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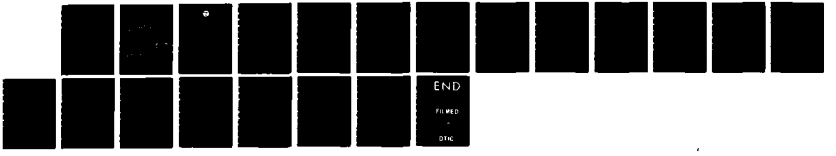

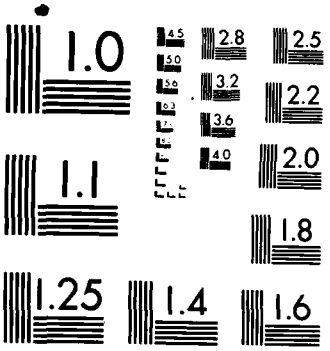

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THE EFFECTS OF BIS(TRI-N-BUTYL TIN) OXIDE ON THREE SPECIES OF MARINE PHYTOPLANKTON

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The effects of three concentrations of bis(tri-n-butyltin) oxide (TBTO) (1.5, 3.0, and 6.0 ppb) on three species of marine phytoplankton were assessed. Parameters measured were in vivo fluorescence and DCMU induced fluorescence. Fluorescence ratios were calculated from these values. The effects of TBTO exposure varied considerably from species to species. Gymnodinium splendens was the most sensitive, affected by all concentrations of TBTO. Dunaliella sp. demonstrated inhibition of growth at 3.0 and 6.0 TBTO after 3 days. No measurable effects were detected for Phaeodactylum tricornutum at any of the TBTO exposures. These data indicate the effect of TBTO on marine phytoplankton may be species specific.

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EXECUTIVE SUMMARY

OBJECTIVE

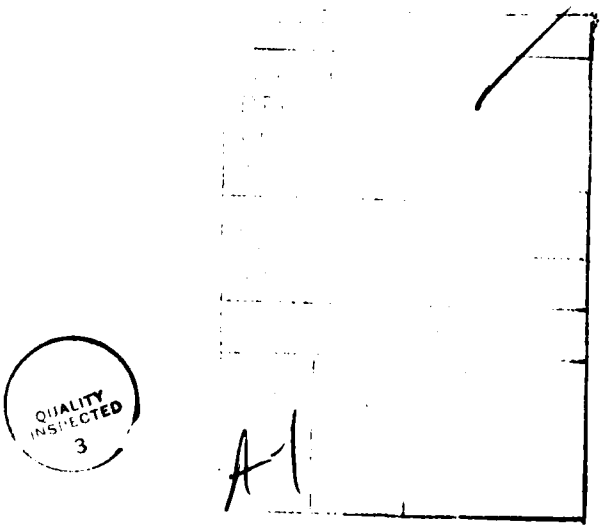
Assess the effects of three concentrations of bis(tri-n-butyltin) oxide (TBTO) (1.5, 3.0, and 6.0 ppb) on three species of marine phytoplankton. To better define the toxic range of TBTO to phytoplankton, a 72-hour assay was performed in which three species of phytoplankton - Gymnodinium splendens (Division Pyrrophyta), Dunaliella sp. (Division Chlorophyta), and Phaeodactylum tricornutum (Division Bacillariophyta) - were exposed to each of three concentrations of TBTO.

RESULTS

TBTO killed all Gymnodinium splendens cells at each concentration tested; in Dunaliella sp. growth was inhibited at 1.5-ppb TBTO and stopped at 3.0- and 6.0-ppb TBTO after 3 days; no measurable changes in Phaeodactylum tricornutum growth rates were detected at any of the concentrations tested.

CONCLUSIONS

The tolerance of phytoplankton to low levels of TBTO varies considerably from species to species. Dinoflagellates have a minimal tolerance to TBTO. Introduction of this toxicant can result in an immediate response as demonstrated by reduced fluorescence ratios. The green flagellate Dunaliella sp. is more tolerant to TBTO and requires more time to produce measurable effects. An estimated EC_{50} of 1.5 was obtained for Dunaliella sp. Of the three species tested, diatoms are the most resistant. No measurable effects were obtained for P. tricornutum at any of the TBTO concentrations used. After 72 hours, however, there was a reduction in fluorescence ratios. This reduction cannot be easily attributed to TBTO exposure as all other measurements suggest no deleterious effects. This study does indicate potential toxicity to some species of marine phytoplankton at low-level TBTO exposure.



INTRODUCTION

As part of the Navy's research program on the effects of organotin-based antifouling coatings on marine organisms, three species of phytoplankton were assessed for their tolerance to low levels of bis(tri-n-butyltin) oxide (TBTO). TBTO is the toxic component of many organotin-based antifouling coatings. Previous work with TBTO and the green flagellate Dunaliella sp. resulted in no measurable effects at 0.5 ppb, reduced growth rates at 2.0 ppb, and death of the entire culture at 10.0 ppb.[1] Our earlier studies with Dunaliella sp. suggest 5.0-ppm TBTO is lethal. The gap between concentrations assessed in these two initial tests was too great to determine the actual differences between sublethal and lethal concentrations.

To better define the toxic range of TBTO to phytoplankton, a 72-hour assay was performed in which three species of phytoplankton, each representing a different taxonomic division, were exposed to each of three concentrations of TBTO.

METHODS

TEST ORGANISMS AND CULTURE PREPARATION

The organisms used in this study were Gymnodinium splendens (Division Pyrrophyta), Dunaliella sp. (Division Chlorophyta), and Phaeodactylum tricornutum (Division Bacillariophyta). Unialgal cultures of each species were obtained from the Southwest Fisheries Center, La Jolla, California. Stock cultures were routinely maintained at the Naval Ocean Systems Center Marine Science Laboratory in exponential-phase growth on Guillard's F/2 medium under constant temperature (18 °C) and illumination (1.89 milliwatts/cm^2). Standard culture medium was prepared by enriching 0.45-micron filtered seawater, obtained through the laboratory seawater system, with nutrients, vitamins, and trace metals according to the procedure of Guillard and Ryther (1962). After enrichment, the medium was sterilized by filtering through 0.22-micron Millipore filters.

For each species, 500 ml of media were inoculated with 5 ml of stock algae 5 days prior to the start of the test. Since growth rate during exponential phase was to be the parameter monitored after TBTO exposure, this 5-day period allowed the newly inoculated cultures to progress from lag-phase to exponential-phase growth. Initial cell densities of 2.5 by 10^3, 5.0 by 10^4, and 5.0 by 10^5 cells/ml were used for G. splendens, Dunaliella, and P. tricornutum, respectively.

The effect of TBTO on phytoplankton was determined by comparing growth rates and photosynthetic capabilities of control organisms to treatment organisms. The growth rate for each species at each TBTO concentration was

[1] This previous work was accomplished under a 1981-82 independent research program entitled the Fate and Effects of Organotin in the Marine Environment.

determined from measurements of in vivo fluorescence. In unstressed organisms, cells use only a portion of available light, the excess light being reemitted as fluorescence. An increase in in vivo fluorescence with time indicates an actively photosynthesizing culture that is healthy and growing.

The photosynthetic capability of phytoplankton was determined by comparing the yield of (3-(3,4-dichlorophenyl)-1,1-dimethly urea) (DCMU)-induced fluorescence to in vivo fluorescence, as a fluorescence ratio (FR). DCMU is an herbicide that inhibits photosynthesis by blocking electron transport at the level of photosystem II. This blockage results in a constant level of fluorescence directly proportional to the amount of chlorophyll-a present (Bannister, 1967; Slovacek & Hannan, 1977). This is in contrast to in vivo measurements, which can fluctuate according to the physiological state of the algal cells, and is less dependent on chlorophyll-a concentration.

The FR, defined as the ratio of DCMU-induced fluorescence to in vivo fluorescence, reportedly can be used to estimate the following: (1) chlorophyll-a levels (Slovacek & Hannan, 1977); (2) physiological condition of phytoplankton (Blasco & Dexter, 1972; Halldal & Halldal, 1973; Cullen & Renger, 1979); (3) efficiency of photochemical activity (Kiefer & Hodson, 1974); and (4) photosynthetic activity (Roy & Legendre, 1979). Roy & Legendre (1979) indicated that for a given level of light, the FR can be a useful indicator of nutritional stress. The in vivo fluorescence output may vary according to the physiological state of the organism, the amount of light received, and other physical and biological parameters. Thus, in vivo fluorescence measurements must be coupled with another indicator of plankton physiology to delineate the effects of toxicants. Since the physical parameters were constant in this experiment (i.e., temperature and light), the evaluation of both in vivo fluorescence and the FR should provide an accurate estimate of the effects of TBTO on phytoplankton growth rates and photosynthetic capability.

Both in vivo and DCMU-induced fluorescence measurements were made at 24-hour intervals with a Turner Designs fluorometer (Model 10-000R).

TEST CONDITIONS

Test cultures were maintained in glass-stoppered, 10-ml (13 by 100 mm) KIMAX glass tubes. The tubes were soaked for 24 hours in RBS-35 biological cleaning solution, rinsed six times with hot tap water, and then rinsed six times with deionized water. A 24-hour soak with filtered seawater followed the washing regime.

For each species, 15 controls and 15 replicates per TBTO concentration were prepared by delivering 6.5 ml of phytoplankton culture to the clean tubes. The stock cultures were continuously mixed with a magnetic stirrer to maintain homogeneity during delivery. After distribution of phytoplankton to the test tubes, the test was initiated by adding appropriate volumes of the TBTO stock to all treatment tubes. In vivo fluorescence measurements were then made on all replicates. For each condition, three tubes were randomly selected for DCMU-fluorescence measurements. These replicates were discarded after the addition of DCMU and fluorescence measurements.

The remaining replicates were held in a wire test tube holder and suspended above a bank of fluorescent bulbs to provide even illumination (1.52 milliwatts/cm^2). Bottom lighting is superior to side lighting because it eliminates most problems associated with side lighting like shadowing, decreasing illumination, and filtering selected wavelengths. The temperature was maintained at 18 °C.

PREPARATION OF TBTO

A TBTO-saturated seawater stock solution was prepared by adding 0.1 ml of TBTO (M&T Company) to 500 ml of 0.45-micron filtered seawater. This was placed on a magnetic stirrer for 24 hours to yield a saturated solution. Solutions were filtered through two layers of premoistened, fluted filter paper, and the concentration of TBTO was determined by methyl isobutylketone (MIBK) solvent extraction and Graphite Furnace Atomic Absorption Spectrophotometry. From this stock a measured solution of 260-ppb TBTO was prepared. Appropriate volumes of this 260-ppb stock were added to each tube to obtain test concentrations of 1.5-, 3.0-, and 6.0-ppb TBTO.

DATA ANALYSIS

The procedures used in the data analysis were the same for each species monitored in this study. The first step in data analysis consisted of graphing in vivo fluorescence values against time. Graphs were generated by an HP-21-MK minicomputer equipped with a Calcomp Model 565 plotter. Trends in the effects of TBTO on growth were visualized by connecting the mean in vivo values over the 72-hour test period. Slopes of the growth curves generated for algae exposed to the three concentrations of TBTO were then compared by visual examination to the slope of the growth curve for the control algae. If significant differences in slope between the control and treatments were not obvious, an analysis of covariance (ANCOVA) was then performed on log-transformed data.

The graphical evaluation and ANCOVA of the in vivo fluorescence data were followed by the calculation and comparison of the FRs. Mean FRs were determined for each species at each concentration and plotted to evaluate trends over time. These trends were then compared to in vivo fluorescence trends to determine the effects of TBTO on a given species of phytoplankton.

RESULTS

Initial cell densities and general responses to TBTO exposure for each species are presented in Table 1. In summary, TBTO killed all Gymnodinium splendens cells at each concentration tested; in Dunaliella sp. growth was inhibited at 1.5-ppb TBTO and stopped at 3.0- and 6.0-ppb TBTO after 3 days; and no measurable changes in Phaeodactylum tricornutum growth rates were detected at any of the concentrations tested. The fluorescence ratios calculated for each control species indicate high photosynthetic activity without stress. The slight decline observed in the FR after 24 hours may be attributable to self-shading due to increased cell density (Roy & Legendre, 1979).

Table 1. Initial cell densities and effects of exposure to TBTO on Gymnodinium splendens, Dunaliella sp., and Phaeodactylum tricornutum.

		TBTO Concentrations Assessed											
		1.5 ppb				3.0 ppb				6.0 ppb			
		Time of assessment (hours)											
	Initial Density Cells/ml	0	24	48	72	0	24	48	72	0	24	48	72
G. splendens	2.3E+03	-	*	*	**	*	**	**	**	*	**	**	**
Dunaliella sp.	5.4E+04	0	-	-	*	0	-	**	**	0	-	**	**
P. tricornutum	5.0E+05	0	0	0	0	0	0	0	-	0	0	0	0

KEY:

0 = No effect
- = Slight reduction in growth rates and/or fluorescence ratios
* = Significant difference in growth rates and/or significantly lower fluorescence ratios
** = Culture dead

Coupling the in vivo fluorescence data with the FRs suggests that the control replicates were actively photosynthesizing and growing. For each species, the mean in vivo fluorescence values and mean FRs with standard deviation are presented in Table 2.

GYMNODINIUM SPLENDENS

Based on changes in in vivo fluorescence, growth was minimal for the control G. splendens (Figure 1). However, stock cultures routinely maintained at this laboratory have a much longer doubling time than that of other species. Therefore, no increase in cell densities was expected during this short 3-day test. Visual examination of the in vivo fluorescence data shown in Figure 1 indicates this dinoflagellate was highly sensitive to TBTO. Even at the lowest concentration assessed, growth was inhibited within the first 24 hours. No signs of cell recovery were observed for G. splendens exposed to any of the three TBTO concentrations. No statistical analyses were performed on these data because each concentration of TBTO killed all G. splendens cells. In all cases exposure to TBTO resulted in death of the culture within 72 hours.

Table 2. Mean in vivo fluorescence and fluorescence ratios ± standard deviation for *Gymnodinium splendens*, *Dunaliella* sp., and *Phaeodactylum tricornutum* at each TBTO concentration assessed.

	In Vivo Fluorescence			
	0 hours	24 hours	48 hours	72 hours
G. splendens				
Controls	0.340±0.023	0.390±0.018	0.450±0.022	0.480±0.017
1.5-ppb exposure	0.380 0.095	0.070 0.067	0.040 0.067	0.010 0.002
3.0-ppb exposure	0.620 0.056	0.030 0.003	0.010 0.002	0.010 0.000
6.0-ppb exposure	0.670 0.088	0.020 0.004	0.010 0.001	0.010 0.001
Dunaliella sp.				
Controls	0.240±0.017	0.550±0.061	1.290±0.119	1.890±0.133
1.5-ppb exposure	0.200 0.019	0.420 0.042	0.620 0.097	0.230 0.211
3.0-ppb exposure	0.210 0.014	0.330 0.016	0.170 0.037	0.020 0.008
6.0-ppb exposure	0.180 0.028	0.280 0.017	0.050 0.011	0.010 0.002
P. tricornutum				
Controls	0.840±0.018	2.510±0.052	6.030±0.238	7.600±0.559
1.5-ppb exposure	0.840 0.023	2.630 0.197	5.880 0.395	10.320 1.537
3.0-ppb exposure	0.850 0.028	2.610 0.142	5.410 0.428	11.230 0.899
6.0-ppb exposure	0.850 0.044	2.650 0.081	5.040 0.246	10.570 0.840

	Fluorescence Ratios			
G. splendens				
Controls	3.850±0.140	4.110±0.116	4.090±0.175	3.700±0.040
1.5-ppb exposure	2.810 0.760	0.950 0.038	1.000 0.000	1.000 0.000
3.0-ppb exposure	1.000 0.035	0.960 0.028	1.000 0.000	1.000 0.000
6.0-ppb exposure	1.000 0.100	0.990 0.030	1.000 0.000	1.000 0.000
Dunaliella sp.				
Controls	2.830±0.389	2.150±0.965	2.710±0.332	1.840±0.107
1.5-ppb exposure	3.110 0.497	2.100 0.262	2.090 0.100	2.210 1.320
3.0-ppb exposure	2.950 0.196	2.020 0.278	1.000 0.069	1.000 0.041
6.0-ppb exposure	3.040 0.034	1.440 0.080	1.000 0.066	0.950 0.101
P. tricornutum				
Controls	3.260±0.221	3.520±0.115	2.260±0.291	2.310±0.164
1.5-ppb exposure	2.810 0.470	3.230 0.290	2.420 0.236	1.950 0.140
3.0-ppb exposure	3.120 0.133	3.320 0.030	2.190 0.205	1.730 0.830
6.0-ppb exposure	3.220 0.410	2.940 0.144	2.000 0.000	1.700 0.045

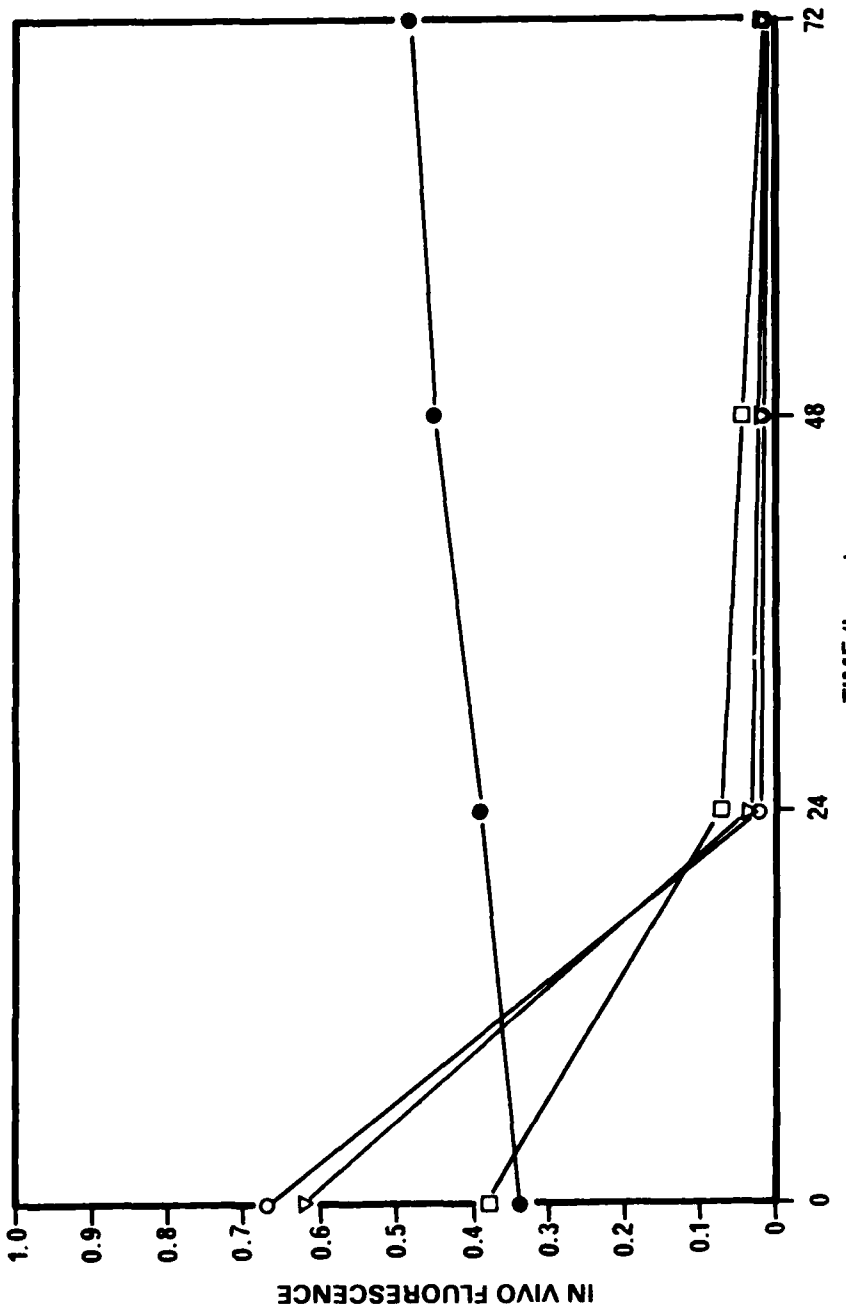


Figure 1. In vivo fluorescence measured during the exposure of *Gymnodinium splendens* to varying concentrations of TBTO (● = Control; □ = 1.5 ppb; ▽ = 3.0 ppb; ○ = 6.0 ppb).

The data presented in Figure 1 and Table 2 for G. splendens demonstrate increases in both in vivo fluorescence and sample variance with increasing TBTO concentration at the beginning of the test. However, after the first 24 hours, both in vivo fluorescence and sample variance decreased considerably. The in vivo fluorescence increase observed immediately after the addition of TBTO was probably a response to stress. By the time the in vivo fluorescence measurements were made, the TBTO had already interfered with photosynthesis. This resulted in elevated in vivo fluorescence upon illumination. The dramatic decrease in in vivo fluorescence 24 hours later was due to the lack of viable cells. G. splendens cells are visible to the naked eye. Visual examination of samples 24 hours after TBTO exposure revealed a cloudy flocculant in lieu of healthy cells. The control samples were characterized by a clear medium in which whole cells could be identified.

The fluorescence ratios for G. splendens (Figure 2 and Table 2) also indicate exposure to TBTO is lethal. The mean value for the control samples is 3.85 with values of 2.8, 1.0, and 1.0 obtained for the 1.5, 3.0, and 6.0 ppb TBTO exposures, respectively. Using the criteria established by Roy & Legendre (1979), this trend indicates some form of stress resulting from photosynthetic debilitation. For the remainder of the experiment, the FRs for G. splendens exposed to all TBTO concentrations remained near 1.0 (Figure 2). These data coupled with the in vivo fluorescence data indicate death of the cells within 24 hours after TBTO exposure.

DUNALIELLA

For Dunaliella sp., exposure to 3.0- and 6.0-ppb TBTO resulted in reduced growth (Figure 3). The control samples exhibited exponential growth over the 72-hour test period. In vivo fluorescence yields over the first 48 hours for Dunaliella exposed to 1.5-ppb TBTO were approximately 50 percent lower than the controls. After 48 hours, in vivo fluorescence for these samples were approximately 90 percent lower than the controls. These data suggest that 1.5-pbb TBTO may be approaching the EC_{50} (concentration affecting 50 percent of the population) for Dunaliella. Exposure to 3.0- and 6.0-ppb TBTO resulted in death of the culture after 48 hours (Table 1).

The FRs (Figure 2 and Table 2) track the in vivo fluorescence data. During the first 24 hours, the FR values for the 6.0-ppb TBTO exposure drop significantly from 3.0 to 1.4. Over the next 48 hours the FR value decreases to 1.0. An FR of 1.0 indicates the lack of viable cells. A 1.0-FR value was also observed for Dunaliella sp. after 48 hours exposure to 3.0-ppb TBTO. The FRs for the 1.5-ppb TBTO exposure decrease from 3.1 to 2.1 over the first 24 hours and then remain nearly constant for the duration of the test. No statistical analyses were performed on these data because visual examination of the data showed that 1.5-ppb TBTO reduced growth while 3.0- and 5.0-ppb TBTO killed all test Dunaliella sp.

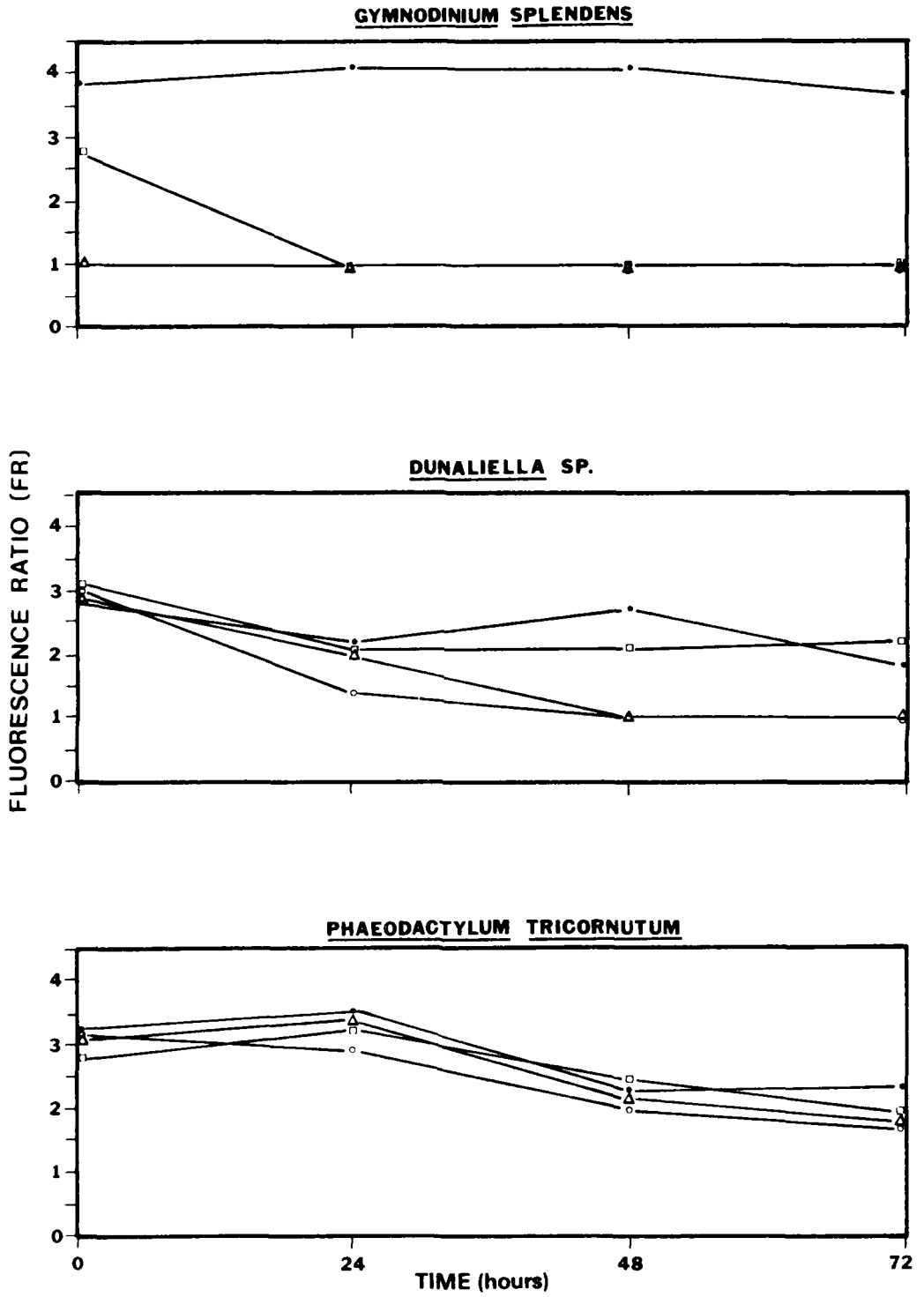


Figure 2. Fluorescence ratios determined for phytoplankton exposed to TBTO (● = Control; □ = 1.5 ppb; △ = 3.0 ppb; ○ = 6.0 ppb).

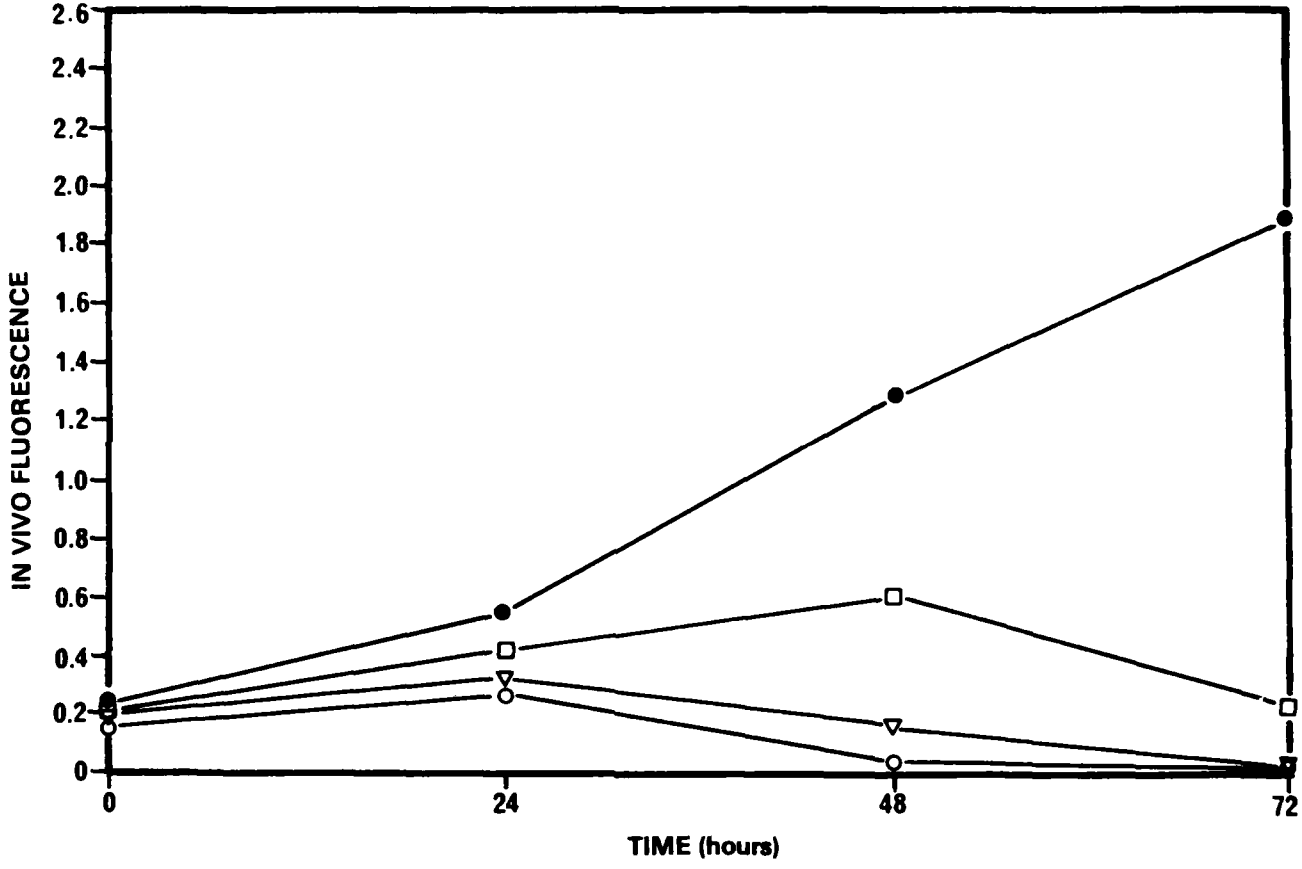


Figure 3. In vivo fluorescence measured during the exposure of *Dunaliella* sp. to varying concentrations of TBTO (● = Control; □ = 1.5 ppb; ▽ = 3.0 ppb; ○ = 6.0 ppb).

PHAEODACTYLUM TRICORNUTUM

P. tricornutum growth rates were not affected by exposure to TBTO at any of the concentrations tested. These trends were observed for both in vivo fluorescence (Figure 4) and the FRs (Figure 2 and Table 2). From the curves presented in Figure 4, TBTO may have stimulated growth or inhibited growth-limiting factors. An analysis of covariance performed on log-transformed in vivo fluorescence data showed no significant difference between controls and treatments (Table 3).

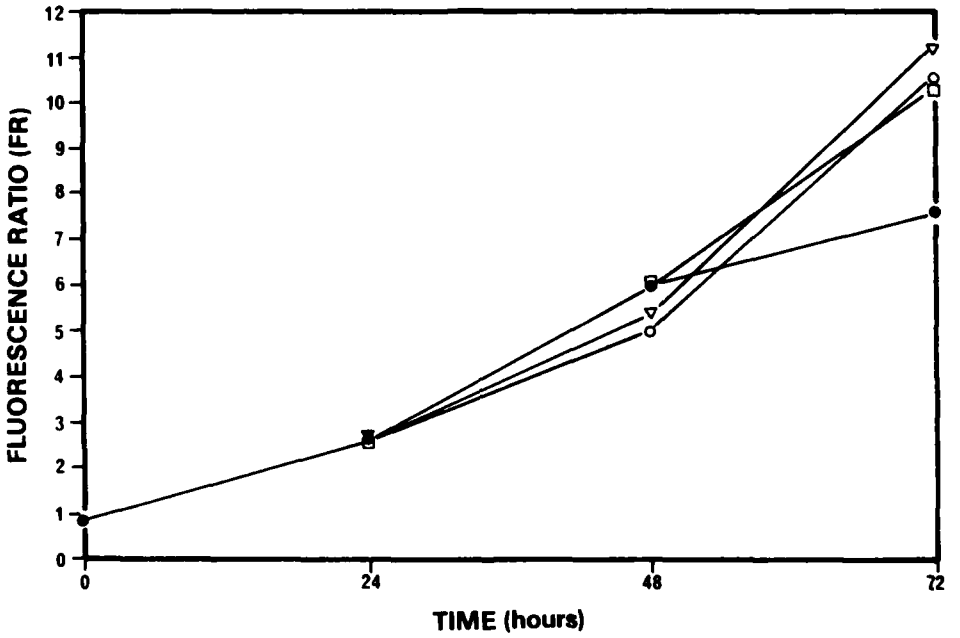


Figure 4. In vivo fluorescence measured during the exposure of *Phaeodactylum tricornutum* to varying concentrations of TBTO (● = Control; □ = 1.5 ppb; ▽= 3.0 ppb; ○ = 6.0 ppb).

Table 3. Results of the statistical analyses performed on the Phaeodactylum tricornutum data. Data were evaluated at the 95-percent confidence level.

Linear Regression Analysis

Controls

Linear regression eq.: $Y = 0.0146X - 0.0213$ $r^2 = 0.9430$
F-calc = 661.85
F-crit = 4.08
Significant linear relationship: Yes

1.5-ppb TBTO Exposure

Linear regression eq.: $Y = 0.0157X - 0.0297$ $r^2 = 0.9705$
F-calc = 1316.65
F-crit = 4.08
Significant linear relationship: Yes

3.0-ppb TBTO Exposure

Linear regression eq.: $Y = 0.0158X - 0.322$ $r^2 = 0.9818$
F-calc = 2156.92
F-crit = 4.08
Significant linear relationship: Yes

6.0-ppb TBTO Exposure

Linear regression eq.: $Y = 0.0154X = 0.0304$ $r^2 = 0.9769$
F-calc = 1316.65
F-crit = 4.08
Significant linear relationship: Yes

Analysis of Convariance Test Results

H_o: There is no difference among the slopes of the regression equation determined for Phaeodactylum tricornutum exposed to various concentrations of TBTO.

F-calc = 1.60
F-crit = 2.66
Conclusion: No significant difference

DISCUSSION

The pure cultures of marine phytoplankton tested showed a range of responses to TBTO exposure. G. splendens, the dinoflagellate, was the most sensitive of the three species tested. It was affected by all TBTO concentrations used.

Mandelli (1969) reported that dinoflagellates are more sensitive to copper than diatoms. This appears to be true for G. splendens and TBTO as well. The increase in in vivo fluorescence measurements immediately after exposure to TBTO and the considerable variance in these measurements suggest this species is highly sensitive to TBTO. In previous studies with other species of phytoplankton, the initial in vivo fluorescence measurement has been used to confirm similar initial cell densities among replicates as well as among treatments. As the method of delivery used in this test assured similar cell densities, this increase in in vivo fluorescence with increasing concentration of TBTO was evidently due to inhibition of photosynthesis in living cells. As TBTO concentration increased, there was an increase in sample variance among replicates for these initial measurements. This wide degree of variance indicates the cells were in different physiological states. As the cells approached death, the variance in measurements decreased. In vivo fluorescence remained elevated for dead cells until the cells lysed and the chlorophyll degraded. In the presence of light and moderate temperatures, (20 °C) this occurred within 24 hours.

The results obtained with Dunaliella sp. were somewhat different than those obtained in other TBTO tests. Earlier tests indicated that TBTO concentrations up to 2.0 ppb reduced growth, and concentrations exceeding 5.0 ppb were lethal. These initial tests were performed with poor quality glass tubes capped with cork, rubber, or polyethylene stoppers. Subsequent tests have indicated that each of these materials absorbed TBTO. Therefore, the actual exposure concentration in these early tests was probably less than expected. The use of Pyrex glass tubes and stoppers in the present experiment probably resulted in smaller losses of TBTO from solution to container walls. The need for nonadsorptive test containers has been stressed by Fitzwater et al. (1982). The approximate EC_{50} of 1.5 ppb for Dunaliella sp. appears to be in line with the results obtained by Henderson (1981) working with microcosms and organotin-containing antifouling coatings. Wong, et al. (1982) observed an IC_{50} (median inhibition concentration) of 3.0 ppb for tributyltins for Lake Ontario algae. Although the differences between fresh and salt water may play a significant role in toxicity, the values reported by Wong et al. are similar to those obtained in the present study.

Although P. tricornutum has been shown to be highly resistant to copper (Bentley-Mowat & Reid, 1977; Braek et al., 1976; Jensen et al., 1976) it was hypothesized that exposure to TBTO would result in inhibition of growth since organotin is more toxic than copper (Evans & Smith, 1975). P. tricornutum demonstrated similar no-effect responses in a test assessing organotin-based leachates. For copper, inhibitory concentrations vary considerably from species to species of phytoplankton. The same is probably true for organotins. Dunaliella sp. growth was inhibited at copper concentrations near 500 ppb (Mandelli, 1969; Erickson et al., 1970). Growth inhibiting copper concentrations range from 250 µg/l (Braek et al., 1976) to 400 µg/l (Jensen et

al., 1976) and exceeding 10^{-4}M (approximately 6.4 ppm) (Bentley-Mowat & Reid, 1977) for P. tricornutum. G. splendens was inhibited by copper concentrations as low as 10-20 ppb (Saifullah, 1978).

The diverse range in tolerance to copper and trace metals reported in the literature was observed for phytoplankton exposed to TBTO in the laboratory. P. tricornutum demonstrated a high tolerance to all concentrations of TBTO, followed by Dunaliella sp. tolerating the lower TBTO concentrations. G. splendens did not tolerate exposure to any of the TBTO concentrations used. When compared to copper, TBTO appears to be approximately two orders of magnitude more toxic to these species of marine phytoplankton. In terms of antifouling coatings, this is beneficial since it may reduce the rate of settlement of fouling organisms. It may, however, pose a problem if the TBTO leaches from the paint matrix and enters the water column without any form of degradation.

CONCLUSIONS

The tolerance of phytoplankton to low levels of TBTO varies considerably from species to species, as seen in Gymnodinium splendens, Dunaliella sp., and Phaeodactylum tricornutum. Dinoflagellates have a minimal tolerance to TBTO. Introduction of this toxicant can result in an immediate response as demonstrated by reduced fluorescence ratios. The green flagellate Dunaliella sp. is more tolerant to TBTO and requires more time to produce measurable effects. An estimated EC_{50} of 1.5 was obtained for Dunaliella sp. Of the three species tested, diatoms are the most resistant. No measurable effects were obtained for P. tricornutum at any of the TBTO concentrations used. After 72 hours, however, there was a reduction in fluorescence ratios. This reduction cannot be easily attributed to TBTO exposure as all other measurements suggest no deleterious effects. This study does indicate potential toxicity to some species of marine phytoplankton at low-level TBTO exposure.

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