The Effect of Polychlorobiphenyls (Aroclor 1242) on Bicarbonate-C^{14} Uptake by *Euglena gracilis*

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Polychlorinated biphenyls (PCB's) are widespread, persistent pollutants of the environment. The ubiquitious nature and pronounced toxicity of these compounds to biotic systems have focused attention on these substances. Since the primary uptake of PCB's into the food chain occurs at lower levels of the aquatic community (Ware and Addisson, 1973), their effect and mode of action on phytoplankton warrants further investigation.

Previous studies have provided evidence of heterogeneous responses of plankton to PCB's (Moore and Harris, 1972). Thus the growth of Skeletonema is inhibited at concentrations as low as 10 ppb (Mosser et al., 1972) whereas Euglena gracilis is unaffected at this level. Such diverse PCB tolerance data have resulted in confusion as to the mode of action of this toxicant in relation to phytoplankton.

To a large extent, postulated mechanisms for PCB inhibition are based a priori on chemical characteristics common to all chlorinated hydrocarbons - affinity to membranes and ability to form hydrophobic membrane complexes. This is apparent from the rationale provided for data obtained from studies of mitrochondria and chloroplasts.

Experiments with mitochondria indicate that the respiratory process is affected during PCB inhibition, a result attributed to involvement of membrane enzyme systems, associated with oxidation, or possibly to the membrane itself (Pardini, 1971). A decrease in phosphate uptake with PCB exposure, to the point where the cells leaked phosphate was noted by Larson and Tillberg (1975).

In vitro investigations of chloroplasts revealed a decrease in the evolution of oxygen associated with photosynthesis, at the 1 ppb level (Luard, 1973). This was interpreted in terms of PCB inhibition of electron transport in Photosystem II of the photosynthesis unit.

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More recently however, Fisher (1975) reported that although there was a decrease in net photosynthesis of a batch culture at the 0.1 ppm PCB level, growth studies indicated no "per cell" reduction in photosynthesis. The implication of these findings is that PCB's affect the cell growth mechanism but not the photosynthetic process of the chloroplast. Fisher suggested that the inhibitory action may be due to interaction of PCB's with the cell's membrane bound nitrogen metabolism enzymes.

Euglena gracilis has been found to be relatively insensitive to PCB's (Mosser, 1972). Ewald et al. (1976), reported that the growth of Euglena gracilis was unaffected by 100 ppm PCB (Aroclor 1242) on 48 hours of exposure.

It is the objective of this study to determine the affect, if any, of PCB (Aroclor 1242) on the population growth rate of Euglena gracilis, and also the kinetics of inhibition upon initial exposure.

Materials and Methods

I. Cell Population Growth

Batch cultures of Euglena gracilis, Krebs strain Z, were used to inoculate 200 mls of culture media (Fisher, 1975) which had previously been spiked with 0.01, 0.1, 1.0 and 10.0 ppm Aroclor 1242 in a 0.5% ethanol solution. Ethanol and ethanol-free controls were included in the run. Cell counts, of the controls and experimental runs, were conducted upon inoculation and subsequently after 4, 6 and 8 days. These counts were made with a "Bright Line" haemocytometer and light microscope (100X). Each control and experimental run was carried out in four replicate flasks. The cultures were maintained, in continuous white fluorescent light of approximately 1200 foot-candles, at 25.2°C.

II. Inhibition Kinetics and Sodium Bicarbonate (C-14) Uptake.

A batch culture was grown to a cellular concentration of 9.5×10^5 cell/ml. The cells were then exposed to Aroclor 1242 for varying lengths of time prior to C^{14} introduction.

Aliquots (10 mls) of suspension (i.e. 9.5×10^6 cell) were added to each of nine test-tubes. Four of these samples were then spiked with a PCB-ethanol mixture to bring the tube culture concentration to 10 ppm PCB in 0.5% ethanol. Four more samples were treated with an equivalent amount of ethanol less the PCB. The ninth

sample was utilized as a control. Immediately, one of each of the PCB-ethanol and ethanol samples as well as the control sample, were treated (T=0 hours) with C-14 labeled sodium bicarbonate (equivalent to 0.1 μc/10 ml tube). Similar pairs of samples were labeled at T = 1/2, 2 and 4 hours. All tubes were incubated with the C-14 for two hours, in white fluorescent light and then killed with 2% formaldehyde solution.

Each time set and control was replicated four times. This entire procedure was repeated for a set of dark controls.

"Light" and "Dark" runs were then centrifuged to pellets, resuspended in fresh solvent and pipetted onto filters which were dried under heat lamps. The latter were then counted by a Beckman LS-100C Liquid Scintillation Counter.

Results

I. Cell Population Growth

The growth data for batch cultures at different PCB concentrations are shown in Table 1, and indicate no inhibition of growth at the 0.01, 0.1 and 1 ppm levels of PCB. At the 10 ppm concentration however, there was a definite indication of growth inhibition, which was still apparent after 8 days, by the absence of logarithmic growth. The percentage of growth was established using the ethanol control as the reference (Fig. 1).

TABLE 1

DAILY CELL COUNTS FOR EUGLENA GRACILIS EXPOSED TO VARYING LEVELS OF PCB IN "LIGHT"

	No. of Days of Exposure			
Sample	0	4	6	8
Control	1.3×10^2	57.4±9.5	242.2±49.1	378.3±100.4
Control & ethanol	"	46.5±11.2	241.3±50.3	431.6±66.1
0.01 ppm PCB	"	60.7±12.7	300.0±81.4	375.8±57.2
0.1 ppm PCB	"	59.9±16.9	280.0±49.7	327.5±71.9
1 ppm PCB	"	42.0±8.0	215.0±78.3	330.0±39.9
10 ppm PCB	"	10.2+3.5	14.1+3.3	26.6+6.6

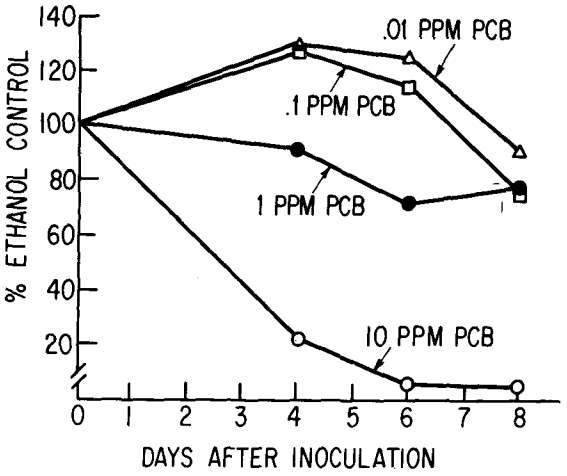

Fig. 1. % Population Growth to Ethanol Control

II. Kinetics of Inhibition

The total C-14 uptake in the "light" tubes, with varying degrees of exposure to the PCB prior to introduction of the labeled bicarbonate, is shown in Fig. 2.

The percentage C-14 uptake with respect to the ethanol control is revealed in Fig. 3 indicating a 30% inhibition on addition of PCB (T=0), increasing to 50% at T=1/2 hr. However on longer exposure to PCB, the inhibition decreases proportionately to 15% at T = 4 hrs.

Absorption (cpm) of C-14 in the "dark" was insignificant in comparison to that for the "light" runs (Figs. 2 and 4).

Nevertheless, increased duration of exposure to PCB in the "dark" caused a 33% decrease in C-14 uptake as compared with the ethanol control (Fig. 5).

Discussion and Conclusions

Population tests indicate that at 10 ppm PCB Aroclor 1242) there is a significant inhibition in the growth of Euglena gracilis, a result which on first glance seems contrary to the findings of Ewald, W.G. (1976). However Ewald reported no evidence of growth inhibition at the end of 48 hours, whereas the present study indicates there is no significant variation from the controls until the fourth day (i.e. 96 hours of incubation)

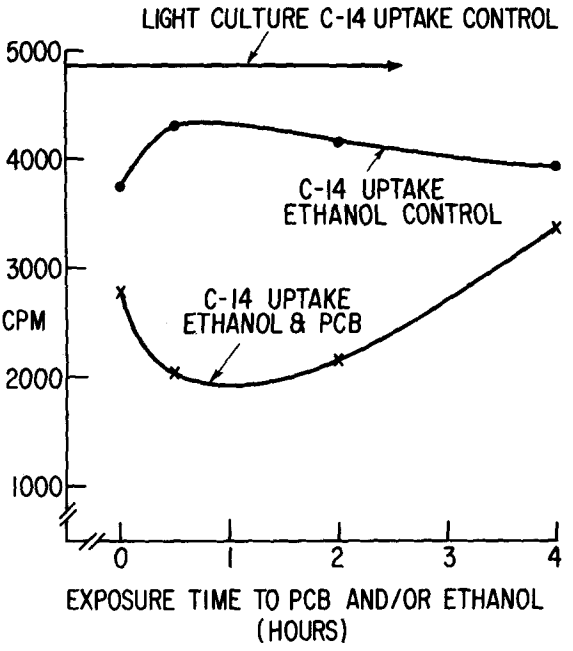

Fig. 2. Uptake of C-14 in "Light" following varying periods of exposure to PCB.

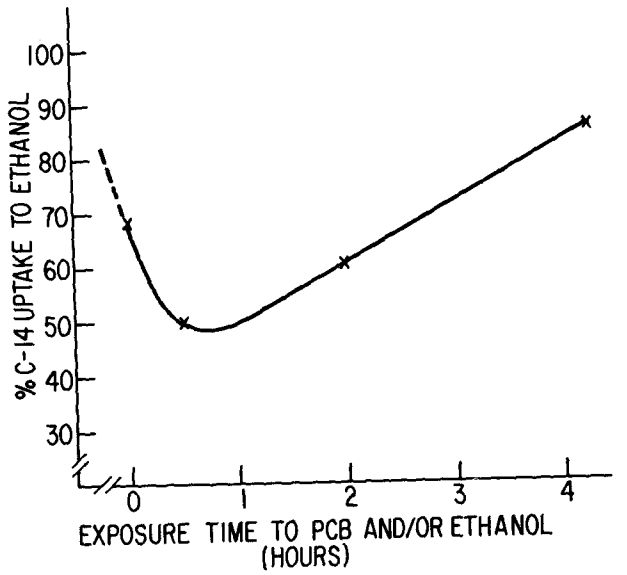

Fig. 3. % C-14 uptake with respect to the ethanol control in "Light".

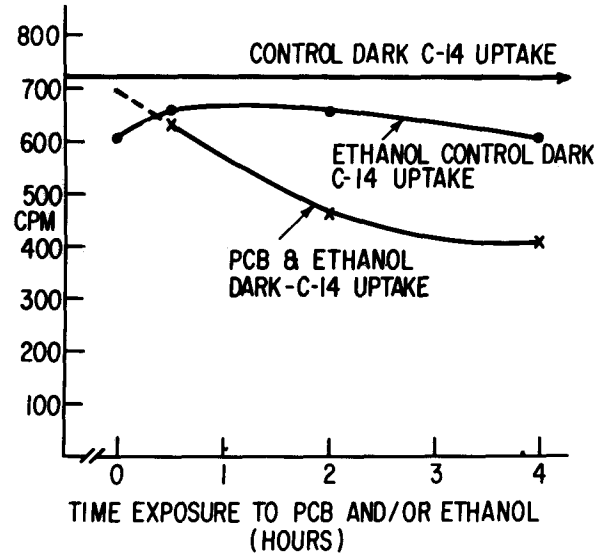

Fig. 4. Uptake of C-14 in "Dark" following varying periods of exposure to PCB.

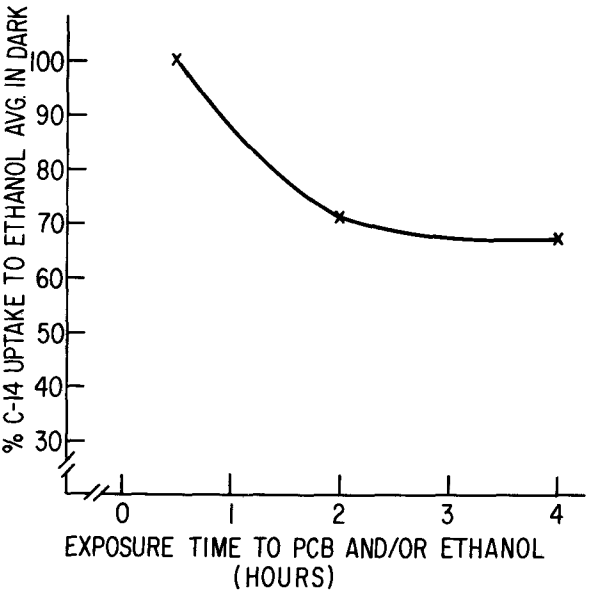

Fig. 5. % C-14 uptake with respect to ethanol control in the "dark".

and this is not completely manifested until completion of the sixth day of the test (Fig. 1). Consequently, it was concluded that 10 ppm levels of PCB are toxic to Euglena gracilis in long term batch growth.

The kinetics of inhibition reveal an interesting phenomenon. On initial introduction of PCB, the photosynthetic rate sharply decreased to 50% of that of the ethanol control (Fig. 3). However, after four hours of exposure, the algae had recovered. These data support the hypothesis of Fisher, N.S. (1975), that in standing cultures with PCB there is no "per cell photosynthetic inhibition."

A clue to the mechanism of PCB growth inhibition may lie in the C-14 uptake of cells in "dark" tubes (Figs. 4 and 5). With increased exposure to PCB's the cells absorb less bicarbonate. This effect can be attributed to:

1) a metabolic change which influences the ease of diffusion between the cells and the medium.
   or
2) direct action of PCB on membrane permeability to bicarbonate.

Indications from other recent experimental work associated with the mechanism for PCB toxicity [Luard (1973); Ware and Addisson (1973); Fisher (1975); Larson and Tillberg (1975)] suggest such an influence on membrane function.

Our experimental findings suggest that inhibition of population growth does not lie directly in the photosynthetic pathway despite the initial inhibition by PCB's on Euglena gracilis in "light". "Dark" absorption of bicarbonate appears to decrease with increased PCB exposure. This decrease may be causally related to the population growth inhibition observed.

Summary

Aroclor 1242 is inhibitory to long-term batch growth of Euglena gracilis at 10 ppm. Exposure to PCB's, subsequent to an initial drop of 50% in the first 30 minutes, does not appear to inhibit photosynthesis on a per cell basis over the time span of four hours. Consequently our experimental findings suggest that inhibition of population growth does not lie directly in the photosynthetic pathway despite the initial inhibition by Aroclor 1242 on Euglena gracilis in "light". "Dark" absorption of bicarbonate appears to decrease with increased PCB exposure. This decrease may be causally related to the population growth inhibition observed.

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