Fate of Polychlorinated Biphenyl (Aroclor 1242) in an Experimental Study and its Significance to the Natural Environment

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Polychlorinated biphenyls (PCBs) and their derivatives are major environmental pollutants. Considerable attention has been given to their persistence and widespread presence in the global ecosystem (14). Reports on possible toxic effects on higher organisms have been reviewed (13) and until recently data on the toxicity of these chemicals to marine and fresh water phytoplankton which constitute the base of the aquatic food chain have been scarce. Recent laboratory studies on marine and fresh water phytoplankton (1, 2, 3, 6, 11, 12), however, indicate that most marine diatoms are very sensitive to PCBs, whereas algae may show variable degrees of resistance. Reduction in growth and disruption of chloroplastic membranes in an aquatic angiosperm (9, 10) and in algae (3), have also been recorded.

In this paper we present data regarding the fate of PCBs under experimental conditions which may have some bearing on natural environments. The experimental organisms were the fresh water algae, Chlamydomonas reinhardi and Chlorella fusca which, in previous studies, have been found to be PCB-pseudo-resistant and highly sensitive, respectively.

MATERIALS AND METHODS

The two species of algae studied were (1) Chlamydomonas reinhardi, strain c137+, commonly used in this laboratory, and (2) Chlorella fusca var. vacuolata (211/8b) obtained from the Cambridge Culture Collection, England.

All algal strains were grown in sterile liquid TAP medium (4). Experiments were carried out in 250 ml Erlenmeyer flasks with side arms, which allowed easy absorbance measurements. Media volume was standardized at 20 ml/flask. Incubation was under continuous light (fluorescent-incandescent mix 6000 lux m^{-2}) at 22-24°C. Culture flasks were agitated by gyratory shaking at 120-150 rpm. PCB-Aroclor 1242(R) was obtained from the Monsanto Chemical Company, St Louis, USA and was dissolved in 100% methanol to make a stock solution of 20,000 ppm from which serial dilutions were made in TAP to get the

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required final concentrations. All glassware was rinsed with methanol prior to normal washing procedures, to remove any possible traces of PCB contamination. Culture vessels were plugged with cotton wool and additionally protected with an aluminium foil cap. All experiments were conducted twice in triplicate. Total cell number was determined by direct count in a hemocytometer.

EXPERIMENTAL

In order to investigate the problem of surface adsorbtion, if any, of the PCBs on the culture flask walls the following procedure was adopted. Abiotic pre-culture flasks containing 20 ml of 5 ppm Aroclor 1242 in TAP were shaken for 3 days under usual growth conditions (see Materials and Methods). On the third day, the culture solution which will be referred to as 'medium fraction', was transferred into a fresh sterile flask. The empty pre-culture vessel was then washed with 0.2 ml of methanol in order to resuspend the PCB which might have been adsorbed to the glass surface and 20 ml of fresh TAP medium was added. This culture solution was called 'adsorbed fraction'.

A separate solvent control was set up with 0.2 ml methanol in 20 ml TAP. To all these flasks 0.5 ml actively growing cells of Chlorella 211/8b (initial density 2 x 10^5 cells/ml) was added and allowed to grow for 7 days, during which period daily growth measurements and cell number determinations were taken. The results are presented in Fig. 1.

Another experiment of similar design was conducted using 10 ppm Aroclor 1242 instead of 5 ppm with Chlorella 211/8b as well as C.reinhardi c137+ as the indicator organism (Fig. 2).

RESULTS AND DISCUSSION

Earlier studies had indicated the growth behaviour of C.reinhardi (c137+) and Chlorella (211/8b) in TAP supplemented with varying levels of Aroclor 1242. Growth of C.reinhardi (c137+) was characterised by an initial fall in total cell number followed by normal growth after 3 days of culture in 5 ppm Aroclor 1242 in TAP. Conversely Chlorella 211/8b was highly sensitive to Aroclor 1242 and failed to grow above 0.6 ppm, whilst slightly lower levels (0.4 ppm, 0.5 ppm) caused a lag period, which however was quickly overcome.

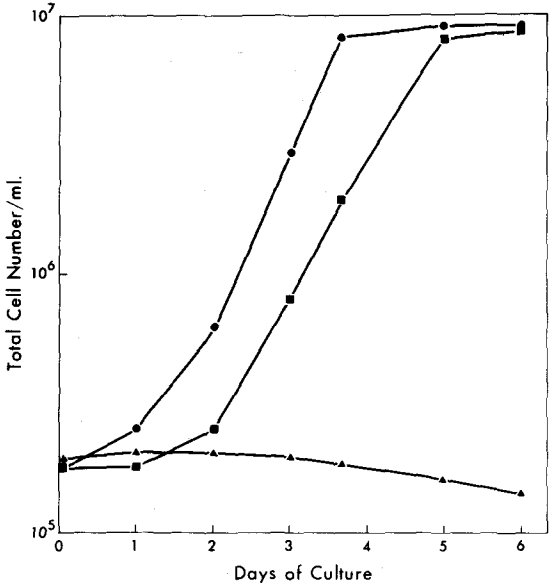

Fig. 1. Growth of Chlorella (211/8b) in 'adsorbed fraction', 'medium fraction' and solvent control.
Aroclor (5 ppm) in TAP was abiotically cultured for 3 days. Adsorbed and medium fraction were obtained as described in text. Incubation as described in MATERIALS AND METHODS.

●——●: solvent control; ▲——▲: adsorbed fraction; ■——■: medium fraction.

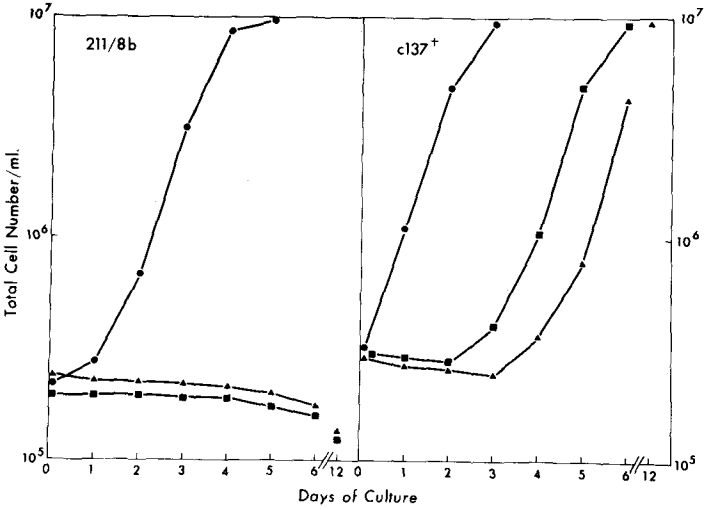

Fig. 2. Growth of Chlorella (211/8b) and C.reinhardi (c137+) in 'adsorbed fraction' 'medium fraction' and solvent control.
Aroclor 1242 (10 ppm) in TAP was abiotically cultured for 3 days. Culture conditions and experimental design as Fig. 1.

In the experiments described above solvent controls had no effect on the growth of either 211/8b or c137+ as these showed normal growth curves (Figs. 1 and 2). Additionally, it was seen that the 'medium fraction' of an abiotic preculture experiment (5 ppm Aroclor 1242) supported normal growth after a slight initial lag of 211/8b (usually highly sensitive to 5 ppm Aroclor 1242). Conversely, the 'adsorbed fraction' did not permit any growth of 211/8b at all. These results indicate that PCBs adsorbed strongly to the glass surfaces from which it was recoverable after 3 days of abiotic culture. Enough toxicity, however, was retained in the medium fraction to cause an initial lag for the sensitive strain of 211/8b. If, however, 10 ppm Aroclor 1242 was used during the preculture period, then both 'medium' and 'adsorbed fraction'were toxic to 211/8b cells. This indicated that the glass surfaces have a limited degree of adsorption, thus leaving sufficient concentration of PCBs in the medium fraction to destroy 211/8b cells.

Using C.reinhardi c137+ as an indicator organism in the 10 ppm fractionation experiment, initial fall along with the standard regenerative-type growth after 3 days were observed in both 'medium' and 'adsorbed fraction'. From these observations we conclude the following:

(1) The major amount of Aroclor 1242 disappears from the biological environment and becomes adsorbed to the glass surfaces. Judging from the present bioassay data derived with 211/8b, we estimate between 0.3 and 0.5 µg Aroclor 1242 to be adsorbed per square centimeter of glass surface.

(2) The above quoted figure represents an upper limit and saturation of adsorption sites occurs, thus leaving certain levels of toxicity within the medium fraction.

(3) This disappearance of a major fraction of the PCB allowed c137+ cells to exhibit their fall/regenerative type of growth curve. When the toxicity was removed, cells were released from their growth inhibition. Furthermore, it should be borne in mind that, although 211/8b is highly sensitive to Aroclor 1242, the inhibition prevails even after most of the toxin is readsorbed onto the fresh glass surfaces of a second culture flask. This indicates that Aroclor 1242 preferentially binds with some target site in 211/8b

and that this binding is irreversible (most likely chloroplastic membranes).

These observations have some implications in not only designing and analysing experimental methods using glassware, but also in assessing the possible fate of PCBs in the environment. From the reports published, it is fairly clear that certain phytoplankton are sensitive to very low concentrations of PCBs (3, 6, 12). Thus PCBs could conceivably contribute to a change in species composition. However, we suggest that this sensitivity is only observable if the toxin is biologically available. Our experiments with abiotic precultures in laboratory conditions clearly indicate that much of the PCB dumped in the environment would have a tendency to adsorb onto 'environmental surfaces' such as rocks and sands, or to be absorbed by resistant organisms. This would explain the vast difference which exists between the estimates of the amount of PCBs dumped and the amount present in the aquatic samples.

It is of interest that HARVEY et al. (1974) have found a 40% decline of PCBs in the North Atlantic waters during the period 1971-73 following the restriction of their use by the USA since 1970. However, this estimate has been challenged (8) on technical grounds. Taking our results into account we support the view taken by HARVEY et al. (1974) and believe that the decline of PCBs in the aquatic environment should be attributed to their dispersal and removal from the water phase on to 'environmental surfaces'. Further study from all types of samples and including biodegradation data is needed to assess the fate of PCB in the environment.

In conclusion, we should strongly oppose the idea that PCBs do not pose an environmental hazard (7) and support our view on the results of toxicity studies on phytoplanktons made so far (see reference). On the basis of our experiments, PCBs once available in the soluble form will severely affect sensitive strains of diatoms and algae and thus would contribute to a possible alteration in species composition of phytoplanktonic communities (12). We also propose that biologically inactive PCBs may be remobilised by other organic pollutants, such as oil or related petroleum products, then in similar fashion to the release of the 'adsorbed fraction' as described above. Thus there may be a latent reservoir of adsorbed PCBs in the environment which will contribute a continuous potential hazard.

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