Bull. Environ. Contam. Toxicol. (1995) 54:389–395

Environmental Contamination and Toxicology

Effects of Triphenyltin Chloride on Growth of the Marine Microalga *Pavlova lutheri* in Continuous Culture

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Received: 24 January 1994/Accepted: 20 August 1994

The toxic effects of organotin compounds on microalgae have been reported by many investigators (Huang et al. 1993; Beaumont and Newman 1986; Walsh et al. 1985; Wong et al. 1982). The type of responses by algae may vary with species and the dose of chemicals, but these responses generally involved reduced growth rate and photosynthesis, and even death of cells. Of the organotin compounds most widely used, tributyl and triphenyl forms appeared to be more toxic on freshwater and marine microalgae (Huang et al. 1993). Several studies showed that microalgae were adaptable to organotins and were able to accumulate some of these compounds at low external concentrations, i.e., close to those occurring in natural water (Huang et al. 1993; Chiles et al. 1989; Maguire et al. 1984). These findings make conspicuous the potential environmental risk related to the biotransfer of organotins to other links in the food chain. This risk appears to be more evident if one supposes that a population of contaminated algae can maintained its long-term growth and productivity at a sublethal contamination level.

All previous studies of toxic effects of organotin compounds have used batch cultures. Obviously, this technic is not applicable to the investigation of long-term effects of the pollutant on microalgae growth since other factors than the toxicant itself (e.g., depletion of nutrients) will cause discontinuous growing. Continuous culture technique appears to be more suitable because a steady-state growth is achieved when fresh supply of nutrients is continuously added at the same rate as the culture medium is withdrawn. It also permits a constant supply of toxicant to a fixed algal population. The objectives of the present work are to evaluate growth and toxicant bioaccumulation characteristics of a continuous culture of *Pavlova lutheri* exposed to sublethal concentrations of triphenyltin. By employing continuous culture with contaminated natural seawater as the inflow nutrient, the experimental protocol allowed a better understanding of the alga response under conditions which may more closely reflect those in the natural environment.

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MATERIALS AND METHODS

Toxic effects of triphenyltin (TPT) for marine microalga *Pavlova lutheri* were investigated in axenic continuous culture in which growth kinetic is expressed by the equation:

$$\mu_e = D + \ln (X_1 / X_0) / t_1 - t_0$$

where X_1 and X_0 represent cell density at times t_1 and t_0 respectively, and μ_e, is the growth rate measured as the number of logarithm-to-base e units of increase per day and D, the dilution rate. The continuous culture was operated as a chemostat in such a way that an inhibitory effect of TPT was reflected by a reduction of cell density and expressed by the ratio μ_e / D. The culture growth chamber of 3.5 L working volume, was supplied with filtered seawater (1 μm cut-off membrane, Gelman Pre-flow sub-micron) at a fixed dilution rate (D) of 0.448 d^{-1} (mean inflow seawater = 1.57 $L \cdot d^{-1}$). The inorganic nutrient concentrations of seawater were 17.1 $\mu mol \cdot L^{-1}$ for NO_3^- + NO_2^- and 1.2 $\mu mol \cdot L^{-1}$ for PO_4^{-3}, with a salinity of 29.2 ‰. In order to compensate for loss of organic growth factors during heat sterilization of seawater, supplemented vitamins were added at one fourth concentration of the recommended F/2 medium (Guillard and Ryther 1962). Illumination was provided by cool-white fluorescent lamps at an incident intensity of 140 $\mu E\ m^{-2}\ s^{-1}$, on a 14:10 LD photocycle. Temperature was kept constant at 17.5°C. Mixing of the culture was provided by the revolution of Teflon-coated magnetic bar (110 r.p.m.) and by bubbling with sterile air at a flow rate of 150 $mL \cdot min^{-1}$. The culture outflow was maintained at temperature of 0° ± 1°C. Four consecutive exposure concentrations of TPT chloride were tested on the same culture by adding the toxicant (23, 28, 40 and 77 $nmol \cdot L^{-1}$ respectively) to the nutrient reservoir (20 L capacity) at periods indicated in Fig.1. Each toxicity test was initiated when the culture had achieved a steady-state (growth rate ≈ dilution rate) and maintained for at least two generation times. A daily sample was taken from the growth chamber, at fixed hour of the photocycle, for measurements of cell density and chlorophyll *a* value. Cells were counted in a Neubauer haemacytometer. Individual counts (n = 5 to 10) differed from the mean value by ± 12%. Chlorophyll *a* values were obtained by the fluorometric method described by Yentsch and Menzel (1963).

Daily overflows were collected for organotin analysis of cells and supernatant medium. The extraction protocol of phenyltins from water and algae was adapted from the anlytical procedure previously described for butyltins (Reader and Pelletier 1992). The detection and the quantification of phenyltins, as hydrides derivatives, were performed by a gas chromatograph equipped with a flame photometric detector (FPD) operated with a quartz tube over the burner replacing the usual optical filter as described by Jiang et al (1991). Limits of detection were 0.5 pmol for triphenyltin (TPT), 0.25 pmol for diphenyltin (DPT) and 0.3 pmole for monophenyltin (MPT). The relative standard deviation (n = 5) was ± 8%, ± 17%, ± 20% for TPT, DPT, and MPT respectively.

RESULTS AND DISCUSSION

During the period preceding toxicity tests (days 0 to 4) a steady-state was achieved at a population density of 362 ± 22 × 10^6 cells · L^{-1} (mean ± SD) (Fig. 1A).

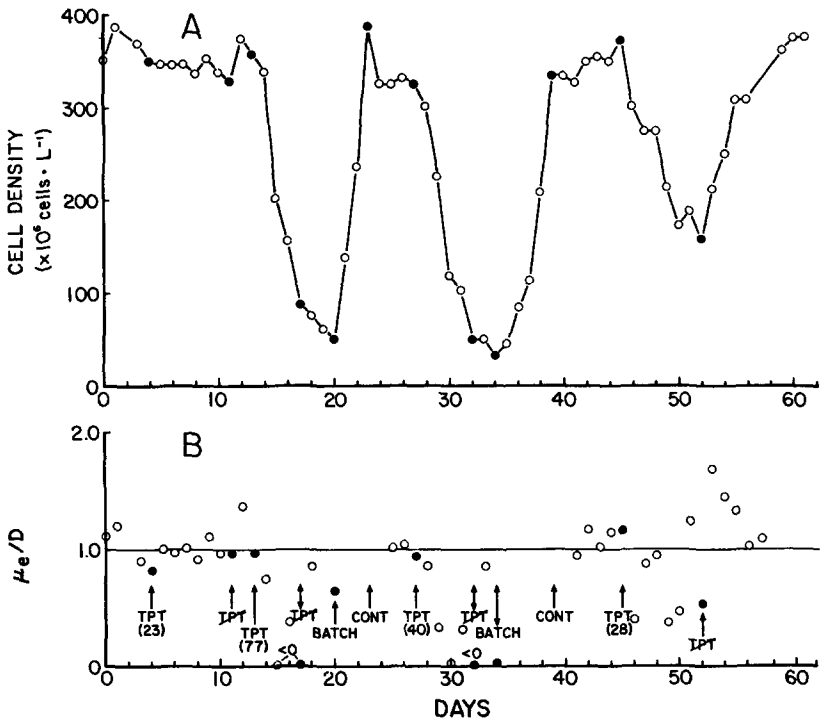

Figure 1. Changes in cell density (A) and μ_e/D ratio (B) for a continuous culture of *Pavlova lutheri* exposed to triphenyltin chloride for various exposure concentrations during 61-d studies. TPT↑ = contaminated nutrient supply. ~~TPT~~↑ = end of contamination. CONT↑ = continuous growth. BATCH↑ = batch growth. The dark dots refer to these events. Numbers in parenthesis indicate concentration of TPT used in nmol · L^{-1}.

At this time, we assumed that the culture established an equilibrium between growth rate and dilution rate as shown by the mean ratio value of μ_e / D = 1.01 ± 0.19 (Fig. 1B). With the addition of 23 nmol · L^{-1} of TPT to the nutrient medium, the cell density (average of 343.4 ± 8.6 × 10^6 cells · L^{-1} (mean ± SD) and μ_e/D ratio (1.00 ± 0.06) did not vary and these biological parameters were still constant throughout the 7-d exposure to toxicant. Such response also occurs for the chlorophyll *a* content of the culture (Fig. 2) which was maintained at mean concentrations of 32.8 μg · L^{-1} ± 2.1 (mean ± SD).

More pronounced inhibitory effects are observed on growth (μ_c/D ratio) after exposures to 40 and 77 nmol · L^{-1} of TPT. As the dilution rate was still constant, a persistent μ_c/D ratio value smaller than 1 determined a biomass washout of the culture which was reflected by a rapid decrease in chlorophyll *a* and cell density (Fig. 1 and 2, days 13 to 17 and 27 to 32).The depression of cell concentration is extended during 2 or 3 days of continuous operating culture, even in absence of toxicant (see days 17 to 20 and 32 to 34, Fig.1). The biomass concentration was returned near to the original steady-state level (average of 339 and 344 × 10^6 cell · L^{-1}) provided that the culture was operating in batch mode (D=0) for several days, (days 20 to 23 and 34 to 39).

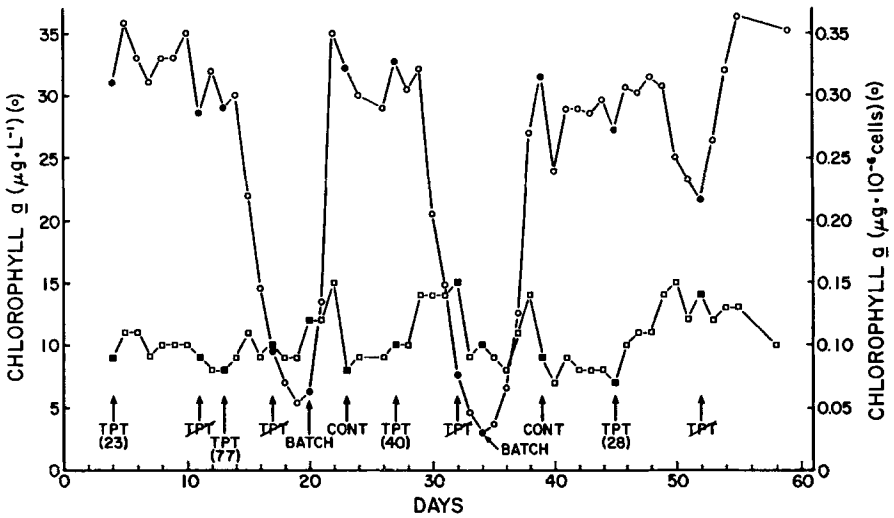

Figure 2. Changes in cellular chlorophyll *a* content (□ – □) and total chlorophyll *a* content (○ – ○) for a continuous culture of *Pavlova lutheri* exposed to triphenyltin chloride for various exposure concentrations. Legend is given in Figure 1.

With the addition of 28 nmol · L^{-1} of TPT in the inflow medium (day 45), cell concentration declined by about 50% within 7 days and then recovered its original level, in absence of TPT (days 52 to 60), while maintaining the same dilution rate to the culture, i.e. operating in continuous mode. These data show that growth rate inhibition of *P. lutheri* begins to take place within a contamination of the inflow medium ranging from 23 to 28 nmol · L^{-1} of TPT. Earlier works using batch culture, reported a toxic effect of TPT on freshwater and marine algae reproduction at starting concentration of 2 to 6 nmol · L^{-1} (Wong et al. 1981; Walsh et al. 1985; Huang et al. 1993). The excellent tolerance and recovery capability of *P. lutheri* in the continuous culture mode may be attributed to an effective biodetoxication of the culture medium as shown by much lower

concentration of TPT in the supernatant compared to the TPT levels observed in the inflow medium (Table 1). It seems likely that this depuration process is a consequence of two combined factors: cellular bioaccumulation (Table 1) and the constant renewal of cells from the culture chamber.

Table 1. Distribution of triphenyltin (TPT) and diphenyltin (DPT) in various fractions of a continuous culture of *Pavlova lutheri* during the first days exposure. (NA = not analyzed).

TPT in inflow medium		TPT in supernatant		Algal burden nmol and $\mu g \cdot g^{-1}$ (dry weigth)			
$nmol \cdot L^{-1}$	$\mu g \cdot L^{-1}$	$nmol \cdot L^{-1}$	$\mu g \cdot L^{-1}$	TPT		DPT	
				$nmol \cdot g^{-1}$	$\mu g \cdot g^{-1}$	$nmol \cdot g^{-1}$	$\mu g \cdot g^{-1}$
23.0	8.1	0.1	0.04	49.3	17.3	3.7	1.3
40.0	14.0	2.0	0.70	116.8	41.0	37.0	13.0
77.0	27.0	NA		509.8	178.9	$\leq$ 1.0	$\leq$ 0.3

This removal of TPT maintained in the supernatant medium concentration values below which toxic effects are generally observed for microalgae (Wong et al. 1981), or even similar to concentrations found in some polluted area (Alzieu et al. 1991; Huang et al. 1993). In this respect, the biotoxicity evaluation of organotins as a function of aqueous concentrations may be not of a great significance for an aquatic environment. Results of our experiment confirms other studies which suggested to relate toxic effects to the cellular level of toxicant instead to the medium contamination (Lederman and Rhee 1981).

Although inhibition has been observed for growth of *P. lutheri* at concentrations ranging from 28 to 77 $nmol \cdot L^{-1}$ TPT, the intracellular concentration of chlorophyll *a* was unaffected by this toxicant (Fig. 2). Hence, during the consecutive toxicity tests corresponding to exposures at 23, 28, 40 and 77 $nmol \cdot L^{-1}$ of TPT, the cellular chlorophyll *a* remained at mean values $\pm$ SD of 10.0 $\pm$ 0.8, 12.7 $\pm$ 1.0, 13.4 $\pm$ 1.9 and 9.4 $\pm$ 1.0 $\mu g \cdot 10^{-8}$ cells, respectively. During free-TPT continuous growth periods, cells exhibit a mean $\pm$ SD chlorophyll *a* content of 9.2 $\pm$ 1.8 $\mu g \cdot 10^{-8}$ cells. This relatively stable cellular chlorophyll *a* content demonstrates that the photosynthetic capacity of the microalgae was preserved as shown by the good recovery of cell density and of μ_c/D ratio after severe contamination by TPT (Fig. 1). Although the nature of inputs and outputs of a continuous culture is limited to a few defined variables, the behavior of *P. lutheri* in such an open system (see also Rhee 1980), may be representative of microalgal response to a discontinuous and acute discharge of pollutant and its dispersion in natural open waters.

Although the bioaccumulation of TPT seems to be evident in continuous culture (Table 1), the ability of *P. lutheri* to degrade the triphenyltin to diphenyl compounds at low level of contamination is rather uncertain. This metabolic process seems to be present chiefly at the intermediate exposure of 40 nmol $\cdot$ L^{-1} of TPT. Even though the biodegradation of TPT has not been previously observed for freshwater and marine microalgae, DPT compounds have been tested for their phytotoxicity and are reported to be 50 to 63 times less harmful than TPT for algae reproduction (Walsh et al. 1985; Huang et al. 1993). On the basis of our results with a continuous culture technique, namely the inefficiency for microalgae *P. lutheri* to metabolize TPT at low exposure concentration and the maintenance of growth rate by contaminated algae, one may expect an important contribution of these algae to be the bioconcentration of TPT into a natural food chain. This conclusion has to be confirmed by evaluating the capability for other phytoplankton species present in a natural algal population to accumulate and degrade TPT in an open system, and secondly the biotransfer the bioaccumulated pollutant into the aquatic food chain.

Acknowledgments. The authors thank Johanne Noël for invaluable help in drafting the figures for this publication, and Hélène Goulet for typing this manuscript. This work was supported by FCAR-Québec.

REFERENCES

Alzieu C, Michel P, Tolosa I, Bacci E, Mee LD, Readman JW (1991) Organotin compounds in the Mediterranean: A continuing cause for concern. Mar Environ Res 32:261-270

Beaumont AR, Newman PB (1986) Low levels of tributyltin reduce growth of marine microalgae. Mar Poll Bull 17:457-461

Chiles TC, Pendoley PD, Laughlin RB (1989) Mechanisms of tri-n-butyltin bioaccumulation by marine phytoplankton. Can J Fish Aquat Sci 46:859-862

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula* confervacea (Cleve) Gran. Can J Microbiol 8:229-239

Huang C, Bai Z, Dai S, Xie Q (1993) Accumulation and toxic effect of organometallic compounds on algae. Appl Organometal Chem 7:373-380

Jiang GB, Maxwell PS, Siu KWM, Luong VT, Berman SS (1991) Determination of butyltins in mussel by gas chromatography with flame photometric detection using quartz surface-induced luminescence. Anal Chem 63:1506-1509

Lederman TC, Rhee GY (1981) The influence of a hexachlorobiphenyl on the growth of Great Lakes phytoplankton. Can J Fish Aquat Sci 39:388-394

Maguire RJ, Wong PTS, Rhamey JS (1984) Accumulation and metabolism of tri-n-butyltin cation by a green alga, *Ankistrodesmus falcatus* Can J Fish Aquat Sci 41:537-540

Reader S, Pelletier E (1992) Identification and determination of butyltin compounds by gas chromatography-ion trap spectrometry. Anal Chim Acta 262: 307-314

Rhee GY (1980) Continuous culture in phytoplankton ecology. In: Advances in aquatic microbiology, vol.2. Droop MR and Jannasch HW,eds, Academic Press London: 151-203

Walsh GE, McLaughlan LL, Lores EM, Louie MK, Deans CH (1985) Effects of organotins on growth and survival of two marine diatoms, *Skeletonema costatum* and *Thalassiosira pseudonana*. Chemosphere 14:383-392

Wong PTS, Chau YK, Kramar O, Bengert GA (1982) Structure-toxicity relationship of tin compounds on algae. Can J Fish Aquat Sci 39:483-488

Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Res 10:221-231