



PII: S0045-6535(97)000369-X

UPTAKE AND TRANSFER OF HIGH PCB CONCENTRATIONS FROM PHYTOPLANKTON TO AQUATIC BIOTA

Juei Shen Wang^{1*}, Hong Nong Chou², Jin-Jia Fan, and Chien-Min Chen³¹ Department of Food Health, Chia Nan College of Pharmacy and Science, Tainan 71710, Taiwan.² Institute of Fisheries Science, National Taiwan University, Taipei, Taiwan.³ Department of Environmental Engineering and Health, Chia Nan College of Pharmacy and Science, Tainan 71710, Taiwan.

(Received in Germany 18 July 1997; accepted 15 August 1997)

ABSTRACT

The uptake and transfer of 2,2',4,4'-tetrachlorobiphenyl (an isomer of polychlorinated biphenyls, PCBs) in the culture of two species of phytoplankton, *Nannochloropsis oculata* and *Isochrysis galbana*, to brine shrimp (*Artemia* sp.) nauplii and hard shell clam (*Meretrix lusoria*) were investigated in a short-term laboratory ecosystem. Three PCB concentrations, 0 ppb, 50 ppb and 500 ppb, were prepared in the algal culture for examining the levels of PCB bioaccumulation and growth effects on these biota. *Artemia* and hard shell clam each when exposed to 500 ppb contaminated *Nannochloropsis* and *Isochrysis* for 4 d could significantly bioaccumulate PCB concentrations up to 318.81 ppm and 22.55 ppm (dry basis), respectively. This may account for the high PCB residues that bioaccumulated in contaminated *Nannochloropsis* (257.52 ppm) and *Isochrysis* (64.22 ppm) through the aquatic food chain. Biomass of PCB treated algae were apparently depressed ($p < 0.05$) relative to that of the control group (0 ppb). Growth conditions (length and larva/mL) of *Artemia* when fed with *Nannochloropsis* under PCB environments also showed stronger inhibition effects compared to that of the control group ($p < 0.05$). ©1998 Elsevier Science Ltd

Keywords: Polychlorinated biphenyls, uptake, phytoplankton, *Artemia*, hard shell clam.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are very stable organic chlorinated compounds and have been extensively used in industry because of their wide range of physical properties and their chemical stability. According to estimates, more than 30% of the 120 million tons of PCB produced since 1929 have been released into the open environment (Tanabe 1988). This persistent pollutant eventually finds its way to the water column and results in toxicological effects on the aquatic ecosystem of a wide variety of species ranging from phytoplankton to marine mammals (Spacie and Hamelink 1985; Phillips 1994). Due to their hydrophobicity and low degradability, PCB could be accumulated as high as thousands- or tens thousand-time concentrations in biota relative to the concentrations present in the aquatic environment (Van der Oost *et al.* 1988; Oliver and Niimi 1988). Their

* To whom correspondence should be addressed.

toxicity to aquatic biota is further enhanced by their ability to bioaccumulate and biomagnify within the food chain due to extremely high liposolubility (Clark and Mackay 1991; van Sprang *et al.* 1991). Serving as a valuable food source for the higher trophic level, phytoplankton plays a very important role in supporting the growth of many aquatic biota. Once in the water column, the lipophilic PCBs partition into the more nonpolar compartments of the aquatic ecosystem or are physically absorbed in particulate matters. Results indicate that the PCBs uptake process on microparticulate materials and into phytoplankton is consistent with partitioning from water into cell lipids (Rohrer *et al.* 1982; Swackhamer and Skoglund 1993). However, the occurrence of organochlorine residues in aquatic organisms of the food chain usually starts with the first link of the marine phytoplankton. The importance of this process is that it may cause a biomagnification of the hydrophobic organic compounds to other higher trophic levels of aquatic biota. Various studies have also reported that most of the contaminants in aquatic biota are accumulated by dietary consumption through the food web rather than by direct uptake from water (Evans *et al.* 1982; Thomann and Connolly 1984; Van der Oost *et al.* 1988). By using the food-chain model, up to 99% of the PCBs is accumulated through dietary consumption rather than uptake from water (Rasmussen *et al.* 1990). Thus, information for the uptake of chlorinated contaminants in phytoplankton and the transfer to higher levels of aquatic biota in the food chain system is needed and should not be ignored.

Previous studies have shown that increased PCB levels in the water column increase the potential for PCB uptake in all trophic levels of the ecosystem (Brown *et al.* 1982; Califano *et al.* 1982; O'Connor and Pizza 1987). Although, most reports of PCB uptake by aquatic biota have been determined or monitored based on ambient concentrations in a natural environment, information of relative high concentrations on the resulting transfer and uptake has been scarce. In the present study, two common microalgae species found in local aquaculture, *Nannochloropsis oculata* and *Isochrysis galbana*, were therefore used in this short-term ecosystem to measure the uptake and transfer of PCBs to other trophic levels of aquatic biota, brine shrimp (*Artemia* sp.) and hard shell clam (*Meretrix lusoria*). Growth effects of these aquatic biota were also measured under the culture with high PCB concentrations.

MATERIALS AND METHODS

A purity >99.0% of 2,2',4,4'-tetrachlorobiphenyl (an isomer of PCBs, purchased from Ultra Scientific, USA) was weighed and dissolved in a series of dilution with acetone to achieve 100 ppb as standard stock in the present study. A salinity of 15‰ Eidschreiber medium was prepared as shown in Table 1 by filtration of 30‰ clean seawater through a 0.22 µm microfilter with the addition of the same volume of deionized water. The prepared artificial medium was then sterilized at 121°C for 20 min and cooled to room temperature for the culture of microalgae. Two pure culture of microalgae, *Nannochloropsis oculata* and *Isochrysis galbana* were provided by the Institute of Fisheries Science, National Taiwan University. *Artemia* nauplii were obtained by hatching the cysts (*Artemia salina*, San Francisco Bay Brand) in filtered seawater (30‰ salinity) under strong aeration for 20 hr. Hard shell clam (*Meretrix lusoria*) with an average diameter 2.5 cm were sampled at a local aquaculture farm.

The collected hard shell clam were then kept in filtered seawater (15‰ salinity) and shipped to the Institute of Fisheries Science, National Taiwan University, for the PCBs uptake study.

Growth conditions of the two algal cultures incubated at 23°C with moderate aeration were recorded daily. At the log phase of the growth curve, two PCB concentrations (50 and 500 ppb) tested in this study were prepared by pipetting 0.5 mL and 5 mL of 100 ppb stock standard to each 1 L of *Nannochloropsis* and *Isochrysis* culture. The control groups (0 ppb) were also set up by pipetting the same volume of acetone as the contaminated groups on each of the culture. Freshly hatched *Artemia* nauplii were then filtered and evenly divided into three portions to different *Nannochloropsis* culture groups with three PCB concentrations. Six hard shell clam were randomly picked up and put into each of 0 ppb, 50 ppb and 500 ppb of PCB treated *Isochrysis* culture. During the 4-d bioaccumulation period, the length (μm) and survival numbers of nauplii (larva/mL) were measured daily. The biomass of each algal species on different PCB treatments was counted every 24 hr by pipetting 50 μL of the culture on a blood cell counter through a microscope. After 4-d incubation, *Artemia* nauplii were filtered on a 106 μm sieve and hard shell clam were also collected from the culture. The algal cells were obtained under a centrifuge (2000 rpm, 2 min). All collected samples were freeze-dried and stored at -20°C until further PCB analysis. Three different PCB concentration treatments on two algal cultures were conducted for two replicates to obtain average values. Significant differences between sample means were determined with Student's *t* test.

Two freeze-dried microalgae and two biota samples were first extracted by using hexane:acetone (1:1 in volume) under ultrasonic disintegration in a Polytron homogenizer to obtain crude lipid. The lipid extracts were then cleaned-up by using 6% deactivated alumina oxide adsorption column (Al_2O_3 , 80-120 mesh, 20 cm x 0.5 cm i.d.) with anhydrous sodium sulfate on top and eluted with hexane for the elimination of lipid and polar compounds. The eluted hexane portions containing PCBs were concentrated and dissolved in hexane for gas chromatography

Table 1. Contents and amounts of Erdschreiber stock medium prepared in the present culture.

Contents	Amounts
Distilled water	1 L
Na_2NO_3	3.5 g
Na_2 glycerophosphate	0.5 g
Tris buffer	5.0 g
Fe-solution*	250 mL
PII-metal solution†	250 mL
Vitamin solution‡	1 mL

5N HCl was used to adjust final pH to 7.8.

* Distilled water, 1 L; Na_2 -EDTA, 600 mg; $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_3 \cdot 6\text{H}_2\text{O}$, 702 mg.

† Distilled water, 1 L; Na_2 -EDTA, 1.00g; H_3BO_3 , 1.14 g; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 49 mg; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 164 mg; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 22 mg; $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 4.8 mg.

‡ Distilled water, 100 mL; Vit B12, 10 mL; Biotin, 5 mg; Thiamine, 500 mg.

analysis. A gas chromatography (Perkin-Elmer Auto System GC) equipped with an ^{63}Ni electron capture detector and a programming integrator (PE Nelson Integrator) were used for the determination of PCB. The fused silica capillary column was a DB-608, 30 m x 0.32 mm I.D., 0.5 μm film thickness (J&W Scientific, USA). The operating temperatures for the injector, column and detector were kept at 280°C, 220°C and 380°C, respectively. High purity nitrogen was used as the carrier gas and make-up gas with flow rates, 29.0 c.c./min and 35.5 mL/min, respectively. One μL each of sample and PCB standard was injected into GC at a splitless injection mode. Quantification of the concentrations was calculated by comparison of peak areas and standard injection. The recovery of PCB after extraction and clean-up procedures was 80-90%. The detection limit of each compound was 1.0 ppb (dry wt basis) in this study.

RESULTS AND DISCUSSION

Upon incorporation into phytoplankton, PCBs have been documented to exert inhibitory effects on photosynthesis and cell motility with direct toxic effects on algae and are readily bioaccumulated into the aquatic food chain (O'Connors *et al.* 1978; Rohrer *et al.* 1982; Evans *et al.* 1991). An as low as 0.1 ppb concentration of Aroclor 1254 (with 54% chlorine of PCBs) has shown to produce growth reductions in marine diatoms and a freshwater algae (*Scenedesmus quadricauda*) and altered the population structure of phytoplankton communities (EPA 1980). Among these sublethal effects, disruption of internal chloroplast membranes and failure of cytokinesis were reported as the major changes observed (Mahanty *et al.* 1983). Furthermore, marine algae exhibited greater-than-expected reductions in photosynthesis when stressed with mixtures of PCBs and DDE (Ernst 1984).

Figure 1 shows the relative biomass percentages (relative to the biomass at day 0) when *Nannochloropsis* and *Isochrysis* were contaminated under three different PCB concentrations (0 ppb, 50 ppb and 500 ppb) with *Artemia* nauplii and hard shell clam for 4 d, respectively. After feeding to *Artemia* nauplii, *Nannochloropsis* of all groups showed a decrease of algae biomass within 3 d, whereas all groups of *Nannochloropsis* had an apparent increase of biomass after day 3. In the *Nannochloropsis* culture with the *Artemia* predator, the relative biomass of control group were significantly higher than the other two PCB groups ($p < 0.05$) at each day. The relative biomass for 50 ppb and 500 ppb PCB treated group were very similar during the whole experiment. However, the increasing biomass for the two contaminated groups after day 3 showed that the algae species was not inhibited up to 500 ppb PCB concentration. According to the study by Fisher *et al.* (1973), the diatoms *Thalassiosira pseudonana* and *Skeletonema costatum* were depressed in growth between 10-25 ppb of PCBs in water, while *Dunaliella tertiolecta* (a green algae) could support concentrations as high as 1000 ppb. Similar response from those phytoplankton species also indicated that over 118-h culture periods the cell densities of the treated cultures relative to control cultures for *Bellerophia*, *Fragilaria pinnata* and *Thalassiosira pseudonana* can decrease to 0.5%, 39% and 34%, respectively. Algal strains, *Thalassiosira nordenskioldii* and *Asterionella glacialis*, resistant to more than 50 ppb of PCBs have been isolated from a highly polluted estuary and shown to enhance their growth in PCB contaminated water (Duncan 1983; Snyder 1985; Cosper *et al.* 1988). In the

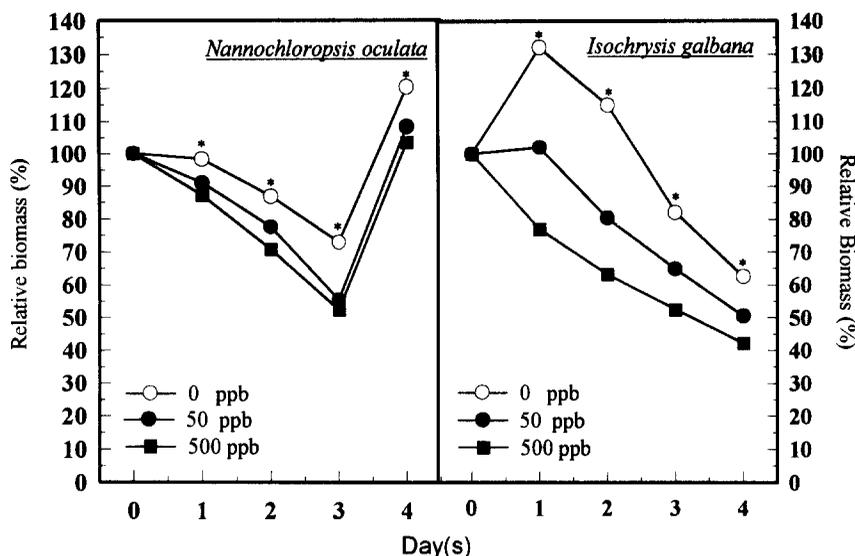


Fig. 1. Relative biomass of *Nannochloropsis oculata* (relative to 3.56×10^6 cell/mL at day 0) and *Isochrysis galbana* (relative to 1.13×10^5 cell/mL at day 0) in the culture with *Artemia* and hard shell clam, respectively, under three different PCB concentrations (0, 50 and 500 ppb) for 4 d. Significant differences between control group and each PCB contaminated group in the same day are indicated by * ($p < 0.05$).

present study, the capability for *Nannochloropsis* to tolerate higher than 500 ppb of PCB and maintain its growth is possible.

For the *Isochrysis* culture, both 0 ppb and 50 ppb PCB groups had an increase of relative biomass (133% and 102%, respectively) compared to that of 500 ppb group (77%) at 1-d experiment (Fig. 1). The result confirms that up to 500 ppb of PCB may be sensitive to *Isochrysis* and cause a growth inhibition in 24 h. A decreasing relative biomass at 2 d (115%), 3 d (84%) and 4 d (62%) for the control group demonstrated that hard shell clam became accustomed to the culture and started consuming the *Isochrysis* species. The significantly lower biomass ($p < 0.05$) on both PCB contaminated groups than control group on each day also demonstrate that both 50 ppb and 500 ppb concentrations result in growth inhibition on *Isochrysis*. However, no mortality was found for any hard shell clam (*Meretrix lusoria*) tested in the present ecosystem under the *Isochrysis* culture with three different PCB concentrations. This indicates that this bivalve may tolerate as high as 500 ppb PCB under the static food chain environment for 4 d.

The growth effect on the length of *Artemia* nauplii during the 4-d period under different PCB conditions in *Nannochloropsis* culture is shown on Figure 2. The control group of *Artemia* had a significant increase of length on the 4-d experiment compared to the other two PCB groups ($p < 0.05$). The highest average length increases were found in the control group, from 751 μm at 1-d to 960 μm at 4-d compared to other two

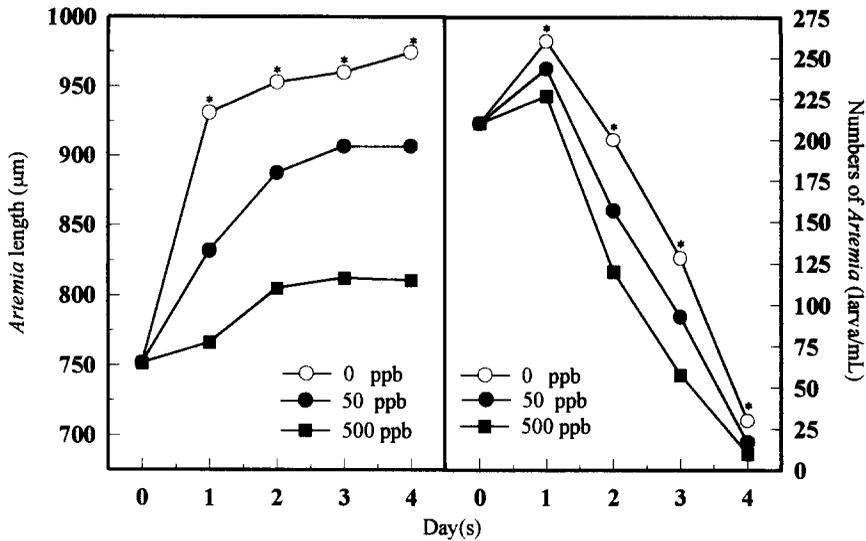


Fig. 2. Average *Artemia* length (μm) and numbers of nauplii (larva/mL) in the culture of *Nannochloropsis oculata* under three different PCB concentrations (0, 50 and 500 ppb) for 4 d. Significant differences on control group and each PCB contaminated group in the same day are indicated by * ($p < 0.05$).

contaminated groups at each day. The 500 ppb PCB group caused the lowest average length increases, from 751 μm at 1-d to 810 μm at 4-d. An elevated level of PCB concentration seems consistent with the growth inhibition on *Artemia* for the first 4-d period. The steady length increases for both PCB groups after 3 d suggest that the two PCB treated cultures apparently exhibited growth reduction on *Artemia* compared to the control group. For the survival conditions of the nauplii as shown in Figure 2, we found that the control group caused the higher number of *Artemia* compared with the other two PCB groups on each day ($p < 0.05$). As a lower number of zooplankton found in an enclosed aquatic environment, a higher biomass of the algae could be observed under normal conditions. The lowest number of *Artemia* nauplii together with the lowest relative biomass of *Nannochloropsis* was found under the 500 ppb PCB environment over the 4-d period (Fig. 1 and 2). Hence, we can conclude that 500 ppb PCB could cause apparent growth reduction and toxic effects on *Artemia* by feeding with contaminated *Nannochloropsis* compared with the other two groups.

Levels of PCB bioaccumulation analyzed for *Nannochloropsis* and *Artemia* under three different PCB concentrations in the culture are shown in Figure 3. In the present study, PCB concentration of *Nannochloropsis* after 4-d inoculation on 500 ppb PCB culture (257.52 ppm) was about 5.5-time than that of 50 ppb PCB culture (46.51 ppm). As the number of algal cells increased in the culture, PCB were partitioned onto the cells and resulted in an increase in the total amount of PCB in the biomass. After 4-d food chain ecosystem, levels of PCB for the *Artemia* in 0 ppb, 50 ppb and 500 ppb groups were 0.83 ppm, 27.02 ppm and 318.81 ppm (in dry basis),

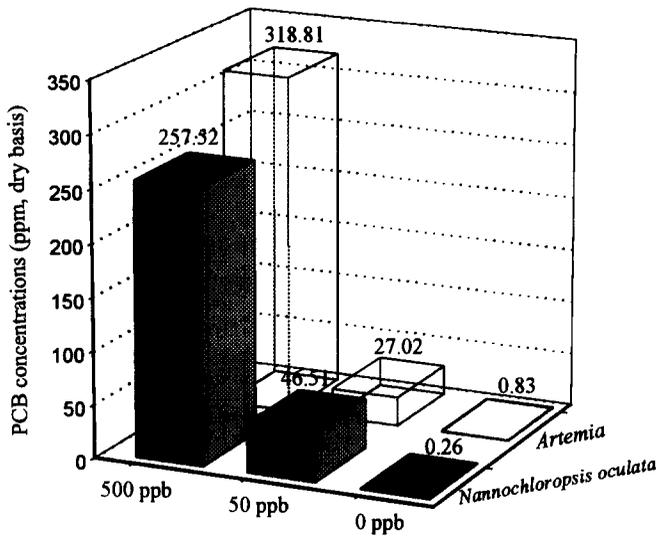


Fig 3. Levels of PCBs (ppm, dry basis) in *Nannochloropsis oculata* and *Artemia* after 4-d culture under three different PCB concentrations (0, 50 and 500 ppb).

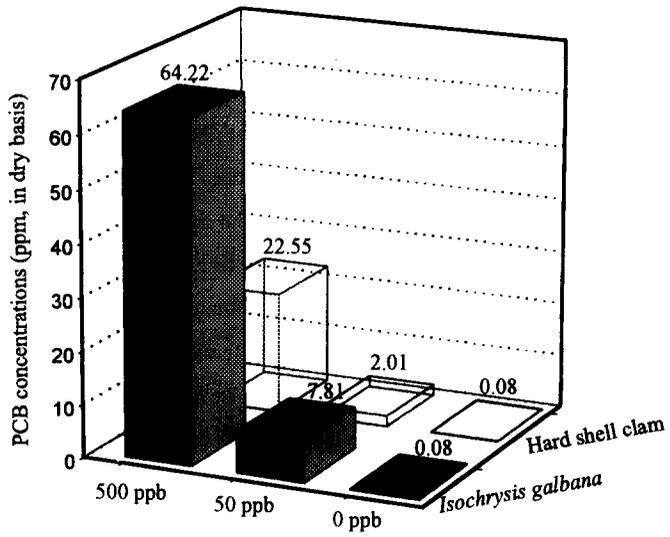


Fig 4. Levels of PCBs (ppm, dry basis) in *Isochrysis galbana* and hard shell clam after 4-d culture under three different PCB concentrations (0, 50 and 500 ppb).

respectively. The value of PCB bioaccumulation on 500 ppb group is 11.8-time than that of 50 ppb group, which is about the ratio of two prepared PCB concentrations on two aqueous solutions. This finding supports the conclusion of Scura and Theilacker (1977) indicating that PCB are concentrated up in each trophic level of a marine food chain consisting of the algal flagellate *Dunaliella* sp., the rotifer *Brachionus plicatilis*, and the larva of the northern anchovy *Engraulis mordax*. The data in that study also suggested that final concentration for the aquatic biota appeared to be dependent on the PCB concentration in the seawater and appeared to be an amplification in a step-wise fashion up the food chain.

The concentrations of PCB bioaccumulated in the other two trophic levels of the aquatic food chain are presented in Figure 4. Hard shell clam and *Isochrysis* on two PCB concentration groups bioaccumulated significantly high amounts of the pollutant compared to that of the control group. Uptake of PCB by the *Isochrysis* after 4 d for the 500 ppb group averaged 64.22 ppm (dry basis) which is about 8.2 times greater than that of the 50 ppb group (7.81 ppm). The levels of PCB in hard shell clam after 4 d for the 500 ppb and 50 ppb groups through the food chain system were determined to be 22.55 ppm and 2.01 ppm (dry basis), respectively. The level of PCB bioaccumulated on hard shell clam from 500 ppb group in the present study was about 11.2 times greater than that of the 50 ppb group.

According to Scura and Theilacker (1977), there appears to be a biological magnification of PCB up the food chain based on a dry weight basis, suggesting that higher members of the food chain may accumulate PCB by eating members of lower trophic levels. Duursma and Marchand (1974) also reported that the PCB whole body concentration increased to 425 ppm for oysters (*Crassostrea virginica*) held in sea water with 5 ppb PCB and to 100 ppm in sea water with 1 ppb PCB. In the present study, the uptake levels on 500 ppb PCB group for *Artemia* and hard shell clam were both found to be about 11 times than that on 50 ppb. However, only 5.9-time and 4-time difference of bioamplification between 500 ppb and 50 ppb PCB groups were found on each of *Nannochloropsis* and *Isochrysis* culture, respectively. This can be explained by the different partition coefficients of PCB on different biological properties of algal species. According to various reports, different levels of accumulation in the aquatic biota are closely associated to different species or biomass of biota, length of time and contamination conditions (Clayton *et al.* 1977; Scura and Theilacker 1977; Borgmann *et al.* 1990). Another reason for the low levels of PCB bioamplification in algae may be that a partition equilibrium between PCB in the aqueous and the phytoplankton has not yet reached.

A more clear correlation of the extent of PCBs uptake by these two phytoplankton species is expressed as the values of bioconcentration factor (BCF) in Table 2. In the present study, the log BCF values from 50 ppb (2.97) and 500 ppb (2.71) in *Nannochloropsis* group are higher than each of PCB treatment at *Isochrysis* group. The static system in this study may cause lower BCF values than the reports by Ernst (1984) who indicated that species of algae could concentrate PCBs over water levels by 10,000. According to Skoglund *et al.* (1996), the PCB uptake by algae is extensive and rapid and remains constant for approximately 24-48 h due to surface

Table 2. The values of log bioconcentration factor (BCF) for two phytoplankton species under two PCB contaminated concentrations (50 and 500 ppb) after 4-d culture.

PCB concentrations	Log BCF*	
	<i>Nannochloropsis oculata</i>	<i>Isochrysis galbana</i>
50 ppb	2.97	2.19
500 ppb	2.71	2.11

$$* \text{Log BCF} = \text{Log} \frac{\text{PCBs (ppm, dry basis) in phytoplankton}}{\text{PCBs (ppm) prepared in culture}}$$

adoption. Increased biomass may also result in proportionately more rapid PCBs uptake and moderate increases in the total amount of uptake (Richer and Peters 1993). This is correlated to the increased *Nannochloropsis* biomass at day 4 that caused higher log BCF than that of *Isochrysis* which had lower biomass at day 4. In the present study the higher PCB concentration that caused the higher mortality on phytoplankton may also result in lower BCF values on the biomass.

The present study reveals that the mechanism of the food chain phenomenon is a significant route for the uptake of high PCB concentrations from lower trophic biota - phytoplankton - to other aquatic predators. Moreover, the significant PCB transfer to other aquatic biota may also depend on different pollutant concentrations.

ACKNOWLEDGMENTS

The research was supported by the National Science Council of Taiwan (Grant No. NSC 83-0115-C-002-0022) and Chia Nan College of Pharmacy and Science at Tainan, Taiwan. The authors are also grateful for valuable comments on this manuscript provided by Dr. Min-Nan Lin from the Tainan Branch of the Taiwan Fisheries Research Institute.

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