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Live algae as a vector candidate for hydrophobic polychlorinated biphenyls translocation to bivalve filter feeders for laboratory toxicity test

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Abstract

Live algae carrying hydrophobic xenobiotics can be an effective vector candidate for the chemical translocation to filter feeders in the laboratory toxicity test, but information on their application is lacking. Time-course uptake and elimination of polychlorinated biphenyls (PCBs) (0, 50, 100, and 500 ng g^{-1}) by two key algal foods, *Isochrysis galbana* and *Tetraselmis suecica*, were measured. Both of the algae achieved maximum concentration in an hour after PCBs exposure regardless the chemical concentrations in our time-course measurements (0, 1, 5, 10, 24, 48 and 72 hrs). Once achieved the maximum concentration, the algae shortly exhibited elimination or eliminating tendency depending on the chemical concentrations. Algae exposed to the chemical for 1 and 24 hrs (hereafter 1 and 24 hr vectors, respectively) were then evaluated as a chemical translocation vector by feeding test to larval and spat *Crassostrea gigas*. In the feeding test the 24 hr vector, which contained lower chemicals than the 1-hr vector, appeared to be more damaging the early lives of the oyster. This was particularly significant for vectors of higher PCBs ($p<0.05$), probably due to algal reduction in food value by the prolonged chemical stress. These findings imply that 1 hr exposure is long enough for a generation of algal vector for laboratory toxicity test, minimizing data error resulted from reduction in food value by longer chemical stress.

Key words

Live algae, PCBs vector, Toxicity test, Bivalve

Introduction

Polychlorinated biphenyls (PCBs) are a group of persistent organochloride compounds that tend to biomagnify in terms of bioconcentration and bioaccumulation in marine ecosystem (Rand *et al*., 1995). Even though some measurements fail to normalize the biomagnification in lipid content (Gray, 2002), it often amounts as high as up to several thousands times relative to the concentration of ambient environment (Van der Ooster *et al*., 1988; Oliver and Niimi, 1988), resulting in overall damages to biological/physiological processes of marine lives and further to human health (McFarland and Clarke, 1989; Safe, 1994; Wania and Mackay, 1996; Mayes *et al*., 1998; Chu *et al*., 2003; Axmon *et al*., 2004; Choy *et al*., 2007). Because of their high damage potential to environment integrity, many studies have focused on the effects of the chemicals, mostly sediments containing the chemicals, on marine lives (Jo *et al*., 2008). Sediment studies may provide environmental realism, but they fail to give specific information. In this regard, laboratory studies can be a useful approach for the information.

Laboratory study needs a vector for translocation of the hydrophobic chemicals to test organisms. Use of a vector is particularly necessary if the test organisms are filter feeders. Flagellates (*Isochrysis* spp. and *Tetraselmis* spp.) and diatoms (*Chaetoceros* spp.) can be the candidate as they have been routinely employed as an algal food for shellfish culture (Bourne *et al*., 1989). But the candidates should be either flagellates or diatoms because of their culture media difference: diatoms need an additional siliceous nutrient (Guillard and Ryther, 1962).

Besides the algal selection, a question still remains. That is from our general understanding that microalgae themselves are

sensitive to PCBs, reducing in nutritional values (Swackhamer and Skoglund, 1993; Stange and Swackhamer, 1994), which in turn can be an additional parameter provoking experimental errors. Earlier works verified algal gains of the chemicals from environment are fast and species-specific (Harding Jr. and Phillips Jr., 1978b, Cosper *et al*., 1988). Therefore, finding an earliest working vector in the time-course is crucial in the laboratory toxicity test. For this purpose we determined earliest working vector for *Isochrysis galbana* and *Tetraselmis suecica* in the time-course uptake and elimination of PCBs and evaluated their effectiveness by feeding tests to early lives of a shellfish.

Materials and Methods

Algal exposure to PCBs: Each of two algae, *Isochrysis galbana* and *Tetraselmis suecica*, was cultured in mass (culture tank volume, 500 l) containing f/2 medium (Guillard and Ryther, 1962) under white light (PAR) at about 140 $\mu molm^{-2}s^{-1}$ and 24L:0D cycle. The cultures at mid-logarithmic growth phase were added with PCBs (Arochlor 1254) to make 0, 50, 100, or 500 ngg^{-1} PCBs and continued at 0, 1, 5, 10, 24, 48, and 72 hr. For a batch harvest, a specially designed algal concentrator (concentrating capacity, 100 l min^{-1}) was used to concentrate each culture to 2 l that was re-concentrated to about 50 ml with an aid of centrifuger at 3000 rpm for 10 min. The centrifuged were freeze-dried for GC analysis. To minimize algal metabolism of PCBs during the concentrating process, all the works were done as quickly as possible.

PCB determination in the alga: One to two grams of the freeze-dried sample were extracted with *n*-hexane in an accelerated solvent extractor (Dionex model ASE 200) equipped with a solvent controller (Dionex Corp, Sunnyvale, CA). The extracts in a rotary evaporator were cleaned up on a multi-layer silica-based adsorbents (70-230 mesh, Neutral, Merck) column (15 mm i.d. × 300 mm long) with 200 ml of *n*-hexane at 10 ml min^{-1} elution flow. The extracts were then passed through adsorbents in the following order; anhydrous sodium sulfate (4 g), silica gel (2 g), 33% sulfuric acid impregnated silica gel (8 g), silica gel (1 g), 2% potassium hydroxide impregnated silica gel (4 g), and silica gel (1 g). The purified samples were concentrated to less than 1 ml, and left at room temperature for 2 days to evaporate to total dryness. The residues were dissolved in 1 ml of *n*-hexane and determined for PCBs. Samples were spiked immediately before injection with solution of 4,4 dibromo-octafluoro biphenyl (DBOFP) as an internal standard.

PCBs extracts were analyzed on a Hewlett-Packard (HP) 6890 series II gas chromatograph (GC)/electron capture detector (ECD) with capillary column DB-5MS (30 m × 0.25 mm × 0.25 μm, J & W Scientific Inc.). An initial oven temperature, 60°C, which was held for 1 min, was increased up to 265°C at 1.2°C min^{-1} via 120°C at 10°C min^{-1}. The temperatures of the injector and detector were 280 and 330°C, respectively. The injection volume was 2 μl at splitless mode. A total Aroclor 1254 was quantified using six major congeners (EPA method 8082A). The certified mussel homogenate (2977, NIST, USA) was analyzed as a standard reference material (SRM). Analysis of the NIST reference material revealed that the PCB analytes quantified were within ±10% of their certified concentrations.

Algal vectors: For feeding test, mid logarithm-phased *I. galbana* and *T. suecica* were cultured in 3 l flask containing different PCBs solutions (0, 50, 100 and 500 ng g^{-1}) for 1 or 24 hrs, respectively, to serve as a vector. Each algal culture was immediately concentrated to make 100 ml vector. The vectors were consumed within an hour after being prepared to minimize physiological change by the chemical burden. Algae exposed to each of four PCBs solutions (0, 50, 100, and 500 ng g^{-1}) for 1 hr and for 24 hr were named 1 hr vector and 24 hr vector, respectively. The two types of algal vectors were commonly prepared from mid logarithm-phased *I. galbana* (Iso) and *T. suecica* (Tetra) in 3 l flask containing different PCBs solutions. The vectors were prepared just prior to use for feeding test to larval and spat *Crassostrea gigas*.

Feeding test: Ten *C. gigas* spawners from each sex of 5 individuals were stripped and separately pooled. Female gametes were rinsed with filtered (mesh size, 0.5 μm) and UV-sterilized seawater, fertilized by adding pooled male gamete, and maintained in the gently aerated culture chamber at 22±0.5 °C until metamorphosis to D-larvae (Park *et al*., 2002). The D-larvae were contained in 30 l culture chamber (water volume, 20 l) at density of 10,000 larvae l^{-1} and supplied with immediately prepared 1 and 24 hr vectors at culture concentration of 1 to 10 x 10^4 cells ml^{-1}, depending on the algal species and oyster larval size/feeding activity. The larval cultures were supplied with the vectors twice a day and subsampled for larval measurements just before every 2 day based water replacement. The cultures continued until first appearance of eye-spotted larvae with abundance about 20% in the control (control ESL_{20}). Measurements were done under a profile projector (Nikon V-12, Japan). The cultures were replicated 3 times in the larval culture room where temperature was fixed to 22±0.5 °C.

PCBs-free ESL_{20} larvae obtained separately distributed into 20 l culture chamber (water volume, 15 l) at a density of 5,000 larvae l^{-1}, served with 50 substrates (oyster shell, 3x3 cm^2) for larval attachment and fed with the same vectors twice a day. Three days after the feeding, all the substrates were carefully removed from the chambers and put into net cages (15x15x10 cm^3) in 30 l culture chamber (water volume, 20 l). Ten substrates were weekly withdrawn from each culture to measure spat attachment using an exaggerating apparatus. All the cultures were replicated 3 times.

Statistical analysis: The statistical analysis was done by student *t*-test in the Sigma Plot Software (Systat Software 9.0, California, USA).

Results and Discussion

Time-course uptake and elimination of PCBs by two candidate vectors, *I. galbana* and *T. suecica*, are shown in Fig. 1. Both the algaes' showed a rapid uptake of the chemicals achieving maximum concentration an hour after exposure. In species

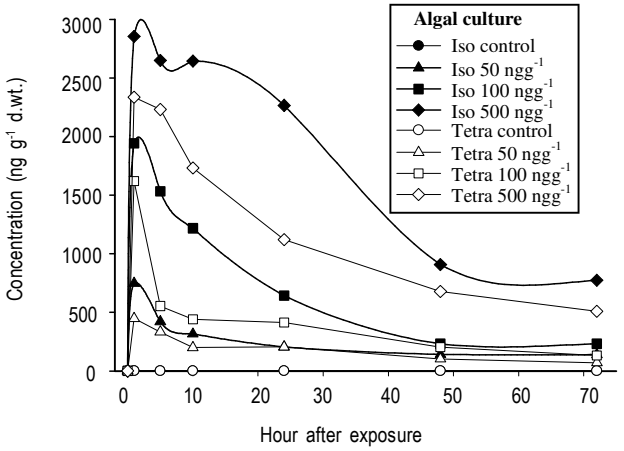

Fig. 1: Time-course uptake and elimination of PCB by *Isochrysis galbana* (Iso) and *Tetraselmis suecica* (Tetra). The algal cultures were exposed to 4 concentrations of Arochlor 1254 (0, 50, 100 and 500 ng g^{-1}) for 0, 1, 5, 10, 24, 48, or 72 hr, respectively

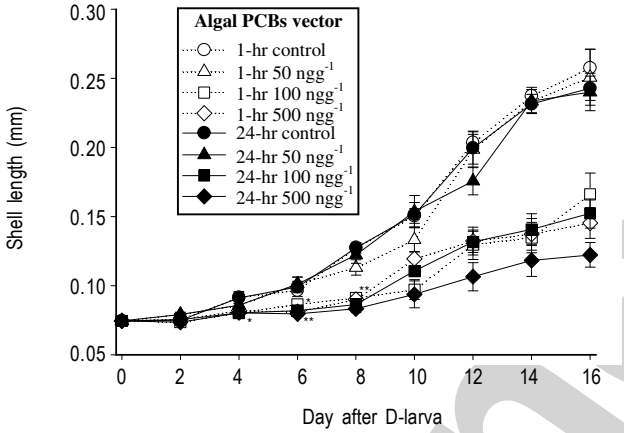

Fig. 2: Growth of larval *Crassostrea gigas* fed on algal vectors carrying PCBs. The vectors were prepared by culturing for 1 or 24 hr in f/2 media carrying four concentrations of Arochlor 1254 (0, 50, 100 and 500 ng g^{-1}), respectively. Abbreviations: 1- hr control, 1 hr exposure at 0 ng g^{-1}; 1- hr 50 ng g^{-1}, 1 hr exposure at 50 ng g^{-1}; 1- hr 100 ng g^{-1}, 1 hr exposure at 100 ng g^{-1}; 1- hr 500 ng g^{-1}, 1 hr exposure at 500 ng g^{-1}; 24 hr control, 24 hr exposure at 0 ng g^{-1}; 24- hr 50 ng g^{-1}, 24 hr exposure at 50 ng g^{-1}; 24 -hr 100 ng g^{-1}, 24 hr exposure at 100 ng g^{-1}; 24- hr 500 ng g^{-1}, 24 hr exposure at 500 ng g^{-1}. Statistical differences: *first different from control with $p<0.05$, **first different from control with $P<0.01$. Error bar represents mean ± SE

comparison, *I. galbana* gained higher magnitude over *T. suecica*. The maximum concentrations of *I. galbana* were 749, 1,942 and 2,853 ng g^{-1} dw for 50, 100, and 500 ng g^{-1} PCBs exposures, while those of *T. suecica* were 448, 1,620 and 2,335 for 50, 100 and 500 ng g^{-1} PCBs, respectively. The species difference in PCBs uptake between *I. galbana* and *T. suecica* might be due to difference in size and structure (Harding Jr. and Phillips Jr., 1978b, Cosper *et al*., 1988; Wallberg and Andersson, 1999). Both of control algae contained PCBs lower than 0.7 ng g^{-1} dw throughout the experiment.

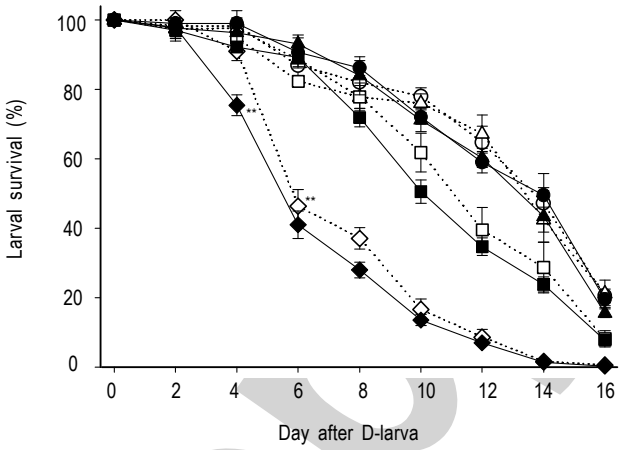

Fig. 3: Survival of larval *Crassostrea gigas* fed on algal vectors carrying PCBs. Preparation and symbols of vectors are as in Fig 2. Statistical difference: **first difference from control with $P<0.01$. Error bar represents mean ± SE

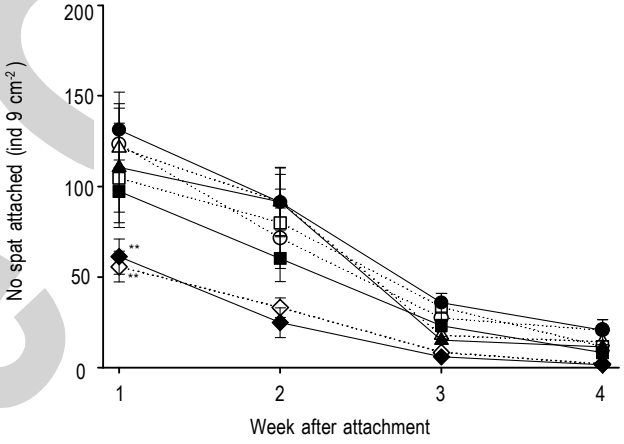

Fig. 4: Survival (attachment) of spat *Crassostrea gigas* fed on algal vectors carrying PCBs. Preparation and symbols of vectors are as in Fig 2. Statistical difference: **first different from control with $P<0.01$. Error bar represents mean ± SE

Live algae have a greater association capacity for PCBs over dead ones and dissolved organic particles, taking a considering part of the chemicals in the aquatic environments (Dean *et al*., 1993; Skoglund *et al*., 1996; Fitzgerald and Steuer, 2006). An earlier work done by Harding Jr. and Phillips Jr. (1978b) measured hour-course uptake for PCB by marine phytoplankton species and found that the phytoplankton uptake for the chemical was so rapid, with equilibrium occurring within 0.5 to 2 hr.

A question is whether the rapid gain is adsorption or absorption. The rapid gain in our study was probably by adsorption rather than by absorption even though we did not deal with the subject. Studies dealing with partitioning of hydrophobic organic compounds (HOCs) into phytoplankton showed that HOCs adsorption to phytoplankton was more rapid than absorption (Wang *et al*., 1982; Autenrieth and DePinot, 1991; Swackhamer and

Skoglund, 1993; Wallberg and Andersson, 1999).

Once achieved maximum concentration the algae exerted an overall elimination soon after the maximum concentration. For example, *I. galbana* eliminations of PCBs 4 hrs after the maximum were 328 (from 749 to 421), 410 (from 1,942 to 1,532), and 204 ng g^{-1} (from 2,853 to 2,649 ng g^{-1}) for 50, 100, and 500 ng g^{-1} algae, respectively. These represent 43.7, 21.1, and 7.2% in eliminating rate for 50, 100, and 500 ng g^{-1} algae. The algal eliminating rate of PCBs 72 hrs after the maximum were 139, 231, and 773 ng g^{-1} for 50, 100, and 500 ng g^{-1} algae, respectively. These correspond to 81.4, 88.1, and 72.9% in eliminating rate for 50, 100, and 500 ng g^{-1} algae, respectively. *T. suecica* elimination of PCBs showed a trend similar to *I. galbana* although, overall, the latter showed a higher magnitude in concentration over the former.

Algal elimination of PCBs is controversial. Some species eliminate the chemicals in hours, some are in days (Harding Jr. and Phillips Jr., 1978b; Scoglund *et al*., 1996; Gerofke *et al*., 2005). Bard (2000) reviewed a cellular defense against xenobiotic chemicals, expressing multixenobiotic resistence (MXR) as a rapid cellular removal system. Although we did not determine any part of the defense system, our results clearly showed earlier gain and earlier elimination for the chemicals. This earlier elimination also has some implication in the laboratory toxicity study for bivalves that need algal vectors.

In the generation of a reliable algal vector, vector damage by test chemicals is worth a deep consideration. Algae have a varying degree of sensitivities to xenobiotic chemicals in terms of reduction in photosynthesis, nutrition, and viability (Harding Jr. and Phillips Jr., 1978a,c; Swackhamer and Skoglund, 1993; Stange and Swackhamer, 1994; Wang *et al*., 1998) which can be additional parameters affecting physiology of test bivalves. Therefore, finding the earliest working vector with least physiological influence might be crucial in laboratory toxicity test. In this regards, 1-hr vector appears best at least in our measurements.

We subsequently tested two vectors (1 and 24 hr vectors) to early life of bivalve. Shown are damages to larval survival (Fig. 2), larval growth (Fig. 3), and spat attachment (Fig. 4). Surprisingly, 24 hr vector was comparably damageable with 1 hr vector or even more damageable with occasional significances ($p<0.05$) in spite of its lower containment of PCBs. This was more evident for the vectors carrying higher PCBs levels.

There are suggested pathways that explain why 24 hr vector is comparably or even more damageable to bivalve receivers. One can be due to occurrence of more toxic metabolites during the cellular metabolism (Kurelec and Pivcevic, 1991; Bard, 2000). Another is probably due to vector itself damage by longer exposure to the chemicals that brings algal reduction in nutrition and viability (Iseki *et al*., 1981; Swackhamer and Skoglund, 1993; Stange and Swackhamer, 1994; Wang *et al*., 1998). To the worse, the algal damage by the longer exposure, in turn, might be an additional parameter for rapid chemical gain into their cellular body (Lynn *et al*., 2007). These findings imply that 1-hr exposure is long enough for a generation of algal vector for laboratory toxicity test, minimizing data error coming from loss of food value by longer chemical exposure.

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