisms were published (Gobas et al., 1987; Lohner et al., 1987; Mailhot, 1987; Kuhn et al., 1990), the bioconcentration potential parameters, including $k_1$, $k_2$ and $BCF$, were not determined well because the methods for determining the parameters were less than accurate (Wang et al., 1996).

The present study aims to obtain more accurate and reliable values for the bioconcentration potential parameters by modelling the time course, instead of steady-state partitioning, for the uptake of hydrophobic organic chemicals (HOCs) in the organism rather than in the water, by taking into consideration growth when the HOC is taken up. This can be done by applying the bioconcentration model of Wang et al. (1996). In addition, a combined bioconcentration and probability model was applied to obtain the bioconcentration potential parameters depending only on acute toxicity test data for percent inhibition $PI(\%)$ ~ exposure concentration of the HOC ~ time.

MATERIALS AND METHODS

Alga cultures

Clonal axenic strains of Chlorella marine, Nannochloris oculata, Pyramidomonas sp.,

* Project 49376271 supported by the NSFC and the study also supported by Natural Science Foundation, Shan-dong Province (No. Q94E0331).

Platymonas subcordiformis, and Phaeodactylum tricornutum Bohlin, obtained from the Microbial Culture Laboratory, Ocean University of Qingdao, P. R. China, were similarly precultured as described by Otsuki et al. (1987).

**Test chemicals**
Tetrachlorobenzene (TeCB, Wako Pure Chemical Industries, Ltd.) was selected to investigate the bioconcentration in, and the toxicity to, the marine algae. Prior to the experiments, a test solution of TeCB was prepared by adding methanol stock to a bottle of double-distilled water.

**Kinetic uptake**
The pre-cultures in a series of 100 ml Erlenmeyer flasks containing 55 ml of sterilized f/2 nutrient medium were incubated at 13±1 °C and 12:12 h LD cycle. When the biomass of the pre-cultures reached the order of 1 g/L, a defined volume of TeCB test solution was added to the pre-cultures at the start of the exposure at a concentration of 17.6 mg/L in methanol carrier(<0.01%), this being below its solubility in water (Miller et al., 1985). Because the concentration exceeded its aqueous solubility, there will clearly be an underestimate of the bioconcentration potential, if only "truly" dissolved HOC can be taken up by the organism (Geyer et al., 1994). Glass stoppers were used to minimize volatilization of TeCB (Lay et al., 1984). Simultaneous pre-culture without addition of TeCB and with methanol (<0.01%) was taken as the control. Batch exposures were conducted at 13±1 °C with 12:12 h LD cycle and the samples were collected at 6, 12, 24, 48 hours. Sub-samples of 5 ml for determination of the algal dried weight were separated into algal and medium phases by filtering through precombusted (450 °C for 4 h) Whatman GF/F glass fiber filter. After the algal phases were dried at 110 °C overnight the dry weight was determined by using an R200D electronic balance (Sartorius, Germany).

Other sub-samples of 50 ml for determination of TeCB concentration in algal phase (C_a) were also separated into algal and medium phases through the GF/F glass fiber filter. The extraction procedures used were similar to the method described in the literature (SOA, 1979). After separation, algal phases were immediately rinsed with 0.45μm filtered seawater, and then homogenized in 1 ml 1:1 perchloric acid: acetic acid of a glass-stoppered 10 ml test tube immersed in a 70 °C water bath. After sufficient homogenization, an appropriate internal standard (such as trichlorobenzene) in methanol—water was added to the homogenates to check the extraction recovery. The sample was extracted twice with 4 ml aliquots of benzene by agitating in a mixer for 10 minutes. The extracts were purified thrice with 1 ml concentrated H_2SO_4, neutralized with 2% NaOH, dried with anhydrous Na_2SO_4 and then stored at −5 °C pending ECD—GC analysis. The concentration of TeCB in the extracts was monitored by a HP 5890 gas chromatograph (Hewlett Packard, USA) equipped with a 63Ni electron—capture detector and 2m, 5mm i.d. 1.95% OV—17 and 1.7% QF1 Chromosorb WAW DMCS columns. Injector temperature was 250 °C, detector temperature was 270 °C, and column temperature was programmed at 5 °C/min from 70 to 100 °C. The injection mode was splitless; injection volume was 1μl. Standards were prepared from pure chemicals. At each time point, duplicate flasks were taken for measurement of the algal dried weight and TeCB concentration in algal phase.

**Acute toxicity test**
The experimental procedures of incubation in acute toxicity tests were the same as those in kinetic uptake experiments, except that the exposure concentration of TeCB