Interactions Between Polychlorinated Biphenyls (PCBs) and Soil Microfungi. Effects of Aroclor-1254 and Other PCBs on *Aspergillus flavus* Cultures

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In view of their large environmental spreading, polychlorinated biphenyls (PCBs) are currently the subject of thorough studies as xenobiotic compounds likely to interfere in biochemical processes of living things. However, the scope in this field is so extensive that the individual results obtained so far with regard to their accumulation, distribution, metabolism and toxicological aspects are still insufficient to draw general conclusions. This applies specially to the interactions between PCBs and edaphic microflora, both in respect of either the changes these compounds may undergo, or their effects upon the composition and growth of the microorganisms involved.

Because of its scarcely selective permeability to the compounds found in its environment, as well as the main role its plays in the natural processes of hydrocarbon degradation, the microfungi appears to be the most suitable biological material for this study. This contribution discusses some aspects of the interactions both in solid and liquid cultures of Aspergillus flavus under different concentrations of various Aroclors of different rates of chlorination.

MATERIAL AND METHODS

Incubation - For liquid cultures, 250 ml Erlenmeyer flasks with 50 ml of a 3.5 % sterile water solution of Bacto Czapek Dox Broth (DIFCO) were used, the inoculum being 0.25 ml of a suspension of spores in sterile distilled water with 0.1 % of Tween-20. Cultures in solid medium (Bacto Czapek Solution Agar; DIFCO) were prepared in 4 cm diameter Petri plates, seeded with 0.1 ml of the same spore suspension. In both cases, incubation took place during 200 hours at 29±1 ºC. Liquid cultures were continuously shaked.

PCBs were added to liquid cultures dissolved in 200 µl acetone. On solid substrata they were added by evaporating hexane solutions of PCBs over the surface

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of the gel medium. In the first case, 5, 10, 25 and 50 ppm of Aroclor were used; and 1.6, 4, 8 and 20 $\mu g/cm^2$ in the second case. A similar treatment was applied to the controls, using pure dissolvents.

Extraction, purification and analysis of PCBs - After separating medium and mycelium through filtration, the former was extracted three times with an equal volume of hexane each time. The mycelium, previously dried over P_2O_5 at a slightly reduced pressure, was divided into three aliquot portions, one of which was homogenized with quartz sand and extracted – for tests conducted with higher chlorine PCBs (Aroclor 1254 & 1260) – in a Soxhlet, using 40:60 hexane:acetone. For lower chlorine PCBs (Aroclor 1232, 1242 & 1248) a better recovery was attained by placing the homogeneous material into a 2 cm diameter column, provided with a filtering plate, and eluting with 200 ml hexane. For the extraction of solid cultures, after homogenization of the entirety of the gel of each plate with quartz sand and anhydrous Na_2SO_4, the same procedure used for homogenized mycelium was applied.

The extracts were concentrated to a convenient volume in a Kuderna-Danish evaporator and purified by adsorption chromatography on 10 x 2 cm Florisil columns, using 150 ml of 95:5 hexane:ethyl eter as eluting solvent. Analyses were made by GLC electron-capture detector and columns with 1.2 % E-301 on 80-100 mesh Chromosorb W-HP as stationary phase. Working temperatures at column, detector and injector were 165, 190 and 200º C, respectively. The flow rate of the gas carrier (N_2) was 35 ml/min.

Quantitative evaluation of PCBs, after having checked that no changes had occurred in individual Aroclor, was done by correlating the heights of five significative peaks in standard and analysis injections showing substantially identical signals.

The absence of changes was verified by the criterion of equality of the chromatographic profile, and considering as unchanged Aroclor extracted those whose chromatograms showed the same ratios between each peak height and those of all the other peaks as the ones of a standard chromatogram.

Fractionation and determination of fungal components - It was carried out starting from the second aliquot of the dried mycelium, according to the procedure of SHIBKO et al. (1967), except for the determination of proteins, for which the third aliquot was used once

homogenized with 5-8 ml of 1N NaOH and kept during 10 min. in a boiling water bath. After centrifugating the suspension (10,000 g / 15 min.), and washed the sediment with 5 ml 1N NaOH, the supernatants were collected and their protein content determined by the microbiuret method of GOA (1953). DNA was determined by the diphenylamine reaction according to the modification of GYLES and MYERS (1965) and RNA with the orcinol reagent (OGUR and ROSEN, 1950).

RESULTS AND DISCUSSION

No metabolic changes of PCBs have been observed, as well as no differences in the accumulation of individual components of a single Aroclor were found at the end of the incubation periods. However, on comparing the accumulation of various Aroclors with different rates of chlorination, it was found that, in liquid cultures, their final concentrations in the mycelium were linearly correlated (coefficient: 0.992) with an index: I = initial PCB concentration in the culture x % PCB chlorination (fig. 1).

On the other hand, the presence of PCBs in the culture deeply alters the growth of the microorganism. The weight of the dry mycelium from liquid cultures drops progressively during the first 120 hours of incubation as the dose rates of the Aroclor-1254 rises from 5 to 50 ppm (fig. 2), while the relative RNA content generally varies in the opposite direction (fig. 3). The relative DNA content remains unchanged in all the cases assayed. In cultures on solid media appears an obvious sporulation delay and a decrease of diameter in the colonies developed from punctual inocula.

It was also found that the magnitude of the above mentioned effects was the highest for Aroclor-1232 and the lowest for the 1260 type, when the microorganism was grown in individual liquid cultures in the presence of 25 ppm of the Aroclor series of 32, 42, 48, 54 and 60 percent chlorine (Table I). Intermediate values showed discontinuity in the case of Aroclor-1254, whose effects were similar in intensity to those produced by other PCB mixtures of lower chlorine percentage.

The characteristics of the post-exponential drop of RNA levels – whose relation with the proteins synthesis is well known – suggest that, similarly to what has been described for tissues of higher animals (TURNER and GREEN, 1974; LITTERST and VAN LOON, 1974), PCBs may stimulate in microorganisms the production of

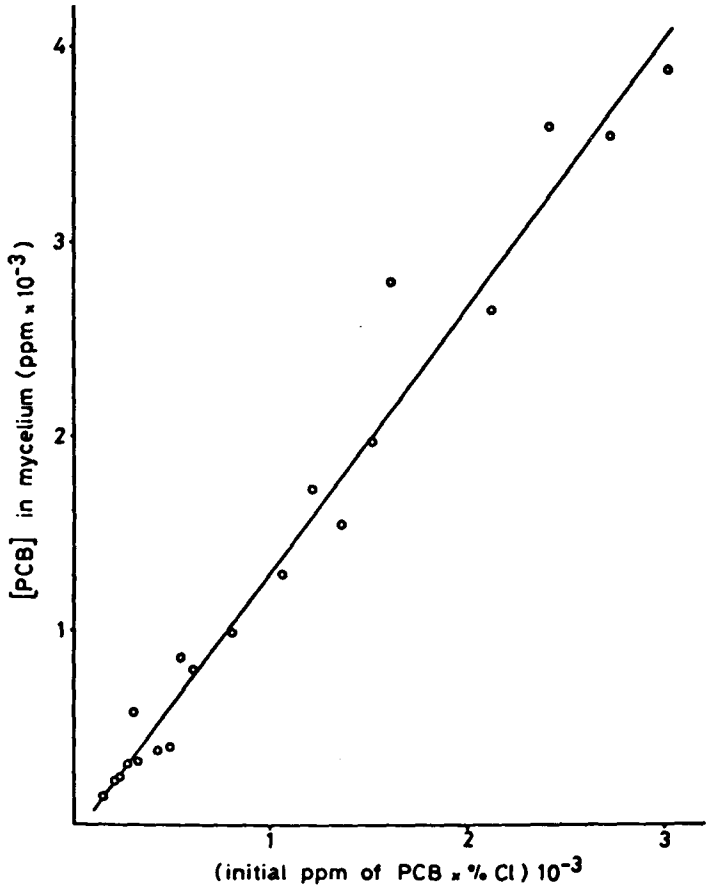

Fig. 1 - Correlation between mycelial accumulation of different Aroclors at different concentrations and the "I" index.

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enzymes involved in some general mechanisms of the metabolism of xenobiotic compounds. Having then verified the absence of incidental transformations affecting PCBs, we investigated whether their presence in the culture could affect the metabolism of another xenobiotic substance susceptible of being attacked by the microorganism. For instance, such is the case of aldrin (HHDN), which various species of Aspergillus are able to oxidize to dieldrin (HEOD) and other derivatives.

TABLE I

Effects of different Aroclors (25 ppm) on the dry weight and the relative RNA content of A. flavus at two different times of incubation.

	dry w. (mg)		dry w. (%)		µg RNA/mg dry w.	
	75 h.	150 h.	75 h.	150 h.	75 h.	150 h.
CONT.	289.0	612.1	100	100	5.9	3.4
A-1232	4.0	327.3	1.4	53.4	18.6	4.3
A-1242	9.9	492.3	3.4	80.4	18.5	3.2
A-1248	11.3	458.2	3.9	74.8	13.2	4.1
A-1254	9.7	301.4	3.3	49.2	18.0	4.0
A-1260	158.6	594.1	54.6	97.0	6.2	4.6

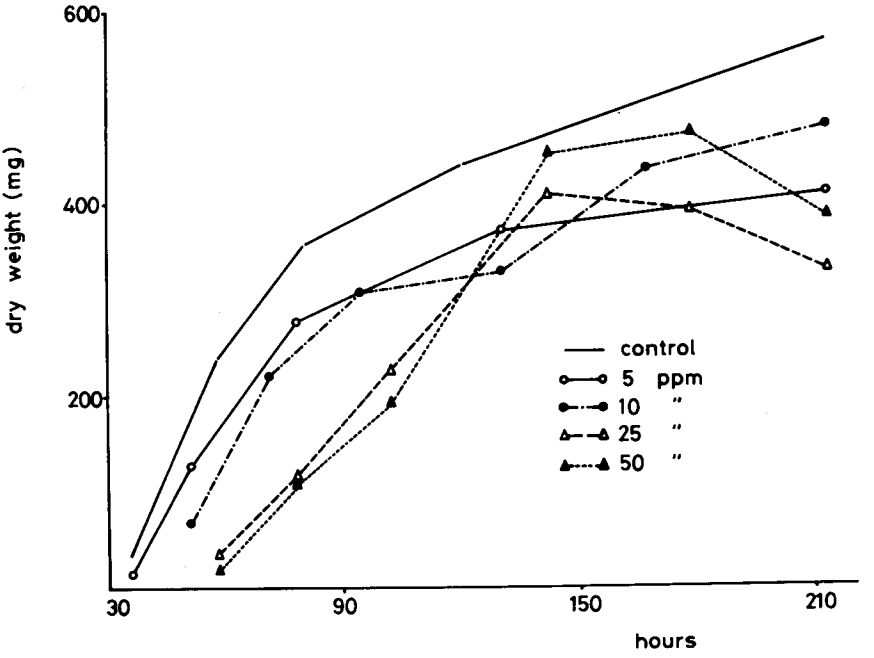

Fig. 2 - Effects of different concentrations of Aroclor-1254 on the dry weight of mycelium. (Liquid cultures).

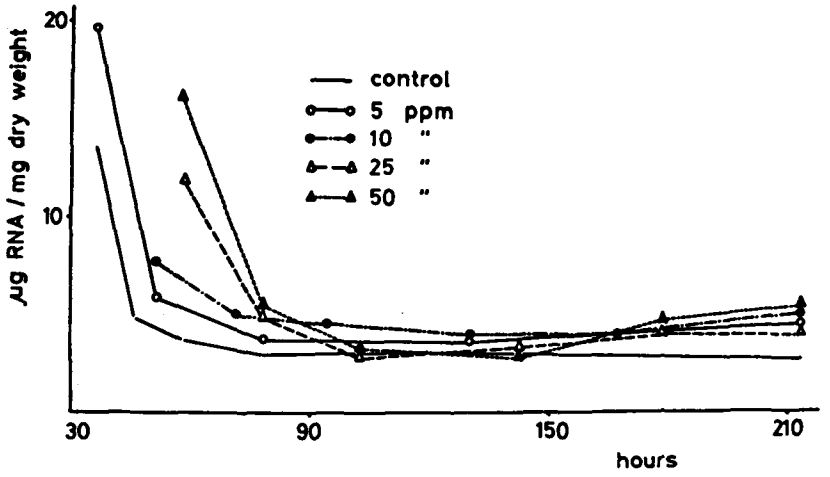

Fig. 3 - Effects of different concentrations of Aroclor-1254 on the relative mycelial RNA content. (Liquid cultures).

The results obtained after adding separately 25 ppm of each of the tested Aroclors to A. flavus cultures containing 5 ppm of aldrin (Table II), are consistent with the assumption of the enzymic induction and also with the relations among the relative RNA contents given in Table I.

TABLE II

Metabolism of aldrin by A. flavus in the presence of 25 ppm of different Aroclors (A_t: aldrin transformed at the end of the incubation period; Myc: dry weight of the mycelium).

	added aldrin (ppm)	added PCB (ppm)	A_t (µg)/ Myc (mg)
CONT.	5	--	0.306
A-1232	5	25	1.225
A-1242	5	25	0.928
A-1248	5	25	0.509
A-1254	5	25	0.910
A-1260	5	25	0.479

The first results of the micromanometric tests which are being conducted, seem also to support the above hypothesis, and work is being carried on to obtain concrete data on the involved enzymic system.

The induction by certain substances of enzymic systems which subsequently are not able to act on the inducting molecules, is not an unknown phenomenon. For instance, the β -galactosydase induction by some synthetic lactose analogous not sensitive to the action of the enzyme, has been largely studied and documented in well known works.

According to such a schema, the effects of PCBs on A. flavus described herein could be interpreted on the basis of a not very specific action of the enzymic systems induced on the endogenous fungal metabolites involved in the growth of the microorganism. Furthermore, having in mind other results, as those obtained by PEAKALL (1967) in relation to the DDE effects on the reproductive mechanisms of birds, it could even be assumed that this type of alterations of the cell regulation is one of the most significative aspects of the environmental impact of xenobiotic compounds.

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