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PCB Toxicity to Phytoplankton: Effects of Dose and Density-Dependent Recovery Responses

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Polychlorinated biphenyls have been discharged into the environment for over 40 years, but comprehensive investigations of the effects of these substances on aquatic organisms have only recently begun (AHMED 1976). Detection of PCB's in phytoplankton has been of considerable concern, as its apparent toxicity is implicated in food web alteration and resultant damage to commercially important fisheries (O'CONNORS et al. 1978).

PCB's of a particular chlorine content and at a particular concentration will elicit different responses in different species, and even clones of the same species of phytoplankton (FISHER & WURSTER 1973, HARDING & PHILLIPS 1978a). Phytoplankton vary with respect to the amount of PCB they accumulate over time, (HARDING & PHILLIPS 1978b), and the response to that dose is a function of the susceptibility of the cell to toxicity, determined partially by the suitability of the environment, and partially by characteristics of the cell itself.

In this paper, we examine susceptibility of phytoplankton to PCB toxicity through the relationships between the dose which the cell receives, and the number of cells receiving the dose. We believe that toxicity must be viewed at both cellular and population levels.

MATERIALS AND METHODS

Skeletonema costatum (Greville) Cleve, a cosmopolitan centric diatom, was cultured axenically in 10 ml of STP enriched seawater medium (PROVASOLI et al. 1957) in 150 x 15 mm screwcap testtubes. Cultures were incubated at 20°C and illuminated (approx. 10 klux) from the sides and below on a 14 h photoperiod, in Forma® diurnal growth chambers.

Since the PCB was to be dissolved in acetone, we first tested for acetone toxicity. Three inocula of different cell densities were prepared from logarith-

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mically growing stock cultures. Testtubes containing 10 μl of acetone + STP or STP without acetone were inoculated with 0.1 ml of each inoculum resulting in 10 ml cultures having cell densities of 3.8×10^2, 3.8×10^3, or 3.8×10^4 cells$\cdot$ml^{-1} (low, medium, and high inoculum density groups). The cultures were counted daily for 5 days using a 0.1 ml nanoplankton counting chamber (PALMER & MALONEY 1954).

To ensure precision of inoculation, 5 cultures at each cell density were counted immediately following inoculation. Student's t-test (IBM 1975) indicated that mean inoculum densities computed from cell counts were the same as expected cell densities.

To test the response of S. costatum to PCB, 0.1 g of Aroclor® 1254 (mixed isomers), dissolved in 10 μl acetone, was added to cultures at each of the three cell densities. Final culture volume was 10 ml; PCB concentration per unit volume was 10 ppb. The cultures were agitated, and cells counted once daily for 5 days. The experiment was repeated at low and intermediate cell densities using 0.01 μg PCB (in 10 μl acetone), the final concentration being 1 ppb PCB in 10 ml of medium. In all cases, 5 - 8 replicate cultures were counted daily.

Student's t-test was used to determine whether mean cell densities in control and treated cultures differed from each other. Differences between means were considered significant at the 1% probability level.

RESULTS AND DISCUSSION

Effects of Dose on Cell Density. Since the response of a phytoplankter to a particular form of PCB is determined by both environmental and physiological factors (MOSSER et al. 1972, HARDING & PHILLIPS 1978a), we attempted to optimize environmental parameters such as light, temperature, and nutrient availability, and to assess solely the responses of S. costatum cultures at different cell densities to Aroclor® 1254. The effect of cell density may be two-fold: 1.) To determine the dose of PCB received by each cell, and 2.) To determine the response of the population to the PCB. We sought to identify the importance of each effect.

The dose of PCB per cell was estimated using the Freundlich isotherm:

$$C_p = KC_{eq}^{1/n} \qquad (1)$$

where, C_p = the PCB concentration (μg) associated with a mass of cells in 1 ml of medium,

K = a constant (ml$\cdot$cell^{-1}),

C_{eq} = the PCB concentration (μg) in 1 ml of medium at equilibrium,

and, 1/n = a constant, equal to $\frac{1}{1}$ in this case.

The total PCB content, C_T, ($\mu g \cdot ml^{-1}$ of medium) is:

$$C_T = (C_p \times cells \cdot ml^{-1}) + C_{eq} \quad (2)$$

which, by solving for C_{eq} in Equation(1), and substituting into Equation(2), C_p can be solved for C_p:

$$C_p = C_T/(cells \cdot ml^{-1} + 1/K) \quad (3)$$

K was determined empirically to be 3.27×10^{-6} $ml \cdot cell^{-1}$, using ^{14}C-PCB kinetics experiments under conditions similar to those described above (KLEPPEL et al. 1979). Because of the magnitude of K relative to cell density, the PCB dose$\cdot cell^{-1}$ was similar for all three inoculum groups at each PCB concentration(wt/vol) tested (Table 1), suggesting that differences in responses by the culture to PCB at a given wt/volume concentration should be due to physiological response of the inoculum.

TABLE 1

The * dose of PCB per cell for each inoculum group

Inoculum Density ($cells \cdot ml^{-1}$)	μg PCB$\cdot cell^{-1}$	
	1 ppb	10 ppb
3.8×10^2	3.26×10^{-9}	3.26×10^{-8}
3.8×10^3	3.23×10^{-9}	3.23×10^{-8}
3.8×10^4		2.91×10^{-8}

*Dose$\cdot cell^{-1}$ was computed using the Freundlich isotherm

The dose computation was simplified by ignoring the interaction of PCB with the testtube walls which amounts to about 12% within an hour of PCB addition (KLEPPEL et al. 1979).

The effects of cell density upon PCB toxicity are shown in fig.1. Acetone had no effect on the growth of the cultures. However, the growth pattern of the controls varied as a function of inoculum density. For instance, the lag phase of the intermediate density group was longer than that of low and high density groups. Addition of 1 ppb PCB to low and intermediate density cultures had no effect on growth relative to the controls

Initially, 10 ppb PCB was inhibitory at all cell densities. Recovery of growth followed inhibition, the degree of recovery varying with inoculum group. Again, the growth patterns of the PCB groups resembled those of their respective controls.

Fig.3 shows how the dose of PCB that each cell re-

ceived changed with time. Since the amount of PCB released to the medium by dead cells was unknown, we assumed that only culture growth altered the dose per cell. As the high density culture grew, the dose of PCB per cell decreased; by day 4 the dose·cell^{-1} was reduced 46 percent. Cell densities in the other groups were too low to affect the Freundlich isotherm, and the dose per cell was determined primarily by the values of K and C_T (Equation 3).

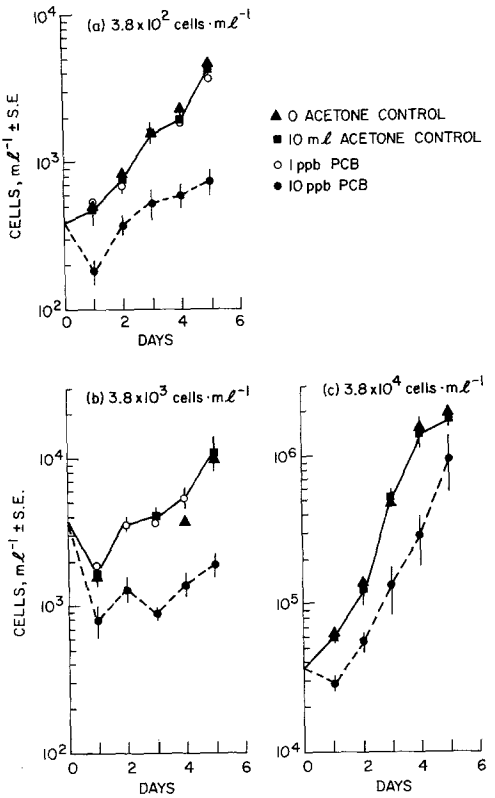

Fig. 1 Growth of S. costatum (± standard error) in cultures containing 0 or 10 μl acetone, and 1 or 10 ppb PCB (in 10 μl acetone) at 3 inoculum densities.

Inoculum Density and PCB Effects of Growth Rate. Growth rates for control and 10 ppb PCB-treated cultures were computed using:

$$N_t = N_o e^{rt} \qquad (4)$$

where, N_t and N_o = cells·ml^{-1} on days t and o,

t = time (days over which the rate was measured), and, r = the intrinsic growth rate of the culture.

Growth rates in PCB-treated cultures decreased relative to controls from days 0 - 1, evidenced recovery from days 1 - 2, and fluctuated around control values thereafter. Data for days 1 - 5 were simplified by regressing a line through the natural logarithms of daily cell densities for control and PCB groups at each inoculum density. The slope of each line was taken as the fitted four-day growth rate.

Fig.3a illustrates the relationships between PCB and control growth rates at the three inoculum densities for days 0 - 1. The numbers between points represent the differences between control and PCB group growth rates, denoting the extent of inhibition. Maximum inhibition occurred in the low inoculum group; approximately equal effects were observed in intermediate and high inoculum groups. The fitted rates (Fig.3b) for high density PCB and control groups differed by only 0.06 $\cdot day^{-1}$; intermediate and low density PCB groups differed from their controls by 0.22 and 0.25 $\cdot d^{-1}$, respectively.

Fig.3c is a plot of one minus the differences between control and PCB group growth rates for days 0 - 1 and 1 - 5. The distance between the rate differences for days 0 - 1, and those for days 1 - 5 indicates the extent of growth rate recovery following initial suppression. Recovery of the low density cultures from an initial suppression of 0.96 (fig.3a), to a fitted 4-day growth rate which differed from controls by 0.22 (fig.3b), represents the largest recovery of any group. The intermediate group recovered least, apparently reflecting the combination of PCB toxicity with the normally slow growth of the group over the first three days of the experiment (fig.1).

Dose versus Density Responses. Intuitively, the toxicity of a substance to a culture in lag growth phase will differ from its effect on an exponentially growing culture. Since inoculum density can determine the extent of the lag phase, and hence, bear heavily upon the susceptibility of the cell to inhibition, it seems that the effect of a toxin is strongly influenced by the number of cells initially exposed.

In order for the culture to exhibit a toxic response, the PCB dose must be above a threshold level, which for *S. costatum* under optimal conditions is between 3×10^{-9} and 3×10^{-8} $\mu g \cdot cell^{-1}$. In addition to the threshold effect is the secondary response related to the physiolog-

ical status of the population which appears determinant of the ability of the culture to recover from initial inhibition.

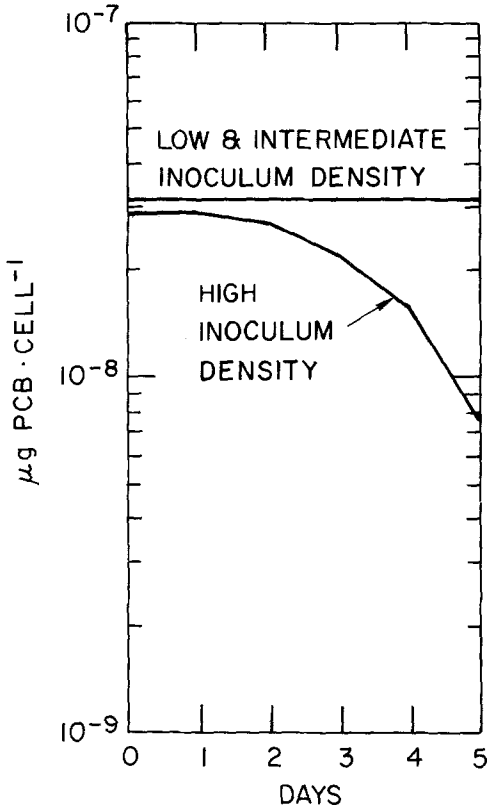

Fig. 2 Changes in PCB dose·cell^{-1} with time.

Implications to Natural Situations. Though this study has emphasized the effects of PCB on cultured phytoplankton, some of our results have implications to natural situations. Since phytoplankton in estuaries are often recruited allochthanously at seasonally varying densities, we might expect different responses to a constant PCB source. For example, the lower Hudson River estuary receives diatoms and chlorophytes from the New York Bight Apex (MALONE 1977), and PCB's sorbed to particulates and dissolved in the water from the upper Hudson River. Since PCB concentrations in Hudson estuarine phytoplankton have been observed at parts per million levels (New York State DEC. unpublished data), well above the toxicity threshold dose for S. costatum (a

periodically dominant Hudson Estuary diatom) under optimal conditions, growth inhibition may occur under some circumstances. The ability of such a population to overcome the inhibition will be partially dependent on the size of the population, and their physiological status at the time of threshold dosing.

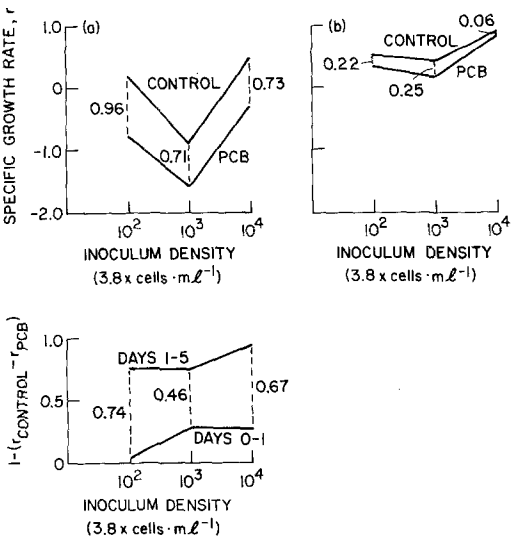

Fig.3 Specific growth rates a) computed for days 0 - 1, and b) fitted for days 1 - 5, for control and PCB groups, at each inoculum density. c) Changes in the difference between control and PCB cultures between days 0 - 1, and days 1 - 5, at 3 inoculum densities.

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