Biodegradation Studies of PCBs

The fate of PCBs in the environment is of considerable interest to ecologists. Among the various natural mechanisms for degrading organic materials, a significant contribution may be expected from micro-organisms. We have therefore studied the effects of selected micro-organism cultures on both single isomers and commercial mixtures.

The basic experimental procedure consists of shaking prepared substrates comprising PCBs, mineral solutions, inocula, inhibitors and etc. for varying periods of time and determining the loss of PCB by GLC analysis of extracts. We have studied only primary degradation and have not identified the products of the metabolic processes involved. The experiments were carried out in shake-flask equipment using standardised techniques to establish reproducible conditions. The culture-flasks were stoppered 250 ml conical flasks which were vented at weekly intervals to ensure aerobic conditions. Normal room temperature (20°C) was used for many experiments, but for later work we standardised on a Gallenkamp orbital incubator operated at 130 cycles per minute controlled at 25°C.

The culture flasks normally contained 100 ml of culture solution, plus 5 ppm PCB. Some flasks also contained biphenyl (50 ppm) and inhibitor (mercuric chloride) was included in some for control purposes. To avoid errors due to absorption effects the total contents of each flask were extracted with pentane for analysis.

The pentane extracts were reduced to a volume of 2 - 3 ml (a concentration factor of 50 to 100 x) on a warm water bath using a nitrogen purge. For analyses using packed column GLC the pentane extracts were used directly, but for capillary column work a further concentration step of about 100 x was obtained by adding a small quantity of n-decane and removing all the pentane by evaporation. Packed column GLC analyses for single isomers and simple mixtures were made using a 4 ft. column packed with 4% Silicone Oil OV 101 on 60 - 80 mesh Gas Chrom Q coupled to a high temperature Ni^{63} electron capture detector. For analyses of complex mixtures and commercial products a 200ft. stainless steel capillary tube (0.030" bore) coated with Apiecon L was used.

By using this capillary system the methods developed by Welti and Sissons (1) could be used for component identification.

Early experiments were made using activated sludge inocula (culture C_1), from the biological effluent treatment plant of Monsanto at Ruabon, N. Wales, pre-adapted to degrade biphenyl. During this phase of the work the experimental techniques were developed and some degradation of PCBs was observed. It was also noted that the addition of biphenyl to the culture flask enhanced the degradation of the PCBs.

During this time we learned that workers at University College of North Wales, Bangor, had isolated from soil a pure biphenyl-degrading culture, reported to be a nocardium of the order atinomycetes. We were able to obtain a sample of this culture and, following the advice given by Professor W.C. Evans, successfully grew our own stock which we designated culture C_2. Later on, the Bangor workers isolated another pure culture from a canal mud (pseudomonas family) and again we were able to grow a stock which was designated culture C_3.

Using cultures C_2 and C_3 we studied the degradation of a number of single isomers and simple mixtures both with and without the addition of biphenyl. The results of this work are summarised in the table (Table 1).

The work has since been extended to the study of commercial mixtures like Aroclor 1242, using capillary column GLC to identify and quantify the components present before and after degradation for varying periods of time. The complexity of these commercial mixtures complicates the problem of analysis and interpretation and we cannot yet give a complete assessment of the results.

TABLE 1

Isomer	Culture	With or without biphenyl	Time to Degrade to 50%
2,4'-dichloro-	C_2	With	2 days
	C_2	Without	5 days
4,4'-dichloro	C_2	With	>50 days
	C_2	Without	>50 days
	C_3	With	<10 days
	C_3	Without	<15 days
2,3-dichloro	C_2	With	4 days
		Without	4 days
3,4-dichloro	C_2	With	2 days
		Without	3 days
2,5,2'-trichloro-	C_2	With	3 days
		Without	7 days
2',3',4'-trichloro-	C_2	With	<2 days
		Without	3 days
2,3',4'-trichloro-	C_2	With	6 days
		Without	6 days
2,4,5-trichloro	C_2	With	>50 days
		Without	>50 days
2,2',4,4'-tetrachloro	C_2	With	>50 days
		Without	>50 days
2,2', 4,6-tetrachloro	C_2	With	>50 days
		Without	>50 days
2',3',4',5',2,3-hexachloro*	C_2	With	<15 days
		Without	>50 days

* Mixed with 2',3,2' and 2,3',4',-trichlorobiphenyls to improve solubility.

[illegible]

The results of the work reported here indicate quite clearly that the lower chlorinated isomers of P.C.Bs can be degraded by enzyme hydroxylation induced by micro-organisms. The results with single isomers seems to support the work of Evans and Lunt (22) and their suggested mechanisms for biphenyl degradation with the specific organisms which they isolated. It seems reasonable to explain our findings in terms of chlorine substitution interfering with the hydroxylation steps or subsequent ring opening stages. This kind of theory would indicate that the culture C_2 would not degrade 4,4'-dichlorobiphenyl, whereas culture C_3 could. It also suggests that C_2 could degrade 2',3',4',5',2,3-hexachlorobiphenyl. These indications were supported by the experimental results obtained with single isomers and simple mixtures. The conclusion to be reached from these results, coupled with the proposed mechanisms of Evans and Lunt is that the intractable low chlorine isomers (<5Cl) of P.C.Bs would be those chlorinated in the 2,6 positions on one ring, and the 2,5,2',5' and 3,5,3',5' tetra-chlorobiphenyls. The total number of intractable low chlorine isomers would, on this basis, be 20 out of a theoretical total of 82. Of these, only 7 are likely to be present in significant quantities, based on probability considerations.

When commercial mixtures of P.C.Bs are exposed to these biphenyl-degrading cultures a somewhat different pattern of degradation appears to emerge. The most noticable feature is that many of the low chlorine isomers which appeared to be intractable when present alone, degrade comparatively readily when present in the [illegible]. Thus 4-4'dichloro-biphenyl degrades to 50% in approximately 2 days when exposed to culture C_2 as a component of **Aroclor 1242.**

The general conclusion from the study of these commercial mixtures is that most isomers present in significant amounts in Aroclor 1242 degrade with time constants [illegible] 20 days; [illegible] small percentage, consisting of [illegible] degrade [illegible] with [illegible]

MONS 040411

The curves showing the pattern of degradation for some isomers in Aroclor 1242 in figures 3 and 4 tend to fall into three groups. Those isomers containing only one or two chlorines degrade rapidly, in less than 10 days. Another group consisting mainly of trichloro-biphenyls seems to degrade rapidly, after an initial induction period, but then tends to a slower rate when the level has dropped to 10% or so. The third group, primarily tetrachloro-biphenyls, degrades more slowly, but does not show such a pronounced induction period.

This pattern of degradation can possibly be explained on the basis of adaptation and reversion. For the mono- and di-chloro-biphenyls the biphenyl-degrading cultures probably need little adaptation and will readily degrade these components. For more highly chlorinated components however, some adaptation will be necessary and the ease with which this is accomplished may depend on the concentrations of precursors available. As the precursors are degraded, however, the organisms may suffer reversion and the rate of degradation thereby decrease. As the chlorine content in the molecule is increased the rate of degradation may be expected to decrease as a result of reducing the available molecular sites for oxidation. However specific tetrachloro isomers, for which suitable precursors are available, may not need a long adaptation period and could therefore follow the type of curve indicated.

References

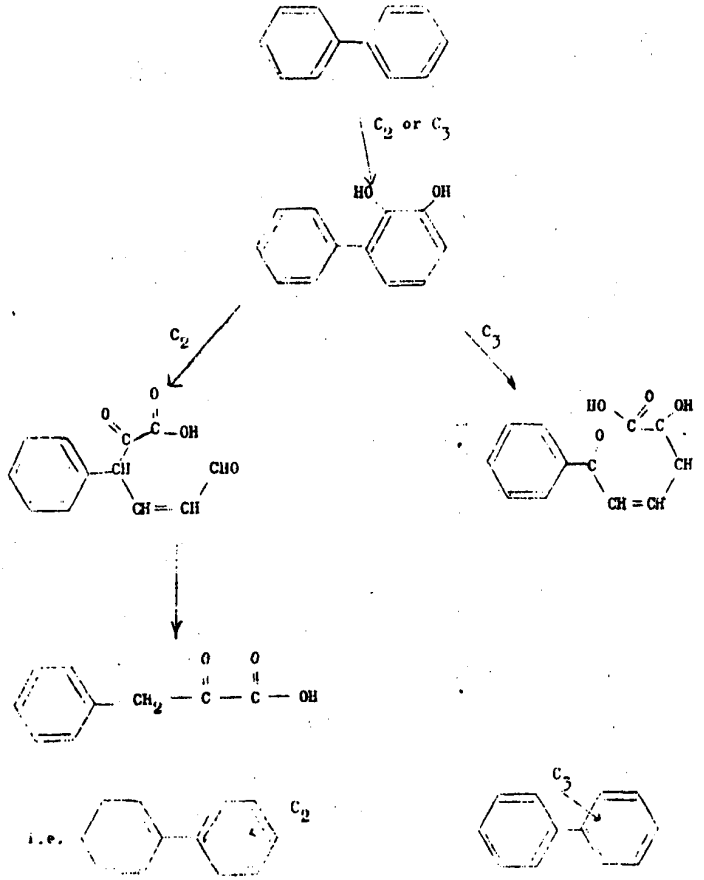
(1) Sissons, D., and Welti, D., (1971) J. Chromatog., 60, 15.

(2) Lunt, D., and Evans, W.C., (April 1970) Proc. Biochem. Soc.

fig 1

1242, 1ppm

FIG. 2 Metabolic pathways for the biodegradation of biphenyl with C_2 and C_3



MONS 040415

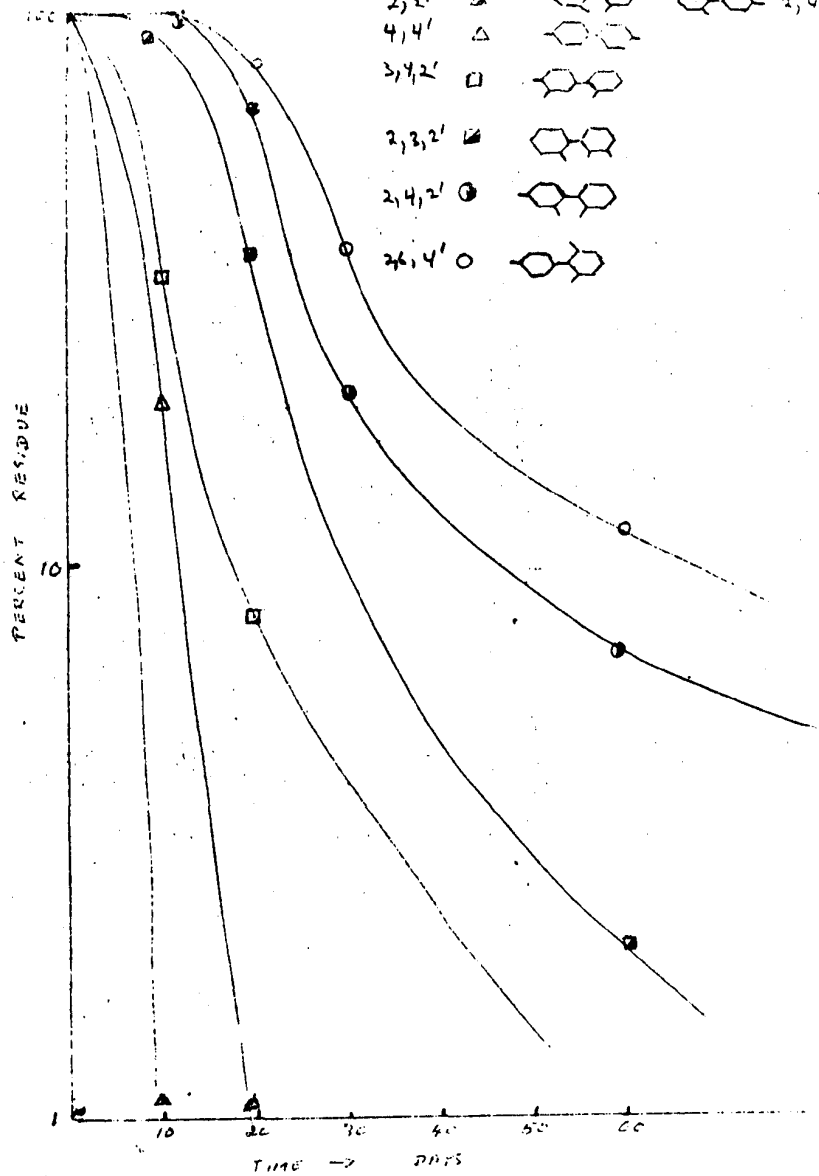


FIG. 3

MONS 040416

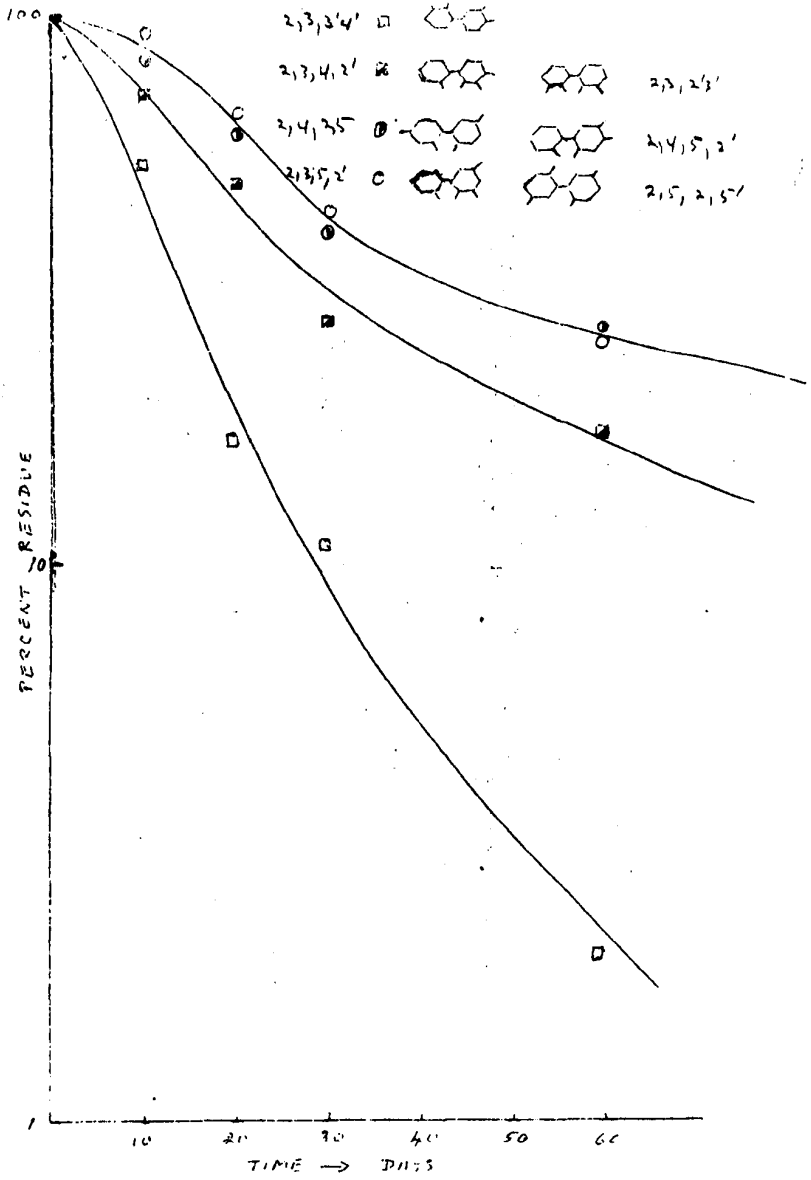


FIG. 4

MONS 040417