Bioconcentration of Four Pure PCB Isomers by *Chlorella pyrenoidosa*

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Introduction

We have shown (HAWES, et al., in press, KRICHER et al., in press) that the commercial polychlorobiphenyl (PCB) preparations called Arochlor, inhibit both the growth and productivity of pure cultures of Chlorella pyrenoidosa. In this paper we report studies on the concentration of four pure PCB isomers by Chlorella and their effects on the growth of Chlorella.

Materials and Methods

Growth and Organisms:

Chlorella pyrenoidosa ATTC #7516 was obtained from the American Type Culture Collection and maintained axenically in liquid Bristol's medium. Cultures were maintained in continuous log growth phase by shaking under continuous flourescent illumination of 165 foot-candles at 20-25°C. Cell numbers were determined either by counting in a hemocytometer or by measuring the amount of light absorbed at 540 nm.

Extraction and Analysis of PCB's:

Cultures were harvested under suction on a Whatman GF/A filter and frozen. The used Bristol's filtrate was extracted with two 5 ml portions of petroleum ether. The Chlorella were extracted for 20 minutes with dimethylsulfoxide (DMSO): methanol (1:4 v/v) and re-extracted for another 10 minutes with DMSO/methanol. Two parts of water was added and the mixture was extracted twice with five parts petroleum ether. Methanol was added as necessary to break the emulsion. The pet. ether extracts were stored at -20°C. Extracts were cleaned up by shaking twice with concentrated sulfuric acid and the petroleum ether layer containing the PCB's was stored until assay.

Samples were analyzed on a Varian Model 2100 gas chromatograph equipped with an electron capture detector. Six foot glass columns containing 5% SE-30 on VarAport 30 (100/120 mesh) were run at 200°C with nitrogen carrier gas at 38-40 ml/min. Standard curves were used to qualitate results.

Chemicals:

Aroclor 1248, 2,2^1, 5, 5^1-tetrachlor, 2, 2^1, 4, 4^1, 5, 5^1 hexachloro, 2,2^1, 3, 3^1, 4, 4^1, 5, 5^1 octachloro, 2, 2^1, 3, 3^1, 4, 4^1, 5, 5^1, 6, 6^1 hexachlorobiphenyl, and 2,6-dichlorodibenzofuran were obtained from Analabs division of New England Nuclear Corp. All solvents were of pesticide analysis quality or the best grade available. The petroleum ether was the 60-80°C. cut.

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Bioconcentration Studies:

All PCB's were added in nanograde acetone at a final concentration of 10 ppb and 0.1% acetone. Chlorella cells were taken from young cultures and added to a final concentration of 2 x 10^6 cells/ml. The cultures were grown under continuous fluorescent illumination on the shaker. For studies on dead cells, the Chlorella was added to boiling Bristol's medium for one (1) minute, cooled and the PCB added. Samples of boiled cells were placed on the shaker for six days to check for living cells.

Results and Discussion

As shown in Table I, Chlorella cells did concentrate each PCB isomer tested within one hour by at least 1000-fold. There was no significant difference in the amount of concentration of the different isomers.

Table I

Bioconcentration of PCB isomers at 10 ppb exposure in one hour by Chlorella

Isomer	Extracted from Chlorella cells (ppm)	Extracted from growth medium (ppb)	% PCB recovered
tetrachloro	32	6.7	85
hexachloro	70	7.0	90
octachloro	16	1.5	22
decachloro	52	6.0	65
acetone only	ND	ND	--

ND = none detected

$$\% \text{ PCB recovered} = \frac{\text{Total PCB Chlorella + Total PCB Bristol's}}{\text{Total PCB added}} \times 100$$

SODERGREN (1968) demonstrated that DDT is concentrated by dead Chlorella cells. The data in Table II show that both the tetrachloro and hexachlorobiphenyl isomers were concentrated by dead Chlorella cells by 6000 and 15,000 times respectively. These amounts are greater than in living cells. Since concentration of PCB's occurs in dead cells, it must be due to a simple chemical partitioning between the water of the medium and the hydrophobic lipids of the cell.

Table II

Concentration of PCB isomers at 10 ppb Exposure by Dead Chlorella

Isomer	Extracted from Chlorella ppm	Extracted from Bristol's Medium ppb	% PCB recovered
tetrachloro	62	5.5	39
hexachloro	156	6.5	62

Studies were performed to determine whether these isomers were metabolized by the Chlorella. No new compounds which could be isolated by our extraction method and which could be detected by the electron capture method were observed. However, as seen in Table III, the amount of PCB isomer recovered from the cells and from the growth system decreased significantly with time of exposure to the chemical. There are three possible explanations of these results: (1) metabolic conversion to compounds not containing chlorine and therefore not detectable using electron capture; (2) metabolic conversion to compounds which would not be extracted into petroleum ether or would be destroyed by sulfuric acid; (3) loss from the system by evaporation or co-distillation. PCB's are chemically inert and loss of chlorines have not been detected generally. Studies by SODERGREN (1975) did not detect any metabolic breakdown of Clophen A50 by Chlorella. HUTZINGER et al., (1972) did not detect metabolic conversion hexa nor octachloro biphenyl in pigeons or rats. The possible conversion to more polar or more labile compounds which would escape our extraction and cleanup is a possibility which cannot be eliminated. The higher recovery of PCB's in dead cells supports the possibility of metabolic conversion co-distillation has not been eliminated as a mechanism. The decrease in concentration with time has not been previously reported. Studies should be performed to determine whether or not the decrease is seen in dead cells.

The effect of the four pure PCB isomers, the commercial PCB Aroclor 1242 and the possible chemical contaminant 2,6-dichlorobenzofuran at 1 ppm on the growth of Chlorella was studied. Cell density was measured spectrophotometrically and growth was measured until the beginning of stationary phase. No significant differences in the growth of Chlorella was detected for any of these compounds.

In contrast, we previously reported inhibition of growth of Aroclor 1242 grown under the same conditions (HAWES et al., in press). This difference may be due to the fact that Hawes measured cell number by counting in a hemocytometer rather than by light absorption. She may have disregarded debris which the spectrophotometer would necessarily measure.

Table III

Bioconcentration of 2,2',5,5'-Tetrachlorobiphenyl After Different Times of Exposure

Sample	Extract from Chlorella cells	gPCB / g cells	Extract from Bristols solution	gPCB / gH_2O	%PCB Recovere
One Hour Exposure					
Acetone Control	ND		ND		--
24 hr. Control	1.11×10^{-5}		4.40×10^{-9}		53.7
10ppb	3.38×10^{-5}		6.53×10^{-9}		81.2
	3.06×10^{-5}		6.88×10^{-9}		88.2
Twenty-Four Hours Exposure					
Control	ND		ND		--
Acetone Control	ND		ND		--
10ppb Control	ND		2.29×10^{-9}		22.4
	Trace		3.95×10^{-9}		38.6
1 ppm Control	1.68×10^{-7}		7.89×10^{-8}		24.2
	3.67×10^{-8}		3.34×10^{-7}		38.7
10ppb	7.64×10^{-6}		3.35×10^{-7}		45.7
	1.51×10^{-5}		2.81×10^{-9}		37.9
1 ppm	3.30×10^{-3}		3.78×10^{-8}		31.2
	3.53×10^{-3}		3.59×10^{-8}		32
Sixy-Four Hours Exposure					--
Control	ND		ND		--
Acetone Control	ND		ND		27.3
10ppb	3.56×10^{-6}		2.84×10^{-9}		

ND = None Detected

$$\% \text{ PCB Recovered} = \frac{\text{Total PCB extracted from Chlorella} + \text{Total PCB extracted from Bristols Sol.}}{\text{Total PCB Added to Culture}} \times 100$$

Experiments were carried out in 250 ml. Erlenmeyer flasks, incubated on a shaker under continuous light. Chlorella cells were present in a concentration of approximately 2×10^6 cells/ml. The volume of each flask was brought to 100 ml. with Bristols solution, and added to each was 0.1 ml. of acetone or PCB. The samples contained the following:

Control - Chlorella + Bristols
Acetone Control - Chlorella + Bristols + 0.1 ml. acetone
10ppb Control - Bristols + 0.1 ml. 10ppm PCB
1ppm Control - Bristols + 0.1 ml. 1ppt PCB
10ppb - Chlorella + Bristols + 0.1 ml. 10ppm PCB
1ppm - Chlorella + Bristols + 0.1 ml. 1ppt PCB

In summary, PCB's are concentrated within Chlorella by several thousand-fold whether the cells are alive or dead, suggesting the mechanism is simple partitioning into cell lipids. However, with increasing times of incubation, the amount of PCB recovered from cells decreases. Our studies failed to detect any inhibition of growth of Chlorella by the PCB isomers despite the large amount taken up by the cells. In addition, neither Aroclor 1242 nor the known chemical contaminant of commercial PCB's, 2,6-dichlorodibenzofuran, affected the growth of Chlorella. Thus, it appears that PCB's are relatively nontoxic to Chlorella although they are concentrated by the cell.

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