**Journal of Industrial Microbiology (1995) 14**, 265–270


# Triphenyltin inhibits photosynthesis and respiration in marine microalgae

H.M. Mooney and J.W. Patching

*The Martin Ryan Marine Science Institute, UC Galway, Ireland*

(Received 29 April 1994; accepted 8 August 1994)

*Key words:* Triphenyltin; Diphenyltin; *Skeletonema costatum*; *Dunaliella tertiolecta*; Photosynthesis; Respiration; Organotins

## SUMMARY

The effects of diphenyltin and triphenyltin (TPhT) on gross photosynthesis and respiration by the diatom *Skeletonema costatum* (Greville) Cleve and the chlorophyte *Dunaliella tertiolecta* (Butcher) were investigated by measuring the rates of change of oxygen concentration in samples which were alternately illuminated and unilluminated. Measurements were carried out for 90 min after organotin addition. Triphenyltin at concentrations in the nM to μM range inhibited photosynthesis and respiration in both ogranisms. Levels of TPhT inhibiting these processes were two to three orders of magnitude higher for *D. tertiolecta* than for *S. costatum*. Photosynthesis and respiration by *D. tertiolecta* were resistant to diphenyltin at concentrations up to its limit of solubility (0.84 mM). With *S. costatum*, inhibitory levels of diphenyltin were one to two orders of magnitude higher than those for triphenyltin. Inhibition was often progressive over the period after organotin addition. This effect varied in intensity and was more noticeable with the more resistant *D. tertiolecta*. Comparison of our results with levels of organotins which have been observed by others in Mediterranean coastal waters indicate that environmental levels of TPhT could influence phytoplankton composition and dynamics.

## INTRODUCTION

Triphenyltin (TPhT) is a trisubstituted organotin of significant commercial importance. Early reports by van der Kerk and Liujten [15,16] drew attention to the use of trisubstituted organotins as antifungal agents. Whilst trialkyltins appeared to be too toxic to the infected plants to be of practical use, triphenyltins were well tolerated [2] and were subsequently used as the active agent in fungicides for application on commercially important crops.

Triphenyltins have also been used as the toxic agent in marine antifouling paints, either as a co-polymer with rubber, or mixed with tributylin (TBT) or copper-based paints [12]. Organotin-based antifouling paints were introduced in the 1970s and found widespread acceptance. It was subsequently noticed, however, that increasing environmental levels of organotins were affecting non-target marine species. As a result, restrictions have been placed on the use of certain antifouling compounds. Whilst legislation in some countries (e.g. Ireland, Japan) attempts to control the use of organotin-based antifouling compounds in general, other legislatures specifically target TBT-based formulations. It is conceivable that restrictions of this type might result in a greater use of TPhT. In addition to TBT, significant levels of TPhT may be found in inshore marine environments. The origin of these organotins seems to be marine antifouling paints [1].

The effects of phenyltin compounds have been studied less than those of TBT, but it would appear that TPhT may be toxic to organisms as diverse as vertebrates (rainbow trout fry [10]: and marine yeasts [17]). Virtually nothing is known about the action of TPhT on phytoplankton, though they usually form the base of aquatic food webs. This paper describes some short-term effects of phenyltins on the respiration and photosynthesis of two marine microalgal species. The organisms studied were a diatom, *Skeletonema costatum* (Grev.) Cleve, and a chlorophyte, *Dunaliella tertiolecta* (Butcher), which were grown in continuous culture in order to have a constant supply of physiologically stable cells.

## MATERIALS AND METHODS

Triphenyltin chloride (TPhT) was purchased from Pfaltz and Bauer (Stamford, CT, USA). Diphenyltin di-chloride (DPhT: 96% pure) was supplied by the Aldrich Chemical Company (Dorset, UK). Neither organotin was further purified. Stock solutions were prepared by dissolving the organotins in absolute ethanol.

Two unialgal phytoplankton species were obtained from the Culture Collection, Regional Technical College, Galway, Ireland. The test species used were *Skeletonema costatum* (Greville) Cleve, and *Dunaliella tertiolecta* (Butcher).

Continuous cultures were grown in 1-L working volume round bottom flasks (Schott, Mainz, Germany). Cultures were aerated at 500 ml $min^{-1}$. Aeration also provided mixing and removed excess culture through the air exhaust. Medium was added by a peristaltic pump. Because of the low flow rates which were required, constant speed pumps (101 pumphead: Watson-Marlow, Falmouth, UK and drive motor 341-696: RS Components, Corby, UK: flow rate, 13 ml $min^{-1}$) were switched on for a short period each hour. Switching was controlled by an asymmetrical recycling digital timer (343-997:

Correspondence to: Dr J.W. Patching, Marine Microbiology Section, The Martin Ryan Marine Science Institute, University College Galway, Ireland.

RS Components). Culture vessels were immersed in a glass-sided constant temperature bath and illuminated by a bank of 18-W 'coolwhite' fluorescent lights (Thorn, Pluslux 4000) providing an irradiance of approximately 53 $\mu$mol $m^{-2}$ $s^{-2}$ on the proximal side of the cultures and 11 $\mu$mol $m^{-2}$ $s^{-2}$ on the opposite side.

Phytoplankton were grown in continuous culture at 17 °C on Guillard and Rhythers' 'f/2' medium [13] with a limiting concentration of silicate added (as 30 mg $L^{-1}$ sodium metasilicate) for *S. costatum*. *D. tertiolecta* was light limited. *S. costatum* was grown at a dilution rate of 0.072 $h^{-1}$ and *D. tertiolecta* was grown at a dilution rate of 0.096 $h^{-1}$. Cell counts were made using a haemocytometer with Neubauer improved rulings (Brand, Wertheim, Germany). Chlorophyll *a* determinations were made according to Lorenzen [18].

Rates of respiration and photosynthesis were measured using a two-channel Biological Oxygen Monitor (Model YSI 5300, YSI Inc., OH 45387, USA) which utilized Clarke-type polarographic oxygen probes in magnetically-stirred sample chambers. The chambers were immersed in a glass constant-temperature bath maintained at 17 °C. When necessary, they were illuminated at an irradiance of approximately 200 $\mu$mol $m^{-2}$ $s^{-2}$ (Philips 11-W, PL-S). Samples of *S. costatum* (40 ml) or *D. tertiolecta* (20 ml) were centrifuged (Mistral 1000, MSE Scientific Instruments, Loughborough, UK) at 3650 × *g* for 1 min and resuspended in 10 ml of sterile 'f/2' medium. This was then divided into two 5-ml aliquots, one of which was exposed to phenyltin. Resulting rates of oxygen evolution or consumption were compared to those obtained with the other aliquot to which an equivalent amount of the carrier substance, ethanol, had been added.

Signals from the biological oxygen monitor were sent to a two-channel chart recorder which produced stright line graphs of changes in oxygen concentration. Rates of net photosynthesis and respiration were calculated from the slope of these lines. Since it was not possible to measure both simultaneously, net photosynthesis measurements were made during the periods 5–15, 30–45 and 60–75 min after phenyltin addition, and respiration measurements were made 15–30; 45–60 and 75–90 min after addition. Rates of gross photosynthesis were calculated by subtracting rates of respiration from the preceding rates of net photosynthesis. Results are expressed as the mean percentage of the control plus or minus the standard error of the mean of at least six separate experiments.

## RESULTS

### *Photosynthesis*

Figure 1 shows dose response curves for gross photosynthesis in *Dunaliella tertiolecta* when exposed to TPhT. The lowest concentration tested (0.84 $\mu$M TPhT) had no significant effect. With higher concentrations of TPhT, delaying the measurement of photosynthesis for a period after addition of the organotin caused a change in the apparent response of the organism. Dose response curves prepared from data collected immediately after addition (0–30 min) showed a slight but significant stimulation of gross photosynthesis by 2.1 $\mu$M TPhT. Higher concentrations produced an increasing inhibition of

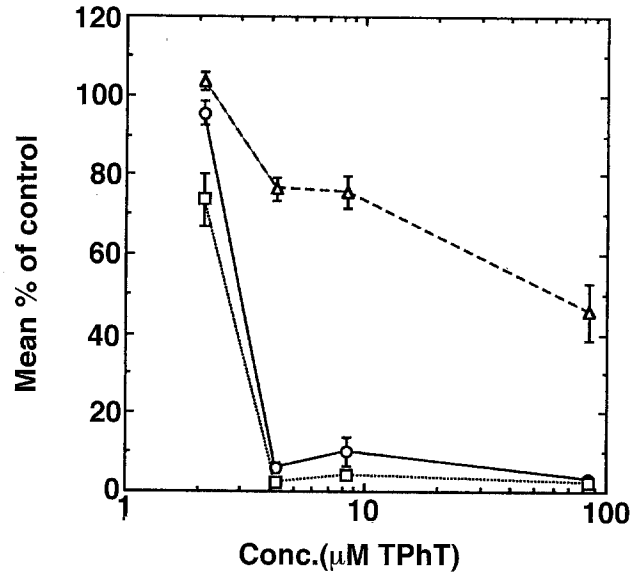


Fig. 1. The effect of TPhT on gross photosynthesis by *D. tertiolecta*: (--△--), 5–15 min; (—○—), 30–45 min; (···□···), 60–75 min after addition.

gross photosynthesis. Inhibition of approximately 50% was produced by exposure to 84 $\mu$M TPhT. Exposure of *D. tertiolecta* to concentrations of TPhT between 4.2 and 84 $\mu$M for a period before measurements were taken, caused a significant increase in inhibition. Rates of gross photosynthesis fell to 10% or less of control values after 30 min exposure and 4% or less after 60 min exposure. With a TPhT concentration of 2.1 $\mu$M the effect of exposure was less marked. An exposure of 60 min resulted in a reduction of photosynthesis to approximately 80% of the control value.

Gross photosynthesis in *S. costatum* was much more sensitive to TPhT (Fig. 2). Exposure did not enhance inhibition in *S. costatum*. The TPhT concentration which inhibited gross photosynthesis in *S. costatum* to 50% of the control value was approximately 80 nM. For comparison, the equivalent values obtained with *D. tertiolecta* were 84 $\mu$M TPhT (immediately after addition) and 2.8 $\mu$M TPhT (60 min exposure).

By comparison with the effects of TPhT, gross photosynthesis in both *D. tertiolecta* and *S. costatum* was relatively resistant to DPhT. Concentrations up to 840 $\mu$M DPhT caused no significant change in gross photosynthesis by *D. tertiolecta*. This was the highest concentration of DPhT which could be used with our methods, since higher concentrations precipitate out of solution [8]. In contrast to the results obtained with TPhT, repression of gross photosynthesis in *S. costatum* by DPhT was enhanced if measurement was delayed for a period after the addition of the organotin (Fig. 3). The DPhT concentration which inhibited gross photosynthesis by 50% in *S. costatum* after 60 min exposure was about 30 times greater than that of TPhT.

### *Respiration*

The effect of TPhT on respiration by *D. tertiolecta* is shown in Fig. 4. These results show several similarities with those

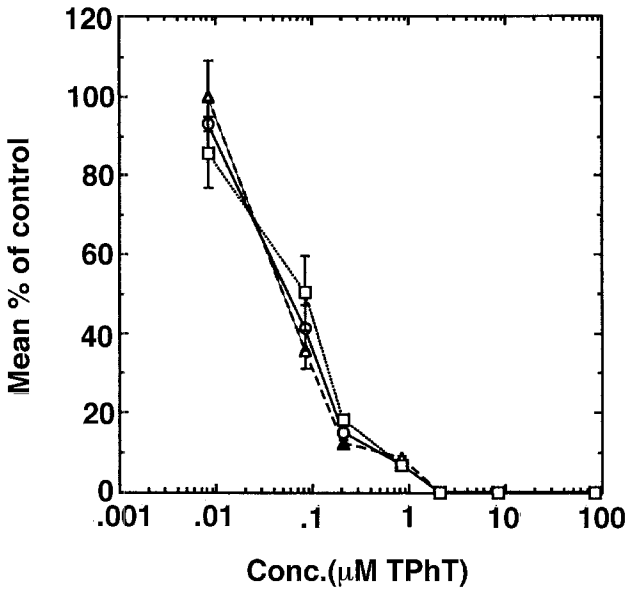


Fig. 2. The effect of TPhT on gross photosynthesis by *S. costatum*: (--△--), 5–15 min; (—○—), 30–45 min; (···□···), 60–75 min after addition.

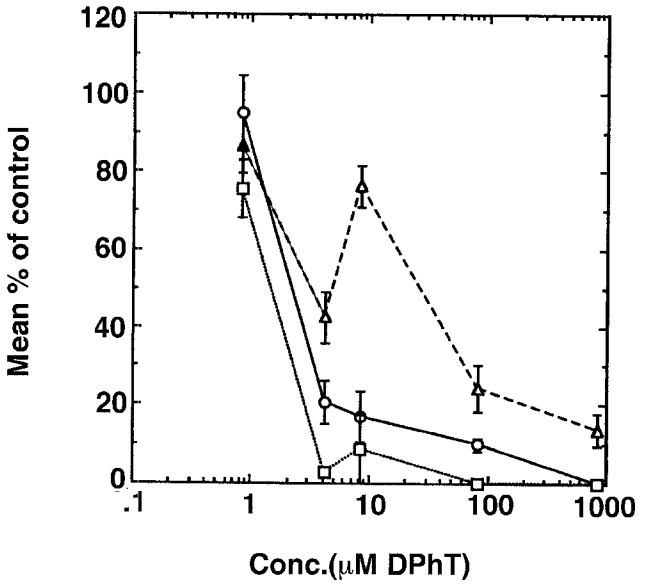


Fig. 3. The effect of DPhT on gross photosynthesis by *S. costatum*: (--△--), 5–15 min; (—○—), 30–45 min; (···□···), 60–75 min after addition.

recorded for gross photosynthesis by the same organism. The range of concentrations over which increasing inhibition was observed was the same. There is evidence of an increase in inhibition with exposure to TPhT. This is shown most clearly by TPhT concentrations between 4.2 and 8.4 μM and to a lesser extent by 2.1 and 84 μM TPhT. Almost the same TPhT concentration inhibited respiration and gross photosynthesis to 50% after 30 min exposure.

Though TPhT concentrations from 8.4 nM to 84 μM produced an overall decrease in the respiration of *S. costatum* from 100% to between 20% to 40% of controls, the decrease

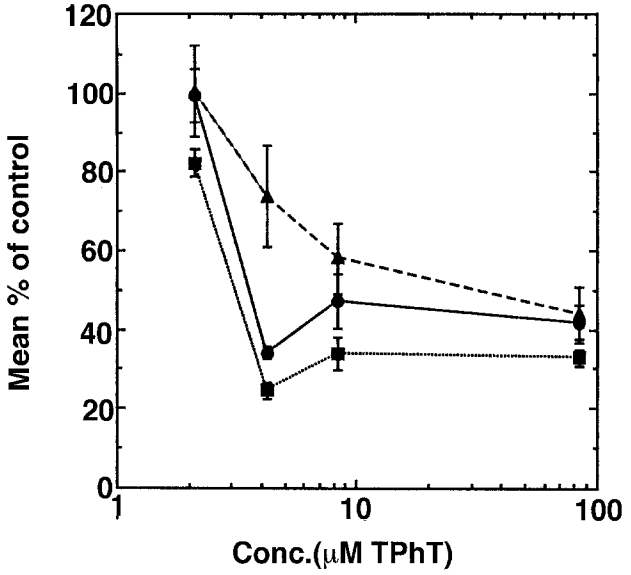


Fig. 4. The effect of TPhT on respiration by *D. tertiolecta*: (--▲--), 15–30 min; (—●—), 45–60 min; (···■···), 75–90 min after addition.

was gradual and showed some irregularities, making precise comparisons with other results difficult (Fig. 5). It would appear, however, that respiration in *S. costatum* is more resistant to TPhT than is photosynthesis, which was completely inhibited by TPhT concentrations greater than 2.1 μM. Unlike photosynthesis, there was some evidence of enhancement of the effects of TPhT if measurement was delayed after addition, but this was less marked than with *D. tertiolecta*, and not found with the lower TPhT concentrations used. As with photosynthesis, respiration was more sensitive to TPhT in *S. costatum* than in *D. tertiolecta*. TPhT concentrations greater than 2.1 μM were necessary to cause significant inhibition in

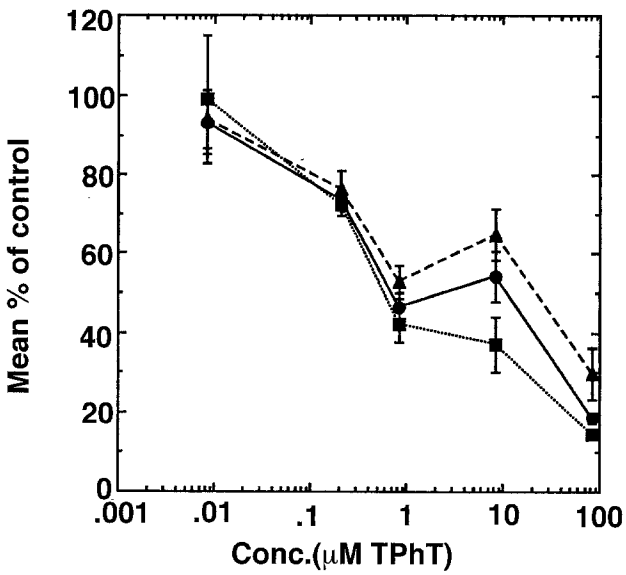


Fig. 5. The effect of TPhT on respiration by *S. costatum*: (--▲--), 15–30 min; (—●—), 45–60 min; (···■···), 75–90 min after addition.

the latter, whereas the same concentration reduced respiration to between 40% and 60% in *S. costatum*.

As was found with photosynthesis, respiration in both *D. tertiolecta* and *S. costatum* was relatively resistant to inhibition by DPhT. Again, concentrations up to 840 μM DPhT caused no significant change in gross photosynthesis by *D. tertiolecta*. Figure 6 shows the effects of DPhT in concentrations between 0.84 μM and 840 μM on respiration in *S. costatum*. Immediately after addition, these concentrations reduced respiration to between 70% and 80% of control values, with no significant correlation between concentration and degree of inhibition. For concentrations between 84 μM and 840 μM DPhT, inhibition was a function of the length of exposure, but at the maximum concentration of DPhT (840 μM) and length of exposure (60 min) used, respiration was still at 30% of the control value.

## DISCUSSION

In general, TPhT at concentrations in the nM to μM range inhibited both photosynthesis and respiration in our test organisms. Exceptionally, photosynthesis by *D. tertiolecta* was subject to a slight but significant stimulation, immediately after the addition of 2.1 μM TPhT. This phenomenon is called hormesis, which has been defined as 'a stimulatory effect of subinhibitory concentrations of any toxic substance on any organism' [20]. Hormesis has also been reported in *D. tertiolecta* exposed to aromatic compounds [11] and in *Pavlova lutheri* exposed to low levels of tributyltin-oxide [6]. Whilst the general trend was for increasing concentrations of either TPhT or DPhT to produce increasing levels of inhibition, irregularities were sometimes noted. The immediate effect of DPhT on photosynthesis in *S. costatum* (Fig. 3) is an example of this. We are unable to explain this, but it would not appear to be due to experimental error. The mean values and standard deviations are derived from completely separate experiments. TPhT solutions were prepared afresh for each experiment to ensure that the effect was not due to errors in preparing dilutions.

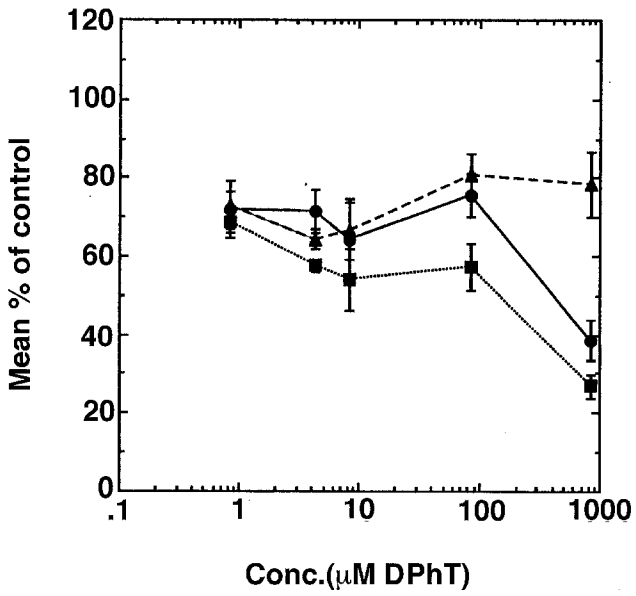


Fig. 6. The effect of DPhT on respiration by *S. costatum*: (--▲--), 15–30 min; (—●—), 45–60 min; (···■···), 75–90 min after addition.

Our results show that levels of TPhT inhibiting gross photosynthesis were two to three orders of magnitude higher for *D. tertiolecta* than for *S. costatum*. Similar results were found with respect to respiration. Comparisons between our results and those of others is difficult, since the latter typically deal with the effects of organotins on the viability and growth of microalgae. Studies on the effects of organotins on phytoplankton growth have shown that *Skeletonema costatum* is more sensitive than *D. tertiolecta* with respect to the effects of tributyltin-oxide (TBTO). Virtually no growth occurred in *S. costatum* cultured in the presence of 3.3 nM TBTO for up to 10 days, whereas growth of *D. tertiolecta* and *Pavlova lutheri* was only slightly reduced by this concentration [6]. The fresh-water chlorophyte, *Ankistrodesmus falcatus* had an $IC_{50}$ value of approximately 0.005 μM on exposure to TPhT [23]. This organism appears to be as sensitive to TPhT as *S. costatum*, and hence considerably less resistant than the marine chlorophyte *D. tertiolecta*, but toxicity can be significantly affected by the physical qualities (Temperature, pH, salinity etc.) of the aquatic environment [9].

Avery et al. [4] studied the effects on photosynthesis in cyanobacteria exposed to TPhT. Of the two species studied, *Plectonema boryanum* appeared to be more resistant to TPhT than *Anaebaena cylindrica*. The $IC_{50}$ for photosynthesis in *P. boryanum* was 13 μM, whereas in *A. cylindrica* it was 5 μM TPhT.

Trisubstituted tins are generally more toxic than mono- or tetra-substituted components [14]. In the present study TPhT also proved to be considerably more toxic than DPhT. Both photosynthesis and respiration in *D. tertiolecta* were resistant to DPhT at concentrations up to its limit of solubility (0.84 mM). With the more sensitive *S. costatum*, inhibitory levels of DPhT were one to two orders of magnitude higher than for TPhT. Determinations of the concentrations of TPhTCl and DPhTCl which inhibited, to 50%, growth ($EC_{50}$) of the diatoms *S. costatum* and *Thalassiosira pseudonana* after 72 h exposure would seem to confirm our findings [22]. $EC_{50}$ values for *T. pseudonana* were 0.11 μM (DPhTCl) and 0.003 μM (TPhTCl). *S. costatum* showed $EC_{50}$ values with respect to DPhTCl and TPhTCl of 0.10 μM and 0.002 μM respectively.

In the present study, inhibition of photosynthesis and respiration was often progressive over a period up to 90 min after the addition of TPhT. This phenomenon may be caused by the need for TPhT to reach its target site within the cell before producing any observable effect. The effect was variable in its extent but was more noticeable with *D. tertiolecta* than with *S. costatum*. This may correlate with the greater resistance of *D. tertiolecta* to inhibition and be related to differences between the two organisms with respect to TPhT accumulation. Blair et al. [7] postulated that the accumulation of either Sn(IV) or TBTO by bacteria is a chemical sorption process rather than active transport. For algae, uptake has also been shown to be an adsorption process, with inactive cells accumu-

lating more toxicant than viable cells and 60% of the bound toxicant being released on treatment with EDTA. 85% of the bound tin was associated with a polysaccharide fraction [24].

Avery et al. [3] showed that the cyanobacteria, *Synechocystis* PCC 6803 and *Plectonema boryanum* and the microalga, *Chlorella emersonii*, biosorb 5 mM TPhT faster than any trialkyltins tested, including TBT. They suggested that the organic moieties of organotins become associated with the surfaces of biological membranes rather than penetrating them. Because the pattern of toxicity of the organotins tested was similar to the pattern of biosorbtion to the same cyanobacteria, it was thought that triorganotin compounds exert their toxic effects primarily through interactions with membrane lipids. This would support the proposal of Cooney and Wuertz [9] that toxicity is related to the total surface area and lipid solubility of trisubstituted tins.

The few investigations which have been carried out on the levels and fate of phenyltins in the environment show that they may be found in aquatic environments at levels which would potentially inhibit our test organisms. Triphenyltin concentrations of approximately 54–240 $\mu$M have been reported in Mediterranean coastal waters, with most of the compound associated with the particulate phase [1].

The persistence of TPhT must also be considered when assessing its potential effects on phytoplankton. Phenyltins degrade in the presence of light [5]. The discovery of a soil bacterium (*Pseudomonas putida* no. C) capable of promoting the decomposition of phenyltins [21] suggests that biodegradation may also influence persistence. In Tokyo Bay, TPhT in mussels has been decreasing exponentially with a half-life of four months since the prohibition of its usage in marine antifouling paints in 1988. It is possible that sediments act as reservoirs for TPhT in marine environments. TPhT levels in the top 5 cm of Tokyo Bay sediment were 4 $\mu$g per kg sediment (wet weight) in 1991 [19]. Though this level was not significantly different from that observed in 1990, the presence of 30 $\mu$g MPhT per kg wet weight of sediment in the same area would indicate that degradation was occurring. Shiraishi and Soma [19] proposed that the rate of TPhT degradation is slower than for TBT and that the TPhT found in Tokyo Bay in 1992 may have leached from antifouling paints before the 1988 ban.

As we have shown, microalgae may display different degrees of sensitivity to phenyltins. In the wild, where species composition and abundance of phytoplankton are constantly changing, the presence of phenyltins could influence the spatial and temporal localization of algal blooms.

## ACKNOWLEDGEMENTS

The authors would like to thank Brian Ottway (Regional Technical College, Galway, Ireland) for supplying the phytoplankton cultures and offering technical advice. We thank Professor Joseph Cooney (Environmental Sciences Program, UMASS at Boston, USA) for his helpful comments and for supplying the triphenyltin-chloride.

## REFERENCES

1 Alzieu, C., P. Michel, I. Tolosa, E. Bacci, L. D. Mee and J.W. Readman. 1991. Organotin compounds in the Mediterranean—a continuing cause for concern. Mar. Environ. Res. 32: 261–270.
2 Ascher, K.R.S. and S. Nissim. 1964. Organo-tin compounds and their potential use in insect control. World. Rev. Pest. Control. 3: 188–211.
3 Avery, S.V., G.A. Codd and G.M. Gadd. 1993. Biosorption of tributyltin and other organotin compounds by cyanobacteria and microalgae. Appl. Microbiol. Biotechnol. 39: 812–817.
4 Avery, S.V., M.E. Miller, G.M. Gadd, G.A. Codd and J.J. Cooney. 1991. Toxicity of organotins towards cyanobacterial photosynthesis and nitrogen fixation. FEMS Microbiol. Lett. 84: 205–210.
5 Barnes, R.D., A.T. Bull and R.C. Poller. 1973. Studies on the persistence of the organotin fungicide fentin acetate (tri-phenyltin acetate) in the soil and on surfaces exposed to light. Pectic. Sci. 4: 305–317.
6 Beaumont, A.R. and P.B. Newman. 1986. Low levels of tributyltin reduce growth of marine micro-algae. Mar. Pollut. Bull. 17: 457–461.
7 Blair, W.L., G.J. Olson, F.E. Brinkman and W.P. Iverson. 1982. Accumulation and fate of tri-*n*-butyltin cation in estuarine bacteria. Microb. Ecol. 8: 241–251.
8 Boopathy, R. and L. Daniels. 1991. Pattern of organotin inhibition of methanogenic bacteria. Appl. Environ. Microbiol. 57: 1189–1193.
9 Cooney, J.J. and S. Wuertz. 1989. Toxic effects of tin compounds on microorganisms. J. Ind. Microbiol. 4: 375–402.
10 Devries, H., A.H. Penninks, N.J. Snoeij and W. Seinen. 1991. Comparative toxicity of organotin compounds to rainbow trout (*Oncorhynchus mykiss*) yolk-sac fry. Science of the Total Environment 103: 229–243.
11 Dunstan, W.M., L.P. Atkinson and J. Natoli. 1975. Stimulation and inhibition of phytoplankton growth by low molecular weight hydrocarbons. Marine Biol. 31: 305–310.
12 Fent, K. and J. Hunn. 1991. Phenyltins in water, sediment, and biota of freshwater marinas. Environ. Sci. Technol. 25: 956–963.
13 Guillard, R.L. and J.H. Rhyther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, *Detonula confervacia*. Can. J. Microbiol. 8: 229–239.
14 Hallas, L.E. and J.J. Cooney. 1981. Tin and tin resistant microorganisms in Chesapeake Bay. Appl. Environ. Microbiol. 41: 446–471.
15 Kerk, G.J.M.v.d. and J.G.A. Liujten. 1954. Investigations of organo-tin compounds. III. The biocidal properties of organo-tin compounds. J. Appl. Chem. 6: 314–319.
16 Kerk, G.J.M.v.d. and J.G.A. Liujten. 1956. Investigations of organo-tin compounds. V. The preparation and antifungal properties of unsymmetrical tri-*n*-alkyltin acetates. J. Appl. Chem. 6: 56–60.
17 Laurence, O.S., J.J. Cooney and G.M. Gadd. 1989. Toxicity of organotins towards the marine yeast *Debaromyces hansenii*. Microb. Ecol. 17: 275–285.
18 Lorenzen, C.J. 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnol. Oceanogr. 12: 343–346.
19 Shiraishi, H. and M. Soma. 1992. Triphenyltin compounds in mussels in Tokyo Bay after restriction of use in Japan. Chemosphere 24: 1103–1109.
20 Stebbing, A.R.D. 1982. Hormesis—the stimulation of growth by low levels of inhibitors. The Science of the Total Environment 22: 213–234.

21 Visoottiviseth, P., K. Kruawan and A. Bhumiratana. 1992. Kinetic studies of degradation of triphenyltin pesticides by *Pseudomonas putida* No. C. J. Sci. Soc. Thailand 18: 67–72.

22 Walsh, G.E., L.L. McLaughlin, E.M. Lores, M.K. Louie and C.H. Deans. 1985. Effects of organotins on growth and survival of two marine diatoms, *Skeletonema costatum* and *Thalassiosira pseudonana*. Chemosphere 14: 383–392.

23 Wong, P.T.S., Y.K. Chau, O. Kramar and G.A. Bengert. 1982. Structure-toxicity relationship of tin compounds on algae. Can. J. Fish. Aquat. Sci. 39: 483–488.

24 Wong, P.T.S., R.J. Maguire, Y.K. Chau and O. Kramer. 1984. Uptake and accumulation of tin by a freshwater-alga, *Ankistrodesmus falcatus*. Can. J. Fish. Aquat. Sci. 41: 1570–1574.