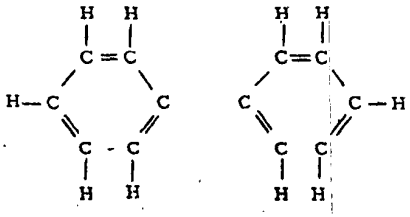
P.C.B.

Biphenyl is an aromatic hydrocarbon consisting of twelve carbon and ten hydrogen atoms. The carbon atoms are arranged in two rings of six, each joined by alternate single and double bonds, the two rings being joined by a bond from a carbon atom of one to a carbon atom of the other. Each of the remaining ten carbon atoms is bonded to a hydrogen atom. The structural formula is:



One or more hydrogen atoms may be substituted by chlorine to form a chlorinated biphenyl. PCBs, or polychlorinated biphenyls, have two or more chlorine atoms. Since from one to ten chlorine atoms may be substituted, there are ten homologs possible and for each homolog, of given chlorine number, several different arrangements, or isomers, of the molecule are possible. The following table shows the number of isomers theoretically possible for each homolog group, making a total of 210 isomers.

TABLE I

Theoretically Possible Chlorobiphenyl Isomers

Homologs No. of Cl atoms per biphenyl molecule	0	1	2	3	4	5	6	7	8	9	10
Theoretical number of isomers	1	3	12	24	42	46	42	24	12	3	1

Commercial PCB products, of which Aroclors are examples, are produced by direct chlorination of biphenyl, the degree of chlorination being chosen to provide desired properties. As the degree of chlorination is increased the products vary from mobile liquids to more viscous liquids and finally to waxes and solids. Lubricity, boiling point and fire resistance also increase with

increasing chlorination. In all cases, however, the products consist of mixtures of homologs and isomers; indeed, many of the desirable functional properties arise because they are mixtures. These materials are inert, chemically resistant and readily soluble in most common organic solvents. They distill in the range 275°C to 450°C and are, in the classical sense, water insoluble.

The most widely used of the series have average chlorine contents of three, five or six chlorine atoms per molecule. Because they have high dielectric strength and dielectric constant and are fire resistant, they are used to insulate capacitors and transformers. Their thermal stability leads to their use as heat transfer fluids. Good viscosity and lubricity characteristics made them useful as hydraulic fluids and lubricant, while solvent power and compatibility resulted in their selection as plasticizers and solvents for carbonless copying papers. All of these uses were reasonable because available evidence indicated no significant toxicity to man or animals except in rare instances of massive prolonged exposure.

Since the discovery that PCBs were accumulating in the environment, other fluids have been substitued for PCBs for heat transfer, hydraulics, lubrication and plasticizer applications and as solvents in carbonless copying papers. This has been done voluntarily by industry without government pressure. However, the PCBs have a unique combination of properties which satisfy our needs as capacitor insulating fluids and for transformers where risk of fire must be minimized. No other known materials fill our needs in these areas.

Our present way of living depends for its existence on the efficient generation, transmission, distribution and use of electrical power. Transformers and capacitors are essential for the last three of these.

Because large electrical generators are rotating machines, they must operate in a gaseous environment. This limits their output to relatively low voltages, whereas efficient power transmission over long distances requires high voltages. Electrical step-up transformers are required for this service, but are usually oil filled since they can be located outdoors and away from people. In some cases, to further improve the efficiency, the power is converted to direct current for transmission. This requires the use of large banks of capacitors. Near the point of use the power is converted from direct to alternating current requiring more capacitors. For local distribution, the power is stepped down to lower voltages at substations which again use oil-filled transformers since they too are outdoors. Since much of the power used is for electric motors, which are inductive, capacitors are used to raise the power factor to avoid high line loses. Distribution transformers are often located inside buildings and in crowded areas. Here fire resistance is necessary to minimize the hazard

to people. Conversion of electrical power to mechanical power for operating appliances, such as furnace blower motors, refrigerators, air conditioners, pumps for compressed air and to supply water from wells, usually involves capacitor start-run motors. Capacitors are required in fluorescent lights and high powered mercury vapor lamps for street lighting and for many other purposes.

No type of fluid other than chlorinated biphenyl with as many chlorine as hydrogen atoms mixed with trichlorobenzene or mixtures of tri- and tetrachlorobenezene gives a transformer insulating fluid which does not burn or form flamable or explosive gases when an arc is struck within it. Fluids of this type called askarels fulfill a vital safety need where transformers must be located in close proximity to people.

It is perhaps less essential that capacitor fluids be fire resistant, but still desirable since failure of a small capacitor could start a fire in a large building with many human occupants. In addition to being fire resistant, PCBs have a higher dielectric constant than other suitable fluids and permit manufacture of smaller capacitors for a given rating which have longer life. Conservation of electrical energy, by using transformers and capacitors in its distribution and use, leads to decreased pollution because of more efficient use of energy converted to electricity.

In 1966, Jensen[1] demonstrated by mass spectrometry that the unknown substances, appearing with organo chlorine insecticides during analyses of environmental speciments by gas/liquid chromatography, were PCBs. Since then, considerable analytical evidence, including much from Monsanto laboratories using increasingly sophisticated techniques (Appendix 1), has accumulated in support of the view that PCBs are widespread in nature and may reach levels which are significant in many aquatic animals and surprisingly high in some species of fish-eating birds[2-8]. The combination of chemical and physical properties necessary for accumulation through a food chain, principally resistance to oxidation and lipid solubility, are characteristic of the chlorinated biphenyls. It is not too surprising that quite low levels in water, perhaps less than 1 part in 10^{10}, can be magnified to more than 1 part in 10^4 when passing through a food chain of aquatic species to predator birds. As stated above, commercial PCB products like Aroclors are not single chemical species, but consist of quite large numbers of homologs and isomers[9] which although similar are not identical in chemical and physical properties. We might expect, therefore, to find variations in the extent to which these various components accumulate in wildlife and the environment and, indeed, many of the published data indicate a predominance of certain homologs, principally those containing 5 or 6 chlorine atoms.[5, 6, 9, 10]

It must be emphasized that with the exception of direct high level controllable contamination near points of manufacture or use, the PCBs found in the environment are the more highly chlorinated homologs (5 or more chlorine atoms). This is true in spite of the fact that the more highly chlorinated homologs consitute only about 35% of the material manufactured. This leads to the conclusion that the less chlorinated materials degrade more rapidly than the more highly chlorinated ones. It also leads to the conclusion that even under conditions of unrestricted use and without special precautions to prevent their entry into the einvornment, these less chlorinated materials degraded rapidly enough to prevent accumulation. Laboratory data (Apendecies I and II) substantiate the fact that they do degrade more rapidly.

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Aroclors are not particularly toxic to small mammals. Acute oral LD_{50} values for rats range from about 6 to 11 g./Kg[11]. For some marine species direct toxicity may be more serious; LC_{50} values ranging from 0-3 to 10 ppm have been reported for brown shrimp (crangon craigon)[12] and even lower concentrations have been reported to be lethal to the Gulf shrimp[13]. In the present context, where accumulation over long periods may be envisaged, the chronic toxicity of Aroclors is probably more relevant. Feeding trials over periods of two years have indicated that in small mammals (rats and dogs) significant effects are not observed at levels of 1 and 10 ppm in food. At levels of 100 ppm liver enlargement has been noted with more highly chlorinated Aroclors[14]. In birds there is evidence that breeding success may be affected by PCBs and feeding trials with Aroclors have indicated the possibility of interference with calcium metabolism leading to thin-shelled eggs and poor hatchability.[4, 8, 14, 15] Generally, however, direct evidence that the present levels of PCBs in the environment are biologically harmful has not been established. There is reason for concern, though, that a continued accumulation of PCBs could eventually generate harmful effects in many species, particularly those near the top of a food chain of aquatic origin.

There have been suggestions that some of the PCB residues have originated from biological or photo-chemical synthesis from other substances present in the environment. It is possible, for example, that DDT can be converted to PCB by such a mechanism,[22, 23] although it seems probable that only the 4-4'-dichloro isomer would be formed in this way and this is not present in significant quantities in the environment.

The fear that unpredictable consequences might follow indefinite accumulation of PCBs in the environment has caused many ecologists to ask how this may be prevented. Some action appears to be necessary, although opinion may be divided on how drastic this action should be.

A simple solution, apparently, would be a total withdrawal of production and use of PCB products. To be effective, such a policy would need to be applied universally and experience shows that multi-laterial negotiations for introducing such measures can be long and protracted. Further, the complete withdrawal of these products would have serious consequences for some industries and the effects on the distribution of electrical power for example could produce serious problems for all users of electrical energy large and small. It is important to balance the social benefits arising from the continued use of such products against the threats posed by the environmental pollution problem. We believe that a satisfactory solution can be achieved by a combination of actions designed to limit the extent to which accumulation can occur, while retaining the use of these products in essential applications. The level reached depends on a combination of factors

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including resistance to degradation, lipid solubility and rate of input into the ecosystem. The present situation has arisen because the rate of degradation of residues is lower than the corresponding rate of input. This high rate of input was due largely to the numerous applications previously discussed and for which substitute products have been introduced except in the electrical industry.

Degradation may occur in many ways, but the principal mechanisms affecting the environmental situation are probably biological and photo-chemical. Evidence for photo-chemical breakdown of PCBs is scanty, but U.V. photolysis of some highly chlorinated PCBs has been reported.[16] Evidence for the biodegradation of PCBs through enzyme catalysed hydroxylation is, however, substantial. Studies of biological degradation arising from exposure of single isomers and commercial mixtures to micro-organism cultures are discussed later and detailed in Appendix 2. These studies show that significantly more biodegradable PCB products are feasible and that virtually complete degradation can be achieved in selected products. Several authors report the results of feeding PCB products to various animals, coupled with tissue analyses.[5, 14, 17, 18] These show substantial metabolism of many of the PCB isomers, especially those with 2 and 3 chlorine atoms per molecule.

The use of products which contain only the more extractable isomers, by increasing the rate of degradation, should reduce the level of residues arising from a given rate of input to the environment. If the rate of input is also reduced by restricting the applications to those such as in the electrical industry, where tight control can be exercised, there is every prospect that the trend to increasing residues can be reversed and that the level of residues in the environment will fall. Monsanto has already restricted sales of products to those applications which are controllable and is supplying more degradable products where they are functionally acceptable. These actions seem appropriate to meet the situation and it is interesting to note that this is regarded as satisfactory and realistic by government representatives in the United States[19] and United Kingdom.[20]

In Sweden, where legislation to control the use of PCBs has been introduced, it is important to realize that although indescriminate use has been banned, a licensing system permits use in well controlled applications such as in electrical capacitors. Similar policies are expected to be adopted in Finland, the Netherlands and other countries.

For such a policy to be successful, cooperation between the industries involved is important to ensure that pollution arising from manufacturing processes and disposal of wastes is minimized. Some of the steps taken to ensure this are discussed in the next section.

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Part of the overall policy for controlling the level of PCB in the environment is to establish a system which effectively limits the rate of entry. It has been emphasized that many of the applications for which PCB products were used formerly were, by their nature, allowing most of the product to find its way into the environment, through aqueous effluent, inadequate incineration, and generally, indiscriminate disposal methods. For many of these applications substitute products are now available. For example in plastics, resins, adhesives, carbonless papers, hydraulic and heat transfer systems and miscellaneous smaller uses, these products, although desirable, are not essential since alternatives can be used, sometimes admittedly at some penalty in cost/performance.

The actions taken to cut introduction of PCBs into the environment to a minimum are the following:

1. Monsanto, the only manufacturer in the U.S.A., decided to sell PCB-containing products only for those uses where two requirements were met:

   a) There was no technically feasible substitute

   b) The use was such that the product did not escape into the environment.

   Uses which met these requirements were as an impregnant in capacitors, as a transformer insulating and cooling fluid and a small group of high temperature heat transfer fluid applications. In all these uses, the fluid is contained in a closed system. In the case of the electrical uses, the containers are sealed and the life of the device is expected to be twenty to forty years. Larger capacitors and transformers are used by public utilities and industrial concerns who could be expected to see to it that any accidental losses from failed or obsolete units would be recovered and disposed of without environmental pollution.

   This single action cut the total use of PCBs by roughly 50%. More importantly, it eliminated those uses which permitted the product to escape into the environment. The company has now restricted sales to electrical uses only to make certain that PCBs are not used in heat transfer systems which, if accidental leakage took place, might introduce PCBs either directly or indirectly into food products.

2. Since there is a considerable amount of experimental evidence (5, 6, 9, 10) showing that the five, six and higher chlorine containing homologs degrade much more slowly than those containing less chlorine, Monsanto has altered the composition of the fluid it sells for capacitor impregnation to cut the concentration of these to a minimum. For many years, the standard fluid for capacitor impregnation had been Aroclor 1242. This fluid has, on the average, three chlorine atoms per molecule. However, as can be seen from the table below, it contains both higher and lower homologs. In particular, it contains nearly seven percent of five chlorine and higher homologs.

TABLE II

TYPICAL HOMOLOG CONTENT OF AROCLOR 1242 AND 1016

	1242	1016
Biphenyl	0.02	0.02
Monochlorobiphenyl	0.72	0.93
Dichlorobiphenyl	15.6	19.4
Trichlorobiphenyl	54.5	64.5
Tetrachlorobiphenyl	22.5	15.0
Penta and higher	6.7	0.16

Aroclor 1016, the sole fluid now sold for capacitor impregnation, contains only a few tenths of a percent of these persistent homologs. The maximum limit has been set at four tenths percent.

The amount of persistent material in fluid sold for capacitor impregnation has therefore been cut by a factor of 15 to 30.

3. Monsanto has instituted stringent procedures in its own plants to eliminate losses during manufacture and handling of PCBs. It has discontinued their use for heat transfer and hydraulic use. Effluent from the plant now contains less than 85 ppb.

4. The company has worked to educate its customers so that they will be aware of the need to prevent escape of PCBs into the environment. Several means are being used for this purpose.

   One of these has been direct consultation with each user to explain the problem. Monsanto salesmen serve as consultants to our customers on methods for reducing losses. Customers with the necessary facilities to carry them out are given Monsanto analytical procedures. Samples are analyzed in the company analytical research laboratory for those not equipped to do their own analyses. Personnel of the laboratory serve as consultants for those doing their own.

   Monsanto and the National Association of Electrical Manufacturers joined in recommending the formation of American National Standards Institute Committee C-107 to standardize proper procedures for handling PCB fluids in the electrical industry. The membership of this committee includes representatives from national groups concerned with the manufacture, use and testing of capacitors and transformers, and groups representing government and the public.

5. PCB fluids used in the electrical industry frequently become contaminated to the extent that the users cannot readily reclaim them. Formerly, these would have been discarded. Now facilities adequate to reclaim seven million pounds of material per year have been set up by Monsanto.

6. Some of the fluid is so badly contaminated, that is, it is contaminated with impurities which cannot be removed by any presently available method, that it must be destroyed. Monsanto has therefore installed an incinerator capable of burning ten million pounds of fluid per year. Waste products from this operation are free from unburned PCBs. To produce this result requires special equipment designed to expose the product to much higher temperatures for longer times than ordinary incineration equipment. The company accepts waste PCB fluid for incineration charging a fee which roughly covers the cost.

   Experiments to determine the feasibility of incinerating solid waste containing PCBs have been run. Results indicate that it would be possible. To date no such facilities have been provided. This is because collection of large numbers of small used capacitors does not seem to be feasible and because of the very large capital expenditure which would be required.

7. For the present, it is recommended that solid waste containing PCBs be buried in sanitary land fills not adjacent to water. This recommendation is based on evidence from a long term test which shows that the rate of migration through soil is very low (27).

The effect of these actions on the amount of less biodegradable or "hard" PCBs reaching the environment can be estimated by considering the information in Table III.

TABLE III

THE EFFECT OF INDUSTRY ACTION ON PRODUCTION OF PCBs CONTAINING MORE THAN 4 CHLORINES PER MOLECULE

Aroclor	Total 1970 U.S. Sales [a]	Fraction > 4 Cl	1970 > 4 Cl [a]	1970 Tr. + Cap. with 1016	1970 >4 Cl Tr. + Cap. with 1016	1970 >4 Cl Cap with 1016
1221	1,476	0.0	0			
1232	260	0.0	0			
1242	48,588	0.07	3,400	6,257[b]	438	
1248	4,073	0.21	855			
1254	12,421	0.72	8,950	3,488[b] 500[c]	2,513 360	360
1260	4,890	1.0	4,890	4,072[b]	4,072	
1262	1,023	1.0	1,023			
1268	330	1.0	330			
1016		.003 ave. .004 max.		26,100[c]	78	78
Total >4 Cl			19,448		7,461	438
Total	73,061			40,417		

a Thousands of pounds
b Transformer fluid
c Capacitor fluid

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The data given are based on 1970 U.S. sales for the following reasons:

1) ~~That was the last year of unrestricted sale~~ not true Sales restricted ...

2) ~~No more environmentally acceptable chlorinated biphenyl products~~ had yet ~~been put~~ on the ~~open market~~ ← not true

3) World wide data are not available

4) It represents the peak sales year.

The first column lists the various Aroclor or PCB types produced. The second gives the 1970 U.S. sales of each product. The third gives the fraction of each product containing more than four chlorine atoms per molecule. The fourth column is the product of the second and third or the amoutn of material containing more than four chlorine atoms. The totals for the 1970 U.S. sales and the content of greater than 4 chlorine material are given.

The fifth column shows the amounts of materials which were sold for electrical use in 1970, substituting Aroclor 1016 for the Aroclor 1242 actually used for capacitors in 1970. The sixth column is again the amounts of homologs containing more than four chlorine atoms. Also, the total of these is given. Thus, substitution of other products for non electrical use and introduction of Aroclor 1016 would have resulted in decreasing the 1970 actual sale of "hard" PCB homologs from 19,448 to 7,461 thousand pounds. This is a substantial reduction. The final column gives the amount of "hard" homologs in the capacitor fluid only which would have been used in 1970. This figure is of interest because it represents that part of the use which is most difficult to collect and most likely to reach the environment.

Two things remain which could be improved:

Reword If 1254 is in use a [illegible] degraded [illegible]

1) A more readily degraded Aroclor should ~~be~~ substituted for 'Aroclor 1254 in DC capacitors. This ~~would~~ result in a ~~further reduction~~ of hard PCBs introduced into ~~the~~ environment.

2) All transformer fluids from failed, obsolete or discarded transformers should be recovered and recycled or destroyed. This would leave only 78 thousand pounds of hard PCBs introduced per year at the 1970 rate. This would cut introduction of hard PCBs into the environment to 0.4% of the actual 1970 rate.

The U. S. Food and Drug Administration is following the PCB situation very closely. Representatives from that department have stated[15] that while it is cause for concern, it is not cause for alarm. This means that the logical course to follow is to take the actions which industry has already taken and to see what results are produced. The reduction in the amount of hard PCB's reaching the environment indicated above will in all likelihood result in a lower equilibrium concentration of these materials in the environment. The precautions now being taken in the manufacture, handling and use of the materials will certainly reduce the concentration close to points of manufacture and use although there may be some delay due to materials held in mud and silt on the bottom of rivers and estuaries. If analytical results on ecological and food samples are more definitive, studies of the toxicology of the PCB's might in the future dictate even more stringent controls could be instituted. Should the results of continuing studies indicate that we have over-reacted, further use of the valuable properties of PCB's might be made to protect consumers from fire hazard.

The concept that an ecologically acceptable PCB product is feasible stemmed originally from the observation that, whereas the use pattern of PCB products covered a very broad spectrum of homologs, the analytical evidence from the literature [5, 6, 9, 10] indicated that most wildlife PCB residues were concentrated in the penta/hexachloro region. This certainly implied that many of the other dominant isomers in the parent materials were not accumulating.

As part of a broad Monsanto study to elucidate the reasons for this, we have investigated the effects of micro-organisms in PCBs under aerobic conditions. Initially our studies were undertaken using activated sludge, taken from one of our biological effluent treatment plants, and pre-adapted to degrade biphenyl (Culture C_1). Although some evidence of degradation of certain single isomers and mixtures was obtained, difficulties were experienced in achieving reproducible results. We were aware that Lunt and Evan at University College, Bangor, N. Wales, had isolated from soil a pure biphenyl-degrading culture, reported to be a nocardicum of the order actinomycetes (Culture C_2). The Bangor workers had studied the breakdown mechanism for biphenyl, using this culture and had demonstrated the metabolic pathway.[21] (Details of this have been included in Appendix 2)

Using this culture for degrading single isomers of chloro-biphenyls, we were able to establish a pattern of degradation broadly agreeing with the metabolic mechanism proposed by Lunt and Evans for biphenyl. Subsequently another biphenyl degrading organism in pure culture was isolated from a canal mud (Culture C_3). This was reported as a being of the pseudonomos family. The metabolic route for degradation by this organism was shown to be different from that of the earlier culture. Subsequent studies of the degradation of chlorobiphenyl isomers showed broad agreement with this proposed metabolic pathway and it was demonstrated that most of the lower chlorine isomers of PCBs ($<$penta) were susceptible to degradation by one or another of these micro-organism cultures.

When commercial mixtures of isomers were subjected to attack by these pure cultures, the analytical results indicated a different pattern of degradation than might have been predicted from the studies of single isomers. For example, the isomer 4,4'-dichlorobiphenyl was not degraded by culture C_2 although it was degraded by culture C_3 in agreement with the proposed pathways. In admixture with other isomers, as in Aroclor 1242, however, the 4-4'-dichlorobiphenyl was degraded by both cultures independently. This implied that in the presence of suitable levels of degradable isomers the organism could adapt to utilize some at least of the more intractable isomers as carbon source. Certain isomer mixtures of practical significance, such as Aroclor 1016, were prime targets of this study and the results indicated virtually complete degradation of this particular product with these two organisms. For Aroclor 1242, very substantial degradation of the significant isomers has been demonstrated and the evidence suggests that some of the more highly chlorinated penta and hexa chloro isomers

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in 1242 can also be degraded through adaptation. More evidence for this is being sought in current experiments in which the more highly chlorinated components are being accentuated to establish better data.

In the work on single isomers it was established that the rates of degradation could be accelerated by including biphenyl itself with the isomer under test. The reasons for this have not been established, but one suggestion is that a mechanism of co-oxidation may be responsible[24] Most of the work done so far has therefore included biphenyl in the study and this does raise the question whether the conditions are too artificial for extrapolation to the natural environment. For single isomers, this probably would be a valid criticism, although nature is well endowed with organic chemical structures not unlike that of biphenyl,[25] which could induce similar mechanisms for degradation.

For mixtures like Aroclor 1016, however, the adaptive mechanism which has been observed for both cultures C_2 and C_3 would probably ensure that adequate nutrient could be derived from the PCB product. Experimentation to confirm this point is currently in progress. It should be understood that degradation has clearly been demonstrated even in the absence of biphenyl: under the laboratory conditions, faster rates are observed with biphenyl present.

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APPENDIX 2

Degradation of Polychlorinated Biphenyls by Micro-organisms

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 the many micro-organisms which abound. As part of a broad study designed to investigate the probable causes for the apparent distribution of PCBs in the environment we have studied the effects of selected micro-organism cultures on both single isomers and commercial mixtures of PCBs.

The basic experimental procedure consists of shaking prepared substrates comprizing PCBs, mineral solutions, inocula, inhibitors, etc. for varying periods of time and determining the loss of PCB by GLC analysis of extracts. Only primary degradation of PCBs have been measured and we have not identified the products of the metabolic processes involved. Since preliminary experiments showed that the rates of degradation were increased by the addition of biphenyl, many of the experiments described have included biphenyl added initially and also subsequently, at intervals, during long exposures. Mercuric chloride was shown to be an effective inhibitor for those micro-organisms and was included at 100 ppm in a number of experiments for control purposes. Although various types of shake-flask equipment were used we finally standardized on an orbital incubator (Gallenkamp) operated at 130 cycles per min. controlled at a temperature of 25°C.

A standard procedure was adopted for the preparation of samples and final extraction for analysis. 5 ml. aliquots of di-ethyl ether solution containing 100 ppm PCBplus 1000 ppm biphenyl (when included) were introduced into 250 ml culture flasks (glass stoppered conical flasks), and the ether evaporated by a slow stream of dry nitrogen, without external heating. To each flask was then added 100 mls of culture solution. To maintain aerobic conditions the flasks were vented at weekly intervals and when appropriate biphenyl (0.005 grms) was added periodically.

We demonstrated that sampling of aliquots could lead to inaccurate results, probably through adsorption effects, and therefore used total contents of each flask for analysis. Residual PCBs were extracted with n-pentane plus small quantities of ethyl alcohol to break emulsions formed in the presence of inocula. 4 x 20 ml pentane extractions were used to ensure total recovery and made up to 100 ml ready for analysis, the details of which are described separately (26).

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Some early experiments were made using activated sludge inocula from the biological effluent treatment plant at Ruabon, N. Wales, pre-adapted to degrade biphenyl. The experimental procedures were developed during this work and some degradation of PCBs was observed, but most of the results discussed here arose from experiments using specific biphenyl degrading cultures isolated by Evans and Lunt (21). Two such cultures have been investigated for the degradation of PCBs. These are a nocardium of the order actinomycetes isolated from a soil sample from the Sudan (Culture C_2) and one isolated from a canal mud sample (U.K.) and reported to be of the pseudonomos family (Culture C_3). Evans and Lunt have described studies of the microbial attack by these cultures on biphenyl and have suggested the metabolic routes shown in Fig. 1.

Stocks of active organisms (C_2 and C_3), received from Professor Evans, have been prepared and maintained according to his recommendations. A prepared growth medium (Table 1) was inoculated with the culture and the mixture aerated continuously in the dark at a temperature of 25°C using scrubbed and filtered air. Inocula for subsequent experimental substrates were withdrawn after stopping the air flow for a brief time to allow excess biphenyl to settle. The medium was prepared in 15 litre quantites. As prepared the pH was about 5.8, but was brought to 7 by addition of NaOH solution before introducing the inoculum. When only relatively small volumes of stock culture (one or two litres) were removed for experiments, the bottle was replinished by addition of an equal volume of solution containing the inorganic constituents of the growth medium. For larger quantities the practice was adopted of starting a fresh culture.

TABLE 1 MEDIUM FOR CULTURE OF BIPHENYL-DEGRADING ORGANISMS

Constituent	Grm. per litre
$(NH_4)_2 SO_4$	1.0
KH_2PO_4	1.0
$MgSO_4.7 H_2O$	0.5
$Fe SO_4$	0.05
Biphenyl	1.0

RESULTS

A number of single isomers and simple mixtures have been studied in contact with Cultures C_2 and C_3, most with C_2. Packed column GLC (26) has been used to measure the loss of PCB. The results are summarized in Table 2.

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TABLE 2

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

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

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Several commercial PCB mixtures have also been studied in contact with cultures C_2 and C_3. Since only a few single isomers were avilable for this study it was hoped that investigations of the commercial mixtures, coupled with high resolution GLC, would extend the range of our knowledge very considerably. In order to obtain adequate resolution, capillary column [200 feet S/S 0.030" bore (in Phase Sep.) coated with Apiezoh L] GLC with Electron Capture detection was adopted. Identification of many of the isomers, separated by the capillary column, was possible using the methods developed by Sissons and Welti (9). Results for two commercial mixtures, Aroclor 1242 and Aroclor 1016, are summarized in Table 3.

TABLE 3

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The results of the work reported here indicate quite clearly that most, if not all, of the lower chlorinated isomers of PCBs can be degraded by enzyme hydroxylation induced by micro-organisms. The results with single isomers seems to support the work of Evans and Lunt (21) 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',2,6-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 ($\leq$5Cl) of PCBs 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. Such isomers would not constitute a significant fraction of commercial PCB.

When commercial mixtures of PCBs 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 mixture. Thus 4-4'dichloro-biphenyl degrades to 50% in approximately 2 days when exposed to culture C_2 as a component of Aroclor 1016 or Aroclor 1242.

The general conclusion from the study of these commercial mixtures is that all isomers present in significant amounts in Aroclor 1016 degrade with time constants (at 25°C) of less than 20 days, and that in Aroclor 1242 a small percentage, consisting of more highly chlorinated homologs, degrades more slowly, probably with some time constants exceeding 50 days.

While it is not possible from our studies to give a detailed explanation of all the results, the differences between the single isomers and mixtures do suggest that adaptation of these organisms is possible when exposed to a range of materials having a structurally related variation.