# Polychlorinated Biphenyl (Aroclor 1242\*): Effects of Uptake on Growth, Nucleic Acids, and Chlorophyll of a Marine Diatom

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# Introduction

Polychlorinated biphenyls (PCB's), similar in structure and effects (7) to certain chlorinated hydrocarbon pesticides have been found in ecosystems of Britain (4), northern Europe (5), and in several sections of the United States (3). PCB's employed as plasticizers, dielectrics, and heat transfer fluids have grown in use since the forties in a manner somewhat paralleling Their seeming ubiquity in the environment and confusion with DDT in chromatographic detection have caused some investigators (2) to suggest that DDT values stated in the literature may be overstated by as much as 90%. Additionally PCB's have been shown to be powerful inducers of hepatic enzymes (8) as well as inhibitors of the carbonic anhydrase system essential to calcium deposition in egg shell produc-These factors alone emphasize urgency in the further investigation of PCB distribution in the global environment, primary sources of release and effects on basic food systems of which marine diatoms play an important part. Because previous work (6) had shown Cylindrotheca closterium to be capable of absorbing, concentrating, and metabolizing DDT, similar work was undertaken with the organism and PCB.

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# Methods

Sixteen one liter erhlenmeyer flasks, each containing 200 ml. of culture media were inoculated with the diatom <u>C. closterium</u>, Reimann and Lewin. Four flasks served as controls, four for each level of PCB (Aroclor 1242) treatment, and four as acetone controls since acetone was the vehicle for the toxicant.

Cell counts were made at the conclusion of a two week incubation period. The contents were then centrifuged, the resulting pellet lyophilized, weighed and assayed for PCB, nucleic acids, and chlorophyll spectra lipids.

PCB (Aroclor 1242) was extracted from the dry pellet with 1 ml. of nanograde acetone. A suitable aliquot was injected into the gas chromatograph for analysis. Two column systems were employed: (1) a six foot X 1/4 inch O.D. glass column packed with 4% SE-30-2% QF-1 on Chromoport XXX and, (2) a six foot X 1.4 inch O.D. glass column packed with 1.5% OV-17-1.95% QF-1 on Chromoport XXX. The operating parameters of the gas Chromatograph were as follows:

Inlet temperature 235°C
Column temperature 200°C
Detector temperature 350°C
Carrier gas flow 60 cc/min

Identification of Aroclor 1242 was accomplished by measuring relative retention times of the five major peaks along with relative peak heights. Quantification was accomplished by comparison of the area under the curve of the five late eluters with the standard Aroclor.

Verification of the peak identities by thin layer chromatography positively identified Aroclor 1242 as the major PCB component of the sample. Some PCB materials not common to the known (Aroclor 1242) mixture were isolated. These materials were, in all cases, early eluters, and possibly metabolic products of Aroclor 1242.

RNA and DNA were assayed using a modification of Agranoff's procedure (1). Chlorophylls and related materials were chloroform: methanol (2:1) extracted and optical density measured at 670 microns. The chlorophyll index which will be referred to is actually the maximum optical density of all chloroform methanol extractible materials read at the maximum absorbance for chlorophyll.

# Results and Discussion

As shown in Table 1, PCB was taken up by C. closterium and concentrated up to 1100 times above levels added to culture media. The higher media level of PCB (.1 ppm) sharply inhibited growth as evidenced

by harvest weights and cell counts. The .1 ppm dosage also significantly reduced RNA synthesis and the chlorophyll index but apparently had no effect on DNA levels. PCB at .01 ppm in the media did not seem to adversely affect growth or seriously alter nucleic acid levels or chlorophyll production.

#### Conclusion

Cylindrotheca closterium absorbed and concentrated the polychlorinated biphenyl (Aroclor 1242) 900 to 1000 times above the level in sea water. At .1 ppm concentration in sea water, PCB's inhibited growth and diminished levels of RNA and chlorophyll of this marine diatom.

Harvest Weight, Cell Counts, Nucleic Acid Levels, and Chlorophyll Index of Cylindrotheca closterium Cultures Exposed to Polychlorinated Biphenyls

TABLE 1

Treatments	Harvest Weight (mg)	Final Cell Count (X 10 <sup>4</sup> )	Chlorophyll Index (OD @ 670)	RNA (mg/mg)	PCB Levels (ppm)
PCB .1 ppm	11.5**	16.5**	.27**	.015**	109.2**
PCB .01 ppm	38.1	113.7	1.26	.033*	4.7*
Control	30.9	116.8	1.52	.039	0
Acetone Control	33.2	98.5	1.37	.040	0

<sup>\*</sup> Significantly different from control at .05 level

<sup>\*\*</sup> Significantly different from control at .01 level

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# References

- 1. AGRANOFF, B. W., Biochem. et Biophys. 72, 251 (1963).
- 2. ANDERSON, D. W., J. J. HICKEY, R. W. RISEBROUGH, D. F. HUGHES, and R. E. CHRISTANSEN, Can. Field Natur., 89, 89 (1969).
- Can. Field Natur., 89, 89 (1969).

  3. DUKE, T. W., J. I. LOWE, and S. J. WILSON, JR.
  Bull. Environ. Contam. Tox., 5, 171 (1970).
- 4. HOLMES, D. C., J. H. SIMMONS, and J. O. TATTON, Nature, 216, 227 (1967).
- JENSEN, S., A. G. JOHNELS, M. OLSSON, and G. OTTERLIND, Nature, 224, 247 (1969).
- 6. KEIL, J. E. and L. E. PRIESTER, Bull. Environ. Contam. Tox., 3, 169 (1969).
- 7. LIGHTENSTEIN, E. P., J. Econ. Ent. 62, 761 (1969).
- 8. RISEBROUGH, R. W., P. RIECHE, S. G. HERMAN, D. B. PEAKALL, and M. N. KIRVEN, Nature, 220, 1098 (1968).