

## Effect of PCBs on the Rate of Carbon-14 Uptake in Phytoplankton Isolates from Oligotrophic and Eutrophic Lakes

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Marine and freshwater phytoplankton are sensitive to various organochlorine pollutants (BIGGS et al. 1978, HARDING & PHILLIPS 1978, OLOFFS et al. 1972, COLE & PLAPP 1974, KRICHER et al. 1979). Most studies are concerned with the effects of DDT and PCBs, and show that laboratory cultures or algae grown *in situ* exhibit a wide range of sensitivity to these compounds. The toxicity and effect of PCBs seems to parallel that of DDE (POWERS et al. 1976, 1977).

It has been inferred that the differential sensitivity of algae to organochlorine residues may alter species composition, decrease diversity and interfere with normal successional patterns (FISHER et al. 1974, O'CONNORS et al. 1978). MOSSER et al. (1972) showed that PCB and DDT caused an imbalance in mixed cultures, while no apparent effects were noted in pure cultures.

PCBs reduce the primary productivity of pure and mixed marine algal cultures as well as natural phytoplankton assemblages grown under different conditions. (HARDING & PHILLIPS 1978, POWERS et al. 1977, HARDING 1976). However, little is known about the response of freshwater phytoplankton communities to such pollutants.

To evaluate the effect of PCBs on phytoplankton from oligotrophic and eutrophic lakes, we added PCBs to natural phytoplankton assemblages and measured primary productivity in the lake as well as under defined conditions in the laboratory.

### MATERIAL AND METHODS

#### 1. The lakes

The effect of PCBs (Clophen A 50) on the rate of  $^{14}\text{C}$ -uptake by phytoplankton was studied in the oligotrophic lake, Skäravattnet, and the eutrophic lake, Bysjön, situated in southern Sweden.

In lake Skäravattnet, pH is about 6.6 and conductivity  $92 \mu\text{S}_{20} \text{ cm}^{-1}$  during summer (Table 1). The Secchi disc transparency is about 4.3 m and isoetides are growing

at a depth of 3 m (Jensen, pers. com.).

Table 1. Physical/chemical characteristics of the studied lakes during summer.

	pH	alkalinity (mekv.L <sup>-1</sup> )	conductivity (uS <sub>20</sub> cm <sup>-1</sup> )	tot. phosphorous (ug L <sup>-1</sup> )
Lake Skäravattnet (oligotrophic)	6.6	0.032	92	15
Lake Bysjön (eutrophic)	9.2	2.314	302	411

The eutrophic lake Bysjön is a small, hardwater seepage lake. During summer, heavy algal blooms usually reduce Secchi disc transparency to less than 0.5 m. The level of phosphorous is high (Table 1) and during stratification, the near bottom water usually becomes anoxic causing a release of nutrient from the sediment.

## 2. Field experiments

Water was sampled at 0.2 m depth and transferred to 130-mL borosilicate glass bottles. After adding PCBs (Clophen A 50) and 14-C bicarbonate (4 uCi), the bottles were suspended at the sampling depth. PCBs were dissolved in ethanol as described previously (SÖDERGREN 1973), yielded a final concentration in the bottles of 26 ug L<sup>-1</sup>. Controls received equal volumes of ethanol (2 uL). All bottles were exposed to natural daylight for 3.5 h.

Dark bottles were included in each set. For the eutrophic lake, the mean value for the dark bottles was 0.93 mg C m<sup>-3</sup> h<sup>-1</sup> (n= 10). For the oligotrophic lake it was 0.08 mg C m<sup>-3</sup> h<sup>-1</sup>. Each experiment and control was duplicated.

After exposure, subsamples of the water were filtered (Sartorius membrane filter 0.2 um) at a maximum vacuum of 50 kN m<sup>-2</sup>, and radioactivity was determined with liquid scintillation. The amounts of PCBs taken up by the algae and remaining in the water were measured according to SÖDERGREN (1973). PCBs adsorbed to the walls of the bottles were removed by washing with hexane.

## 3. Laboratory experiments

Lake water, sampled as above, was incubated at 20C for 2.5 h at a quantum flux of ca. 150 ueinsteins m<sup>-2</sup> sec<sup>-1</sup> (400-700 nm) from fluorescent light. To one set of samp-

les, PCBs were added at the beginning of the exposure, to another set, the addition was made 16 h before primary productivity was determined. In both cases primary productivity was measured for 2.5 h.

Cell density of the isolates may influence the effect of PCBs on the rate of carbon uptake (KLEPPEL & MCLAUGHLIN 1980). The phytoplankton organisms from the two lakes were therefore exposed to PCBs in amounts that were related to their content of particulate organic carbon (POC). As POC of the oligotrophic lake in summer was approximately 1/10 of the eutrophic lake, the amount of PCBs added was decreased accordingly.

## RESULTS

PCBs affected the rate of carbon uptake in both the oligotrophic and eutrophic lake. In the spring, just after an intense bloom of the diatom *Stephanodiscus hantzshii* Grun. in the eutrophic lake (chlorophyll *a* 38.2 mg m<sup>-3</sup>), primary productivity was depressed 15 % by Clophen A 50 compared to the controls (Table 2). The effect was greater in the autumn when phytoplankton biomass was low (chlorophyll *a* 1.7 mg m<sup>-3</sup>), and dominated by two species of cryptomonads.

Table 2. Effect of a mixture of PCBs (Clophen A 50) on the primary productivity of phytoplankton isolates from the eutrophic lake Bysjön. Duplicate isolates were incubated *in situ* at a depth of 0.2 m. Mean of two parallel analyses. Correction was made for dark values. Chlorophyll *a* was 38.2 mg m<sup>-3</sup> in spring and 1.7 in autumn.

	Primary productivity (mg C m <sup>-3</sup> h <sup>-1</sup> )		Changes compared to control (%)	
	Spring	Autumn	Spring	Autumn
Control	34.2	3.7		
Ethanol added	32.9		- 4	
Clophen A 50 added	29.2	2.5	-15	-31

Most of the PCBs added were taken up by algae (Table 3). Of the total amount recovered, 46 % was found in algae during spring and 30 % in autumn. In the filtered water, the spring and autumn concentrations were similar. No degradation of the added substances was observed because the gas chromatographic pattern of the Clophen A 50 for the algae, water and that adsorbed to the walls was identical to the parent substances.

Table 3. Distribution of a mixture of PCBs (Clophen A50) in phytoplankton assemblages from eutrophic and oligotrophic lakes. n = 6.

	PCB added (ng)	PCB/chlorophyll <i>a</i> (ng/ng)	Uptake by phyto- plankton		Adsorbed to glass walls		Amount in water		Total amount recovered	
			(ng)	(%)	(ng)	(%)	(ng)	(%)	(ng)	(%)
Field incubations										
Lake Bysjön, spring	3392	0.4	1560	46	334	10	1064	32	2958	87
Lake Bysjön, autumn	3392	6.0	1021	30	231	7	1391	41	2635	78
Laboratory incubations										
Lake Bysjön, spring	3392	0.6	2262	67	257	8	585	17	3104	92
Lake Bysjön, summer	3392	0.2	1274	38	402	12	789	23	2465	73
Lake Skäravattnet, spring	3392	5.9	1235	37	173	5	1235	36	2669	79
Lake Skäravattnet, summer	3392	3.4	957	28	191	6	1638	48	2786	82
Lake Skäravattnet, summer	339	0.2	62	18	33	10	177	52	272	80

Table 4. Effect of a mixture of PCBs (Clophen A 50) on the primary productivity of phytoplankton from eutrophic and oligotrophic lakes incubated under identical conditions in the laboratory. Dark values are corrected for. Mean of two parallel analyses. Chlorophyll a in the eutrophic lake was 38.2 and 72.6 mg m<sup>-3</sup> in the spring and summer samples, respectively. For the oligotrophic lake the corresponding figures were 2.1 and 2.8 mg m<sup>-3</sup>.

	Primary productivity (mg C m <sup>-3</sup> h <sup>-1</sup> )		Changes compared to control (%)	
	Spring	Summer	Spring	Summer
Bysjön, eutrophic				
Control	115.4	387.7		
Ethanol added		389.6		+ 0.5
Clophen A 50 and <sup>14</sup> C bicarbonate added simultaneously	76.0	396.0	- 34	+ 2
<sup>14</sup> C bicarbonate added 16h after				
Clophen A 50 addition	91.6		- 21	
Skäravattnet, oligotrophic				
Control	5.6	5.3		
Ethanol added		4.8		- 9
Clophen A 50 and <sup>14</sup> C bicarbonate added simultaneously	1.7	2.3	- 70	- 57
<sup>14</sup> C bicarbonate added 16h after				
Clophen A 50 addition	0.9		- 84	
Clophen A 50 (1/10 of original amounts) and <sup>14</sup> C bicarbonate added simultaneously		4.7		- 11

An addition of ethanol alone only slightly reduced the rate of carbon fixation compared to PCBs dissolved in ethanol (Table 2).

When exposed to fluorescent light in the laboratory, phytoplankton isolates from the oligotrophic lake were more sensitive to PCBs than those from the eutrophic lake. When PCBs and  $^{14}\text{C}$  bicarbonate were added simultaneously, the rate of carbon fixation in the oligotrophic lake was reduced 70 % in the spring and 57 % in the summer (Table 4). The effect was even more pronounced 16 h after the addition of PCBs, which indicated that the phytoplankton community was severely affected.

The effect of PCBs on primary productivity seems to depend on the density of phytoplankton cells in the isolates in relation to the level of PCBs. During summer, phytoplankton isolates from the eutrophic lake that showed a high initial carbon fixation rate ( $387.7 \text{ mg C m}^{-3} \text{ h}^{-1}$ ) and a high chlorophyll *a* ( $72.6 \text{ mg m}^{-3}$ ) and POC concentration ( $15 \text{ mg L}^{-1}$ ), were not affected by the added PCB compounds. However, spring samples, with a fixation rate of about 1/3 and a chlorophyll content of about 1/2 of the summer samples, showed a reduction of 34 % compared to the controls (Table 4). Furthermore, a ten-fold decrease in the amount of PCBs added to the oligotrophic phytoplankton isolates resulted in smaller changes in productivity rates, compared to those receiving original amounts.

As in the field study, phytoplankton organisms incubated in the laboratory sorbed most of the added PCBs (Table 3). Approximately 5-12 % was adsorbed to the walls of the bottles, whereas the concentration in water from the oligotrophic lake was about twice that from the eutrophic lake.

## DISCUSSION

Phytoplankton isolates from eutrophic and oligotrophic lakes, grown *in situ* or under defined conditions in the laboratory, were affected by the addition of Clophen A 50. In eutrophic lake isolates, incorporation of  $^{14}\text{C}$  bicarbonate was only temporarily suppressed and a recovery, although not complete, was noted 16 h after the introduction of PCBs. Similar results were obtained by BRYAN & OLAFFSSON (1978). They showed that, on initial introduction of PCBs to cultures of *Euglena gracilis*, the photosynthetic rate sharply decreased to 50 % of that of the controls but that the algae recovered after 4 h. In the case of isolates from eutrophic lakes, our results support their findings.

Phytoplankton isolates from the oligotrophic lake proved to be more vulnerable to PCBs than those from the eutrophic lake. 16 h after the addition of PCBs, the algae still did not show any sign of adaptation to the foreign substance. Compared to the uptake of  $^{14}\text{C}$  bicarbonate at the start of the experiment, the results indicate a further (47 %) suppression after 16 h exposure to Clophen A 50.

COLE & PLAPP (1974) and HAWES et al. (1976) found a relationship between cell density and productivity of *Chlorella* cultures exposed to various Aroclor fractions. We suggest that a similar relation exists in natural phytoplankton assemblages, on the following grounds: In isolates from the eutrophic lake, the concentration of chlorophyll *a*, a rough measure of the photosynthetic biomass, was approximately 18-26 times that of the oligotrophic lake. Expressed as Clophen A 50/chlorophyll *a*, phytoplankton organisms of the oligotrophic lake contained approximately 10-17 times more PCBs than those of the eutrophic lake. Identical amounts of PCBs added to the different phytoplankton isolates therefore resulted in a pattern of distribution that related to photosynthetic biomass. This may explain the differences in carbon fixation rates between the two lakes.

Consequently, when phytoplankton organisms from the two lakes were exposed to PCBs in amounts that were similar on a PCB/POC basis, the changes in carbon fixation rates were of similar magnitude.

The reduction observed in the oligotrophic lake (11 %) is mainly an effect of the solvent, as ethanol alone decreased the carbon fixation rate by 9 %. Thus, a threshold seems to exist for the amounts of PCBs accumulated per cell below which the carbon fixation rate is only temporarily affected. Similar amounts of PCB compounds therefore effect primary productivity differently, depending on the trophic status of the lake.

The pattern of distribution of Clophen A 50 within the isolates shows that the highest amount was recovered in the algae and a large fraction was found in the water, while only a small part (5 % - 12 %) was adsorbed to the walls of the bottles. It is most likely that the amounts not recovered in the material balance (8 % - 22 %) are to be found attached to the glass walls (SÖDERGREN 1973). As much as 1/3 of the added compounds may therefore be withdrawn the waterphase, a fact that should be considered when evaluating effects in aquatic test systems. The material balance also indicates that a high photosynthetic biomass may store large amounts of PCBs, which underlines the properties of algae as

transfer organisms, facilitating the transport of PCB compounds to higher trophic levels in the food web.

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