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TITLE: BIODEGRADABILITY OF POLYCHLORINATED BIPHENYLS (AROCLORS).

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PROBLEM: To investigate the biodegradability of polychlorinated biphenyls under aerobic conditions, and thus provide evidence of their potential breakdown in the natural environment.

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Introduction

There is mounting analytical evidence that poly-chlorinated biphenyls are accumulating in marine life. Most of this evidence, however, is based on the detection of the higher chlorinated isomers i.e. penta and upwards, there being no reported finding of significant concentrations of the di, tri and tetra chlor-compounds. The failure to detect these lower isomers, of which Aroclor 1242 is largely composed suggests that degradation may be occurring in nature either by microbial action in the marine environment or by metabolism within the animal.

Ruabon research department were asked [1] to carry out laboratory studies to determi the relative ease of degradation of PCB's with particular reference to these constituti Aroclor 1242.

This report gives details of experimental procedures and results to date.

Summary of results

1. Using activated sludge inocula, preadapted to biphenyl degradation, studies have been made of the degradation of single, and simple and complex mixtures of PCB's.

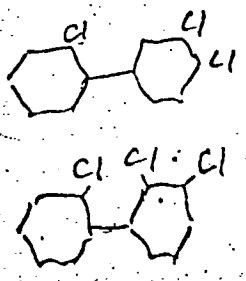
2. Evidence for the degradation of Aroclor 1242 when present as sole carbon source is inconclusive-the loss of one peak only, can definitely be attributed to a biologica mechanism.

3. Significant degradation of PCB's associated with two predominant peaks in the 1242 chromatogram was achieved when biphenyl was present in the substrates.

4. Degradation of 2,4' Dichlor-biphenyl was demonstrated in the presence of biphenyl but not when used as sole carbon source.

5. No degradation of 4,4' Dichlor-biphenyl was detected either in the presence or abs of biphenyl.

6. Conflicting evidence was obtained for the degradation of 2 3'4' Trichlor-biphenyl a binary mixture with the 2,3,2' isomer (biphenyl present). General interpretatio of results indicates that in one run an observed loss of 60% was due to biodegradat Failure to confirm the observations was attributed to some fundamental difference microbial activity.



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1. Experimental Procedures

1.1. General Considerations

A preliminary survey of available literature and abstracts failed to locate any publications giving details of procedures which could be readily adapted for use in PCB biodegradation studies. An attempt was made to apply the method recommended by Bunch and Chambers [2] but was abandoned when it became obvious that the results would be affected by the difficulty of ensuring homogeneous dispersion of PCB's in the aqueo media.

Although much published work exists dealing with the biodegradability of chlorina compounds such as DDT and other pesticides there seems to be little uniformity of experimental approach. In some cases it was considered that methods made inadequate provision for ensuring that apparent degradation was attributable to a biological rather than a physical or chemical mechanism. The published work was, however, of val in defining premises on which subsequent experimentation has been based. These briefl are:

a) Loss of PCB from the biosystem would be measured by specific analysis for the material being studied and not by some associated activity such as oxygen uptake. Thi approach does not preclude the possible formation of stable products of partial degradation which would not be detected by the analytical procedures. However, as the first objective of the work was to demonstrate primary degradation of PCB's it was considered that specific analysis was the most sensitive and conclusive method of measurement.

b) It would be necessary to sacrifice the total contents of experimental units i. culture and substrate for the determination of non-degraded residues. The low solubil of PCB's and the possibility of non-uniform absorption on to the biomass and glass sur would reduce the reliability of results based on the analysis of aliquots.

c) Controls would be included to ensure that PCB loss could be attributed unequivocally to biodegradation.

1.2. Source and Preparation of Inocula

Since the overall aim of the experimental work is to demonstrate whether or not significant biodegradation of PCB's can occur in the natural environment it would seem appropriate to take inocula from universal sources such as surface waters or sewage ef ents. It was considered however, of greater importance initially, to show that degradatio could take place under the most favourable conditions. Accordingly, throughout the work to date, the experimental systems have been inoculated with washed suspensions of activated sludge taken from the Ruabon biological treatment plant which successfully treats liquors containing a wide range of organic contaminants. Also, the practice was adopted of either preadapting the inocula to the degradation of the unchlorinated para biphenyl or of including this material in the substrates.

For the initial preparation of inocula, activated sludge was taken from the plant return stream and washed six times with equal volumes of a mineral base solution (formula given in appendix). The washed sludge was added to the total substrates to give final suspended solids concentration of 700 to 1,000 ppm. i.e. a ten-fold dilutic

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1.3. Introduction of PCB's into culture flasks

The basic experimental procedure described in detail later consisted of shaking prepared substrates comprising PCB's, mineral solution, inocula, inhibitors, etc. for varying periods of time and then determining the degree of PCB loss by the analysis of extracts.

During initial discussions it was decided to carry out the first runs using Aroclor 1242 at a concentration of 5 ppm in the culture flask. This level was chosen as a compromise which would permit the direct analysis of extracts without enrichment and also avoid the possible toxic effects of higher concentrations. For some of the later work on a simple binary mixture of trichlor-biphenyls the concentration was reduced to 2 ppm. after demonstrating that this quantity could be satisfactorily measure in simple solvent extracts.

Because of their low solubility it was not possible to introduce PCB's into the culture flasks in aqueous solution, nor could the small quantities required be weighed accurately. Solutions were, therefore, prepared in diethyl ether at concentrations of 40 or 100 ppm (finally 2 and 5 ppm. respectively). 5ml. aliquots of these solutions wer pipetted into the flasks and evaporated by passing a stream of nitrogen over the solven surface, care being taken to avoid less by splashing etc. During this operation, to minimise PCB loss by volatilisation, no external heat was applied

1.4. Control substrates

To show conclusively that PCB loss from the experimental flasks was attributable to biodegradation it was considered essential that control substrates be included in which biological activity was prevented, and from which no, or significantly less PCB loss was detected.

Early considerations showed that heat sterilisation of controls was not practicab and that a more suitable method would be to include an inhibitor in the substrate. For the initial work on Aroclor 1242 copper sulphate was added to give Cu^{++} concentrations of 10 ppm. but when subsequent analyses indicated that chemical action was occurring between component PCB's and the copper salt the latter was replaced with mercuric chlor

Mercuric chloride has proven to be a satisfactory inhibitor when added to controls from which prepared inocula have been excluded i.e. organisms initially present would b those naturally contributed by the non sterile mineral solution and those introduced adventitiously during sample manipulation. At the concentration used(25 ppm as Hg) however, the inhibitory effect has been insufficient to suppress biological activity when the prepared inoculum is added.. General interpretation of results indicates that PCB loss which occurs under these conditions is due to biodegradation and not to, say, irreversible absorption on to the killed inoculum. The most recent work has shown that if a minimum concentration of 50 ppm Hg is used, substantially complete suppression of biphenyl degradation can be achieved.

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1.5. PCB Recovery Procedure

Recovery of residual PCB from the aqueous media involved simple extraction with n-Heptane, a solvent advised by Physical Methods Section. To ensure complete recovery particularly of material which might be adhering to flask walls the following procedure was used:-

a) Add 20 ml n-Heptane to culture flask (250 ml. conical QF. B24) stopper and shake.

b) Transfer flask contents to 200 ml. separating funnel taking care to avoid loss (use n-heptane squeeze bottle to wash stopper and flask mouth)

c) Allow layers to separate - when inoculum was present emulsions were formed whic were difficult to break although some success was achieved by using concentrated sodium chloride solution.

d) Run aqueous layer into culture flask and pour solvent layer into 100ml. volumet taking normal precautions to avoid loss.

e) Transfer aqueous layer back to sep funnel. Add 20ml. n-heptane to culture flas swirl to wash walls and transfer to sep funnel. Shake and again allow to separate.

f) Run aqueous layer into flask and transfer solvent to volumetric flask.

g) Repeat operations (e) and (f) to give a total of 4 x 20 ml. extracts and make volumetric flask to mark with n-heptane.

h) Submit extract for analysis.

After the initial 1242 run it was shown that because of emulsion problems incomplet recovery could occur and the procedure was extended to provide a further 4 x 20 ml. ext (made up to 100 ml) Results were then quoted as the sum of the concentrations found in the two composites. This second extraction was found to be necessary only where inocult was included in the substrate.

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1.6. Gas Chromatographic analyses

The n-heptane extracts prepared in the experimental runs were analysed by G.C. using a Hewlett Packard 402 equipped with Ni^{63} high temperature electron capture detector.

The column used for the earlier work consisted of, 4' 3% Silicone oil OV101 on 80 - 100 mesh Chromosorb W H P. (ex. Supelco). This was later changed for 4' 4% OV 1 on 60 - 80 mesh Gas. Chrom Q.

Analytical conditions for both column systems were:

Flow. 40 ml./min. through column plus 60 ml./min. purge flow in detector Both of Argon/Methane 95/5

Oven Temp. 170° C

Flash Heater 250° C

Detector 250° C

Pulser 150° m. sec.

For the initial work calibration graphs were prepared using reference solutions of Aroclor in n-heptane at concentrations of 5, 2 and 1 ppm. plus internal standard solution of 3 ppm of 2-chlor biphenyl (previously shown to be absent from 1242 traces Subsequently, concentrations were determined by simple comparison of peak areas of reference sample at one level with those of sample extracts, without internal standa

1.7. Expression of results

Substrates for the biodegradation studies were prepared by dispersing evaporated residues in a total volume of 100 ml. By extracting into 100 ml. of solvent analytical results were directly applicable on a concentration basis. Where, to ensu complete recovery, it was necessary to extract the aqueous layer into a further 100 n-heptane, results were quoted as the sum of the individual values.

Thus, for work with single isomers results are given as residual concentrations the culture flasks in parts per million (10^6). These values give only the weight of PCB in relation to the total substrate volume and do not imply that dispersion was homogenous throughout the aqueous media.

For mixtures of PCB's such as Aroclor 1242, results are expressed as per-centag recoveries based on the analysis of predominant peaks in the chromatogram. Since mos of the peaks in the 1242 chromatogram are representative of more than one PCB isomer removal figures (per cent) are not necessarily a direct measure of weight loss as th concentration/detector response relationships will vary with different isomers and chlorination levels.

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1.8. General procedure for setting up experimental runs

The following basic procedure was used throughout the experimental work, variations being made where necessary to suit the requirements of specific runs. In subsequent sections of this report results are shown in tabulated form together with abbreviated descriptions of the relevant procedures.

1.8.1. Adaptation of activated sludge inocula to biphenyl degradation

a) Thoroughly clean the required number of 250 ml. B24 Quickfit Erlenmeyer flasks.

b) Pipette into each flask 5ml. of a 400 ppm solution of biphenyl in diethy ether.

c) Evaporate ether in a fumecupboard by passing a stream of nitrogen throug the flasks. Do not apply external heat - experience showed that significant volatilisation of biphenyl could occur with even gentle warming.

d) Add 90ml. of mineral base solution and 10 ml. of washed inoculum (see section 1.2.) to evaporated residues. Stopper and place on shaker rack.

e) To serve as controls include flasks containing no inoculum but adding recommended inhibitor. Also stopper and shake. Flasks were shaken on oscillating racks at a rate (100 to 120 opm) which gave adequate agitati without excessive splashing.

f) To ensure persistence of aerobic conditions vent flasks at 3 - 4 day intervals. The flasks were stoppered to prevent loss by volatilisation which, for biphenyl, was shown to occur very readily. A calculation, based on the known endogenous oxidation rates of the activated sludge, confirmed that aerobic conditions would be maintained if flasks were vented at the stated frequency. The precaution of stoppering flasks was adopted for all subsequent experimental work.

g) After approx. one week remove control flask and one culture flask and extract by the procedure given in section 1.4. Analyse extracts by G.L.C If analyses show no detectable residue of biphenyl in culture flask, use contents of remaining flasks to inoculate PCB substrates.

The length of the preadaptation period was usually that after which no ode of biphenyl could be detected when the flasks were vented.

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1.8.2. Biodegradation of PCB's using biphenyl adapted inocula

a) As before, prepare required number of clean dry 250 ml. flasks.

b) Pipette into each flask 5 ml. of PCB solution in diethyl ether of a streng which will give the chosen concentration in the final substrates.

c) Evaporate ether.

d) To a predecided number of the flasks add the contents of the flasks from t biphenyl adaptation run. Stopper and place on shaker.

e) To other flasks (controls) add mineral base and inhibitor. Also stopper and shake. Vent flasks at intervals as before.

f) After suitable time intervals extract flask contents into n-heptane and submit extracts to Physical Methods Section for analysis.

It is stressed that the above gives only a basic outline of the experimental approach. Variations were made in the number and nature of controls, and also for the more recent work both biphenyl and PCB have been included in the same substrates

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2. Results and Conclusions

2.1. Run 1. Degradation of Aroclor 1242 by biphenyl-adapted inocula

Results of this run are given in Fig.1. together with abbreviated experimental details. This method of presentation of results is used to reduce the need for excessive cross referencing between report text and tabulated data.

Copper sulphate was added to the control substrates to give Cu^{++} concentration of 10 ppm.

The significance of the results is obscured by the considerable loss of PCB's which occurred in the copper-doped controls. As no prepared inoculum was added to these flasks it is unlikely that the low recoveries reflect the failure to inhibit biodegradation. It is more probable that the loss is due to the chemical or catalytic effect of the copper ions.

Results up to 25 days are based on the analysis of single (4 x 25) 100ml. extracts and low values may thus represent inefficient recovery. The later results give recoveries as the sum of concentrations found in first and second extracts. Amounts found in the second extract are usually between 5 and 10% of those recovered in the first.

As shown in Fig.1. provision was made to assess the potential loss of PCB's which could occur during sample preparation procedures (A, B and C). Within acceptable limits no significant loss was detected.

Conclusions which can be drawn from these results are:

a) PCB'(s) represented by Peak1 in the 1242 chromatogram (see Fig.10.) is rapidly degraded almost certainly by a biological process.

b) After 131 days contact with the biphenyl adapted organisms between 50 and 60% of PCB's represented by peaks 2, 3 and 4 are <u>not</u> degraded. Until chromatogram with greater resolutions are available it is not possible to determine whether the reduction in peak areas is attributable to complete removal of selected PCB's or to a variable reduction in the concentrations of all components.

c) Because of the unsatisfactory nature of the controls the 40-50% loss of mater represented by peaks 2,3 and 4 cannot be unequivocally attributed to biodegradation. More recent work (Run 5) indicates that if mercuric chloride had been used as inhibitor in Run 1 higher PCB recoveries would have been obtained from the control and hence removals measured in the inoculated subst would have been considered to provide more convincing evidence of biodegradat

d) If measured losses are due to biodegradation there is no evidence of any long organism adaptation occurring, results after 131 days not being significant different from those after 25 days - figures after 8 days are anomalous indicating a greater removal than found in any subsequent samples. It is possible that the failure to degrade more than 40 to 50% could be attributed to a delayed toxic effect exerted by Aroclor 1242 at the chosen concentration In future runs provision should be made to ensure that working concentrations of PCB's are not toxic i.e. by the inclusion of a readily degradable material in the substrates.

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2.2. Run 2 Assessment of suitability of mercuric chloride as inhibitor for biodegradation studies

In view of the apparent reaction which occurred in run 1, between copper ions and PCB's other possible inhibitors were considered and mercuric chloride selected as a suitable alternative. Before carrying out further biodegradation work the procedure outlined in Fig.2. was used to provide information on any potential PCB loss which might occur in the presence of the mercuric salt. To check on possible losses which could result from sample manipulation, volatility etc. the evaporated Aroclor residues were treated as described briefly for samples A, B and C in Fig.2. The tabulated results of G.L.C. analyses show that significant loss occurred simply by evaporating the ether solution and redissolving the residue in n-heptane. This result is in conflict with the findings of Run.1. where within analytical limits complete recovery was achieved. As the same technique and precautions were observed in both cases the difference cannot be explained. From the results for sample C where the dry residue was shaken in a stoppered flask for 14 days it appears that significant further PCB loss can occur under these conditio

Comparison of results for samples D, E and F, representing Aroclor recovery after shaking in the presence of 10, 25, and 50 ppm Hg (as $HgCl_2$) respectively with th for sample C indicate that little or no loss occurs by chemical reaction.

Whilst the results of this run were perturbing because of losses which can occur during sample preparation procedures it was concluded that mercuric chloride would be a satisfactory inhibitor for use in biodegradation work.

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2.3. Run 3. Biodegradation of selected dichlor-biphenyls and of a binary mixture of trichlor biphenyls

The results of Run 1 were inconclusive due to the unsatisfactory nature of the controls and also to the difficulty of interpreting chromatograms in terms of individu PCB removal. It was felt that more basic information on the fate of PCB's under the experimental conditions would be obtained from the study of single isomers and simple mixtures. Samples of pure isomers were obtained from M.C and M.C.L. sources of which two were selected for biodegradation studies, namely 2,4' Dichlor biphenyl and 4,4' dichlor biphenyl. A binary mixture of trichlor biphenyls (2,3,2' and 2,3'4') coded high DK sample No. A.2090 was obtained from the Ruabon Research sample museum.

2.3.1. Run 3.1. Preliminary assessment of efficiency of sample preparation and recovery procedures Fig.3.

In view of the apparent losses which occurred during sample preparation in Run 2 it was considered advisable to carry out a preliminary work to provide similar information before embarking on any longer term biodegradation studies.

The run outlined in Fig.3. was designed to provide this information and also to define suitable working concentrations to be used in the culture systems. As shown in the tabulated results in Fig.3. the degree of recovery of each PCB sample was measured at concentrations of 5,2 and 1 ppm. For the mixture of chlor-biphenyls results are expressed as %age recoveries, as in the case of Aroclor 1242. In this sample the trichlor-isomers are present in approx. equal concentrations.

From this work it was concluded that in subsequent biodegradation runs suitable initial working concentrations would be 5 ppm for the single dichlor-isomers and 2ppm for sample A2090. This latter concentration was chosen because of the possible increased toxicity which the tri-chlor isomers might exert at higher levels.

2.3.2. Run 3.2. Pre-adaptation of inocula to biphenyl degradation

The experimental procedure used in this run is given in detail in section 1.7. and also outlined in Fig.4. The analytical results show little loss of biphenyl during the sample preparation steps. Also satisfactory recovery was achieved from the contro flask(s) after shaking for one week in the absence of inoculum but with 25 ppm Hg adde As stated earlier more recent work has shown that in the presence of inoculum 25 ppm H is insufficient to suppress biphenyl degradation and under these conditions mercury concentrations of 50 ppm and higher are necessary. The result for sample E (Fig.4.) confirms the degradation of biphenyl by the prepared activated sludge inocula.

2.3.3. Run 3.3. Biodegradation of selected PCB's

The adapted inocula prepared in Run 3.2. was used to seed substrates containing the chosen concentrations of single dichlor and mixed trichlor-biphenyl isomers. Abbreviated experimental details and results are given in Fig.5. As can be seen the precaution was taken for this run of providing controls equal in number to the cultur flasks, one of each being extracted after increasing time intervals. For each PCB sample a single flask was included (X in Fig.5.) to which both inoculum and inhibitor were added. If significant PCB loss had been measured in the non-inhibited systems t contents of flask X would have been extracted to confirm that irreversible adsorption on to the inoculum was not occurring and hence being mistakenly attributed to biodegradation. Analyses after shaking for up to 53 days failed to reveal evidence o significant degradation.

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2.3.4. Run 3.4. To determine if failure to degrade PCB's was due to toxic effects at working concentrations

The results obtained in Run 3.3 could reflect the non degradability of the chosen PCB's but may equally be evidence that organism growth was prevented by toxic effects at the concentrations used.

A further run was set up in which both biphenyl and the relevant PCB (s) were included in the same flask. Preadapted inocula and mineral base were added and the flasks shaken for 11 days before extraction. Failure to degrade biphenyl under these conditions would indicate that the associated PCB(s) were toxic.PC3 concentrat used were identical with those in Run 3.3.As shown by the results given in Fig.6 not only was complete removal of biphenyl achieved in all flasks but surprisingly little of the 2,4' dichlor biphenyl was recovered and there was also a significant loss of the 2,3'4' trichlor isomer represented by Peak 2 in the G.L.C. chromatogram.

These unexpected results indicated that degradation of certain PCB's can be'triggered'by the presence of biphenyl. No evidence has been found in available literature that similar mechanisms can result in the degradation of other 'intractab materials. The literature survey has however been limited to publications dealing wi aspects of water pollution and it is possible that similar observations may have bee reported in the more specialised journals of microbiology. This type of 'triggering action was suggested during verbal discussion with Professor Hughes of Cardiff University a few years ago although no specific evidence was quoted at the time.

In view of the above results two further runs were planned.

1. To confirm that PCB removal in Run3.4 was definitely associated with the presence of biphenyl.
2. If results of 1 were positive; to determine whether the observed influence of biphenyl on PCB degradability can be exerted by other organic materials

2.3.5.Run 3.5. Confirmation of effect of presence of biphenyl on PCB degradati

In this run comparisons were made of the degradability of the di- and tric PCB isomers in the presence and absence of biphenyl. The concentrations of PCB's an biphenyl used were the same as those for Run 3.4;experimental procedures and results are given in Fig. 7.

Analysis of extracts from the flasks containing 2,4' Dichlor biphenyl show that in the presence of biphenyl (sample B) the concentration of the PCB isomer was reduced by 94%. Removal from the substrate from which biphenyl was excluded (D) was only 14% thus confirming the result obtained in Run 3.4.The 44% loss which still occurred when mercuric chloride was included (C) is further evidence that the toxic effect of mercury at the concentration used (25 ppm) was insufficient to completely suppress biological activity, when the prepared inoculum is added. No loss of PCB occurred when inoculum was excluded and mercury added (E) showing that loss in C wa due to reaction between the PCB and mercury salt.

No significant removal of 4,4' Dichlor biphenyl occurred under any of the imposed conditions.

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The results for sample A2090 (Mixed trichlor isomers) showing no significant loss of either component are in conflict with the findings of Run 3.4 where only 40% of 2,3',4', trichlor biphenyl was recovered (biphenyl added). The difference was confirmed by a repeat analysis of the extracts. As there is no evidence to suggest that the two isomers can be disproportionately extracted from the aqueous media and 85% of the 2,3',2 trichlor biphenyl was recovered in Run 3.4 it is concluded that loss of the 2,3',4' isom in that run was attributable to biodegradation. The failure to confirm the observation Run 3.5. must therefore be associated with some fundamental difference in organism activity. Experimental procedures for similar substrates were the same in each run exce that in Run 3.5 the preadaptation to biphenyl stage was omitted for two reasons:

1. To expedite the work generally.

2. As biphenyl was to be included in the substrates of major inter adaption would have proceeded in situ. Results in Fig 7 show that biphenyl was satisfactorily removed (Sample B).

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2.4. Run 4 Assessment of effect of organic materials other than biphenyl on PCB degradation

If the observed effect of biphenyl could be demonstrated for other organic materials it would provide evidence that degradation of otherwise intractable PCB's might occur in the natural environment. To examine this possibility in the laborat a further run was undertaken, using the established experimental procedures, in whi the effect of biphenyl was compared with that of a synthetic sewage. As the strongest evidence for the effect of biphenyl was obtained in the studies of the degradation of the 2,4' dichlor isomer this material was chosen as reference PCB.

The synthetic sewage was prepared by adding Peptone and Meat extract to the mineral base, actual amounts are given in the appendix with the mineral base formul This source of organic material was chosen as being typical of that with which PCB' might come into contact when released to the natural environment.

Experimental details are given in the usual abbreviated form in Fig.8. The analytical results also given in Fig.8. show that after seven days shaking approx. 80% of the 2,4' dichlor isomer was removed in the presence of biphenyl (C) but only about 10% when the synthetic sewage was added (analysis for B compared with A). The latter figure is probably not analytically significant.

As shown in Fig 8 each experimental flask was set up in duplicate. After extraction and analysis of the first group after seven days shaking further additio of peptone and meat extract and biphenyl were made to the relevant flasks in the second group which were then shaken for a further seven days. Results for this second group are included in Fig.8. and again show no significant PCB removal in the presence of synthetic sewage.

In this run the Hg (as $HgCl_2$) concentration in the controls was increased to 100ppm at which level degradation of both diphenyl and the 2,4' dichlor isomer was satisfactorily suppressed.

2.5. Run 5 Effect of presence of biphenyl on biodegradation of Aroclor 1242

If the mechanism by which biphenyl promotes the biodegradation of selected PCB' cannot be demonstrated for more universally organic materials it is unlikely that a case can be argued for similar degradation occurring in nature. The effect of biphenyl could, however form the basis of a sorting test for PCB isomers if it is assumed that degradation (of PCB's) under these conditions generally reflected a greater ease of microbial attack and that compounds responding to the test would be the most likely contenders for formulation of a degradable Aroclor.

The only confirmed evidence for the influence of biphenyl on PCB degradation related at this stage in the work to 2,4' dichlor biphenyl. Before embarking on any further studies of single isomers it was considered that a repeat investigation of the degradability of Aroclor 1242 would indicate whether PCB's other than 2,4' dichlor isomer could be similarly attacked. Accordingly, the run outlined in Fig.9. was carried out which was designed to compare the degradability of 1242 constituents in the presence and absence of biphenyl.

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The following conclusionsto date can be drawn from the tabulated results:

1. Peak 1 is completely lost in seven days from the substrate containing both biphenyl and A1242 in the presence of inoculum (Solution B in Fig.9.)

2. Under the same conditions but with biphenyl excluded (D) the loss was approx. 60%. Reference to the first A1242 run (Fig.1.) shows that Peak 1 was completel degraded in 8 days (biphenyl absent). The reason for the reduced rate of removal in Run 5 under the same conditions and the persistance of approx. 50% after 37 days cannot really be explained as preadapted inocula were used in bot cases. The results in Run 4 indicate that the rate and degree of removal of PCB(s) represented by peak 1 are influenced by the presence of biphenyl.

3. The results for Peaks 2 and 3 do not show any significant loss which could be attributed to biodegradation either in the presence or absence of biphenyl.

4. Comparison of analyses for Peak 4 of extracts from inoculated substrates with a without the inclusion of biphenyl (solutions B and D respectively) show a significant greater loss with biphenyl present.

5. This run was started before Run 4 and before it had been demonstrated that high concentrations of $HgCl_2$ were necessary to suppress biodegradation. The losses i the controls in Fig.9. (C) therefore reflect the persistence of biodegradation in the presence of 25 ppm Hg.

General Conclusions and Discussion of Results

The results to date have shown conclusively that biodegradation of selected PCI can occur comparatively rapidly in the presence of unchlorinated biphenyl. The evic for degradation when PCB's are present as sole carbon sources is less satisfactory.

The mechanism by which PCB breakdown is promoted by biphenyl is not known but it is suggested that enzymes essential for at least the initial attack on the chlorinated compounds can only be synthesised during the degradation of biphenyl itself. The failure to achieve degradation in the presence of more naturally occurring carbon sources indicates that the mechanism is specific to biphenyl.and unlikely to occur in nature unless of course significant levels of biphenyl are released in association with the PCB(s). However, the specificity of biphenyl shoul be confirmed by investigating the effect of other synthetic organics.

As stated earlier it is considered that the effect of biphenyl could form the basis of a sorting test to select PCB's which are most likely to degrade in nature and also to obtain information on the influence of the sites of chlorine substituti in the PCB molecule on biodegradability. In this context it is important that high resolution chromatograms be obtained for Aroclor 1242 and that isomers associated with particular peaks be identified.

Further Work

1. Study of degradability of selected di- tri- and tetra chlor isomers in the presence and absence of biphenyl.

2. Improve resolution of Aroclor 1242 and PCB chromatograms in general and identification of degraded isomers.

3. Investigate effect of other organic materials as trigger metabolites.

4. Investigate degradability of PCB's using pure culture of biphenyl-degrading bacteria.

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References: 1. Memo from W.R. Richard May 13th 1969, AROCLOR-ANALYSIS IN PESTICIDE RESIDUES.

2. Journ. Water Poll. Control Fed. 39,2,181 (Feb. 1967)

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Appendix

1. Mineral Base Formula

Prepared in accordance with details given by Bunch and Chambers in Journ. Water Poll. Contr. Fed. 39,2,181.

Stock Solutions

a. Dissolve 27.5 gm. $CaCl_2$ in distilled water and dilute to one litre.

b. Dissolve 22.5 gm. $MgSO_4.7H_2O$ in distilled water and dilute to one litre.

c. Dissolve 0.25 gm. $FeCl_3$, $6H_2O$ in distilled water and dilute to one litre.

d. Phosphate buffer : Dissolve 8.5 gm KH_2PO_4, 21.75 gm. K_2HPO_4. 33.4 gm. $Na_2HPO_4.7H_2O$ and 1.7 gm. NH_4Cl in about 500 ml. distilled water and dilute to one litre.

Working solution

To each litre of distilled water add 1ml. of each stock solution in order given mixing well after each addition.

2. Synthetic Sewage

To each litre of above working solution add 0.3 gm. Proteose Peptone and 0.2 gm Lab Lemco (Beef Extract)

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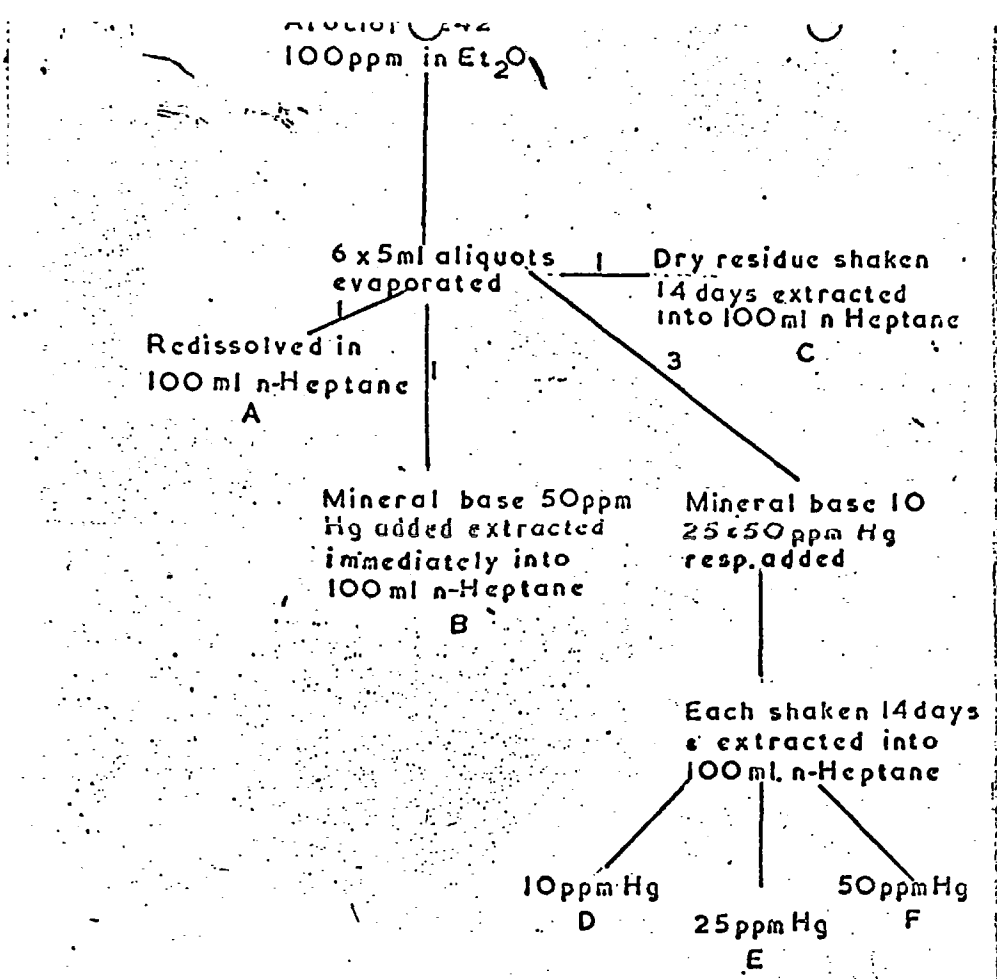

Solution	Aroclor 1242 % Recovered			
	Peak 1	Peak 2	Peak 3	Peak 4
A	85	87	82	
B	82	95	89	
C	71	77	73	
D	68	76	68	
E	71	82	73	
F	63	80	73	

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FIG 2 ASSESSMENT OF SUITABILITY OF MERCURIC CHLORIDE AS INHIBITOR FOR BIODEGRADATION STUDIES.

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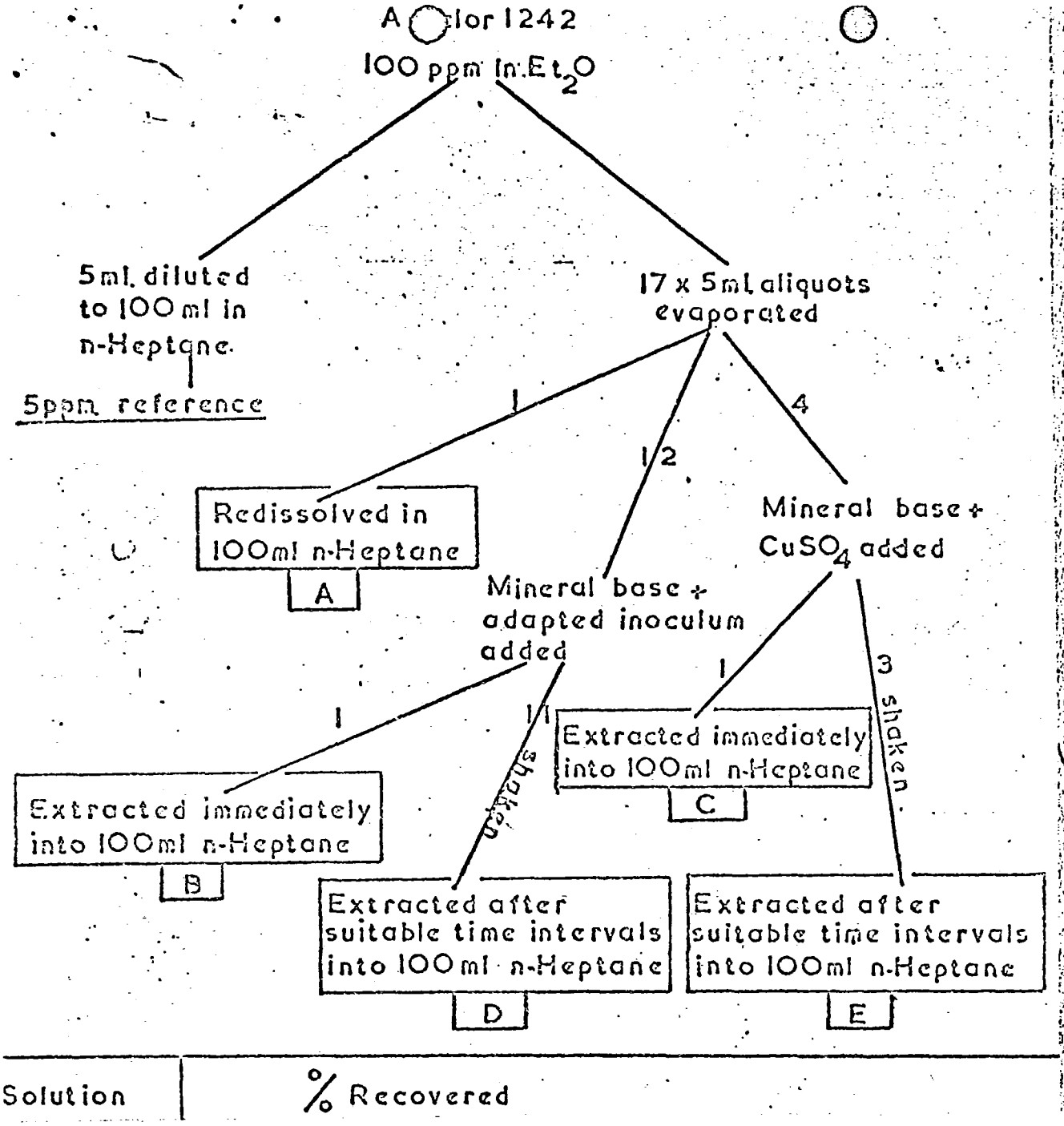

Solution	% Recovered

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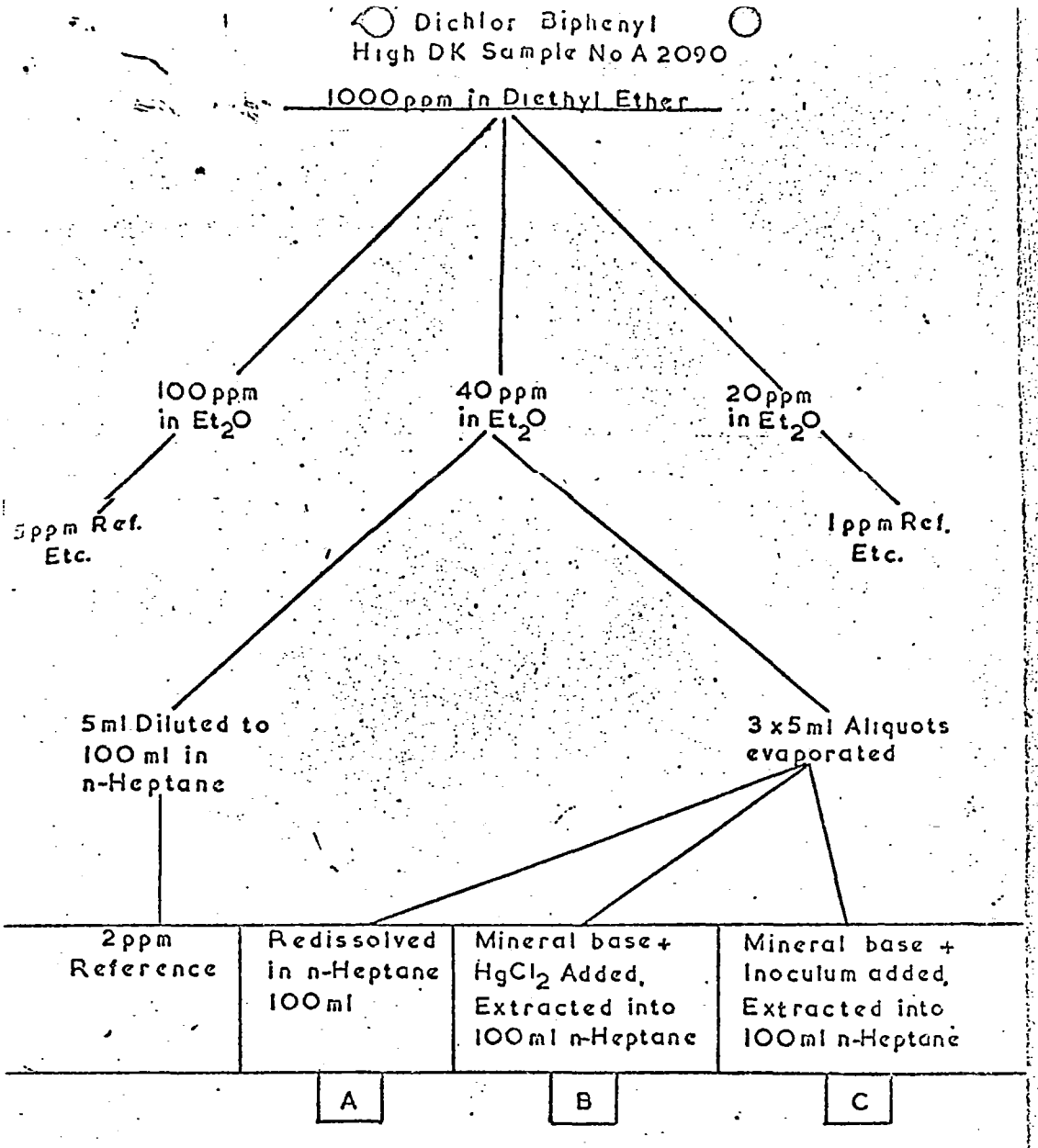

	2,4' Dichlor-Biphenyl ppm			4,4' Dichlor-Biphenyl ppm		
Ref.	5·00	2·00	1·00	5·00	2·00	1·00
A	5·10	1·78	0·94	5·04	1·84	0·75
B	5·10	1·89	0·86	5·04	1·62	0·85
C	4·66	1·78	0·54	5·10	1·74	0·79

High DK. Sample No. A2090

Ref. ppm. Total	5·00		2·00		1·00	
	%Recovered		%Recovered		%Recovered	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
A	92·4	99·2	96·5	101·0	94·0	109·0
B	93·8	94·0	100·0	103·0	91·0	94·0
C	89·6	90·8	90·5	92·5	76·0	96·0

FIG. 3 PRELIMINARY ASSESSMENT OF EFFICIENCY OF SAMPLE PREPARATION AND RECOVERY PROCEDURES

DSW 346715

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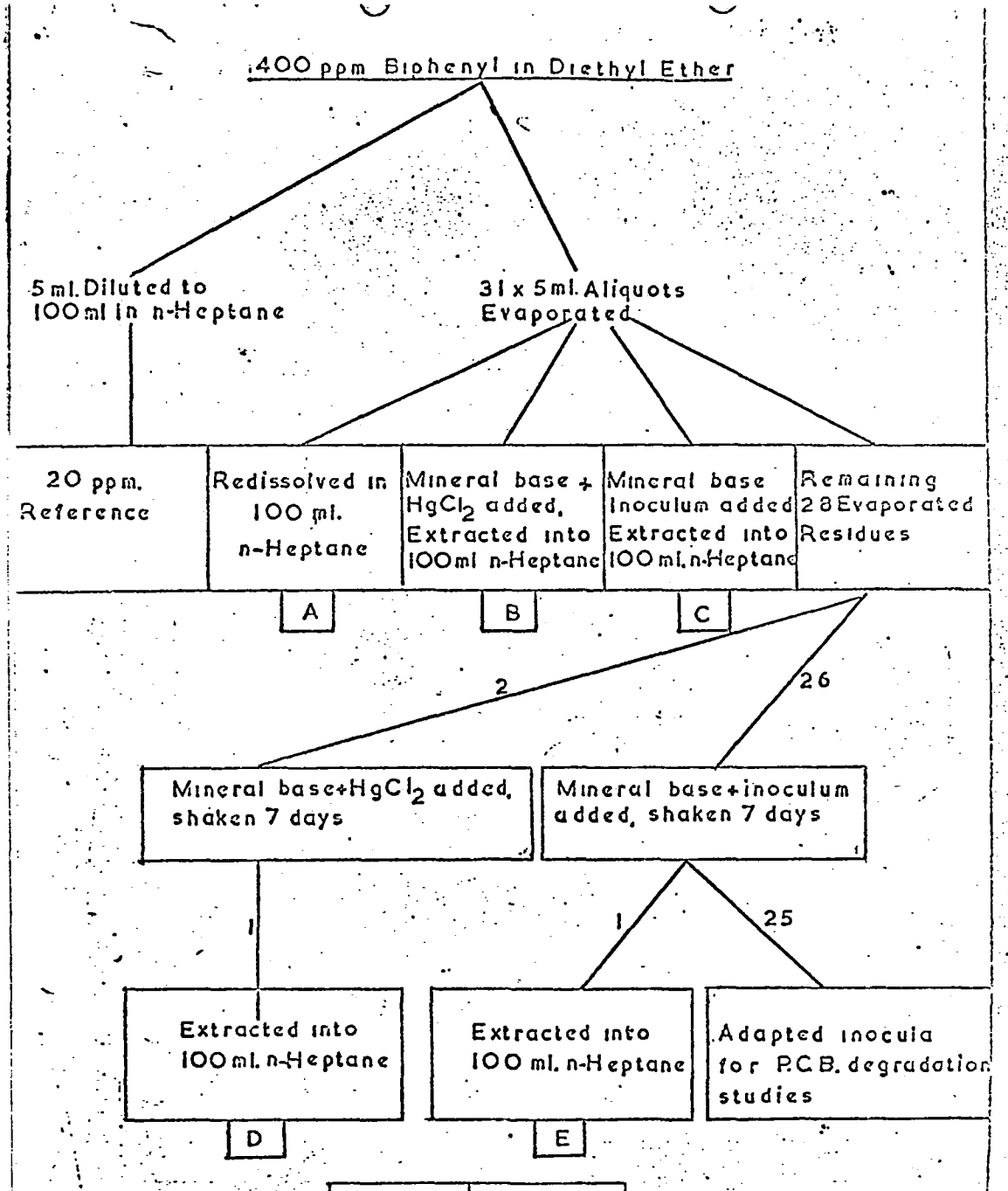

Sample	Biphenyl ppm
Ref.	20
A	18
B	18
C	18
D	18
E	Nil

Fig.4 PROCEDURE FOR PRE-ADAPTATION OF INOCULA TO BIPHENYL DEGRADATION

DSW 346716

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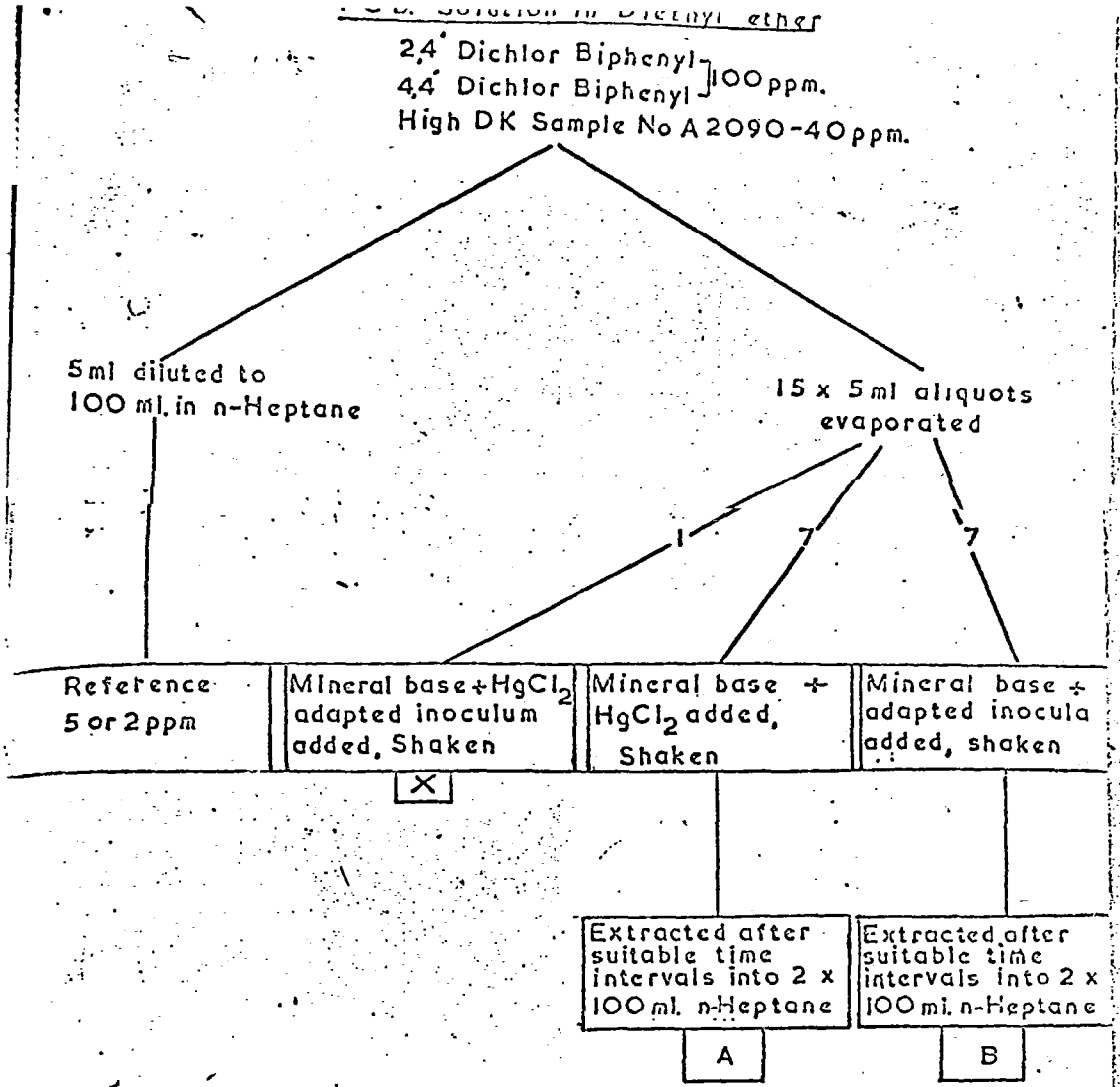

Time shaken days		2,4' Dichlor-Biphenyl, ppm.		4,4' Dichlor-Biphenyl, ppm	
	Ref	A	B	A	B
5	500	4·5	4·6	4·9	4·7
12	500	4·5	4·6	4·7	4·7
19	500	5·1	4·4	5·0	4·8
53	500	4·4	4·5	4·8	4·7

High DK Sample No. A 2090*

Time shaken days	% Recovered			
	Peak 1		Peak 2	
	A	B	A	B
5	95	105	100	100
11	100	90	100	85
18	95	90	95	90
52	90	90	90	85

*Reference 2ppm.

FIG 5. ASSESSMENT OF BIODEGRADABILITY OF SELECTED SINGLE DICHLOR-BIPHENYLS AND BINARY MIXTURE OF TRICHLOR-BIPHENYLS.

DSW 346717

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5ml 2,4' DiCl-BP in Et_2O 100ppm and 5ml Biphenyl in Et_2O 400 ppm evaporated in same flask

5ml High DK A2090 in Et_2O 40ppm and 5ml Biphenyl in Et_2O 400ppm evaporated in same flask

total substrate to

5ml 4,4' DiCl-BP in Et_2O 100ppm and 5ml Biphenyl in Et_2O 400ppm evaporated in same flask

Shaken 11 days, extracted into 2x100ml n-Heptane

A

Shaken 11 days, extracted into 2x100 ml n Heptane

B

Shaken 11 days, extracted into 2 x 100 ml n-Heptane

C

Ref. ppm	A: 2,4' DiCl-Biphenyl total recovered ppm.	B: 4 4 DiCl-Biphenyl total recovered ppm.	C: High DK sample No A2090 total recovered %	
			Peak 1	Peak 2
5·0	<0·1	4·8	85 [2 2'3']	40 [2 3 4']

Reference 2ppm.

N.B. NO DETECTABLE QUANTITIES OF BIPHENYL WERE FOUND IN EXTRACTS A B & C

FIG.6. PROCEDURE USED TO DETERMINE IF PCB CONCENTRATIONS USED IN BIODEGRADATION STUDIES ARE TOXIC

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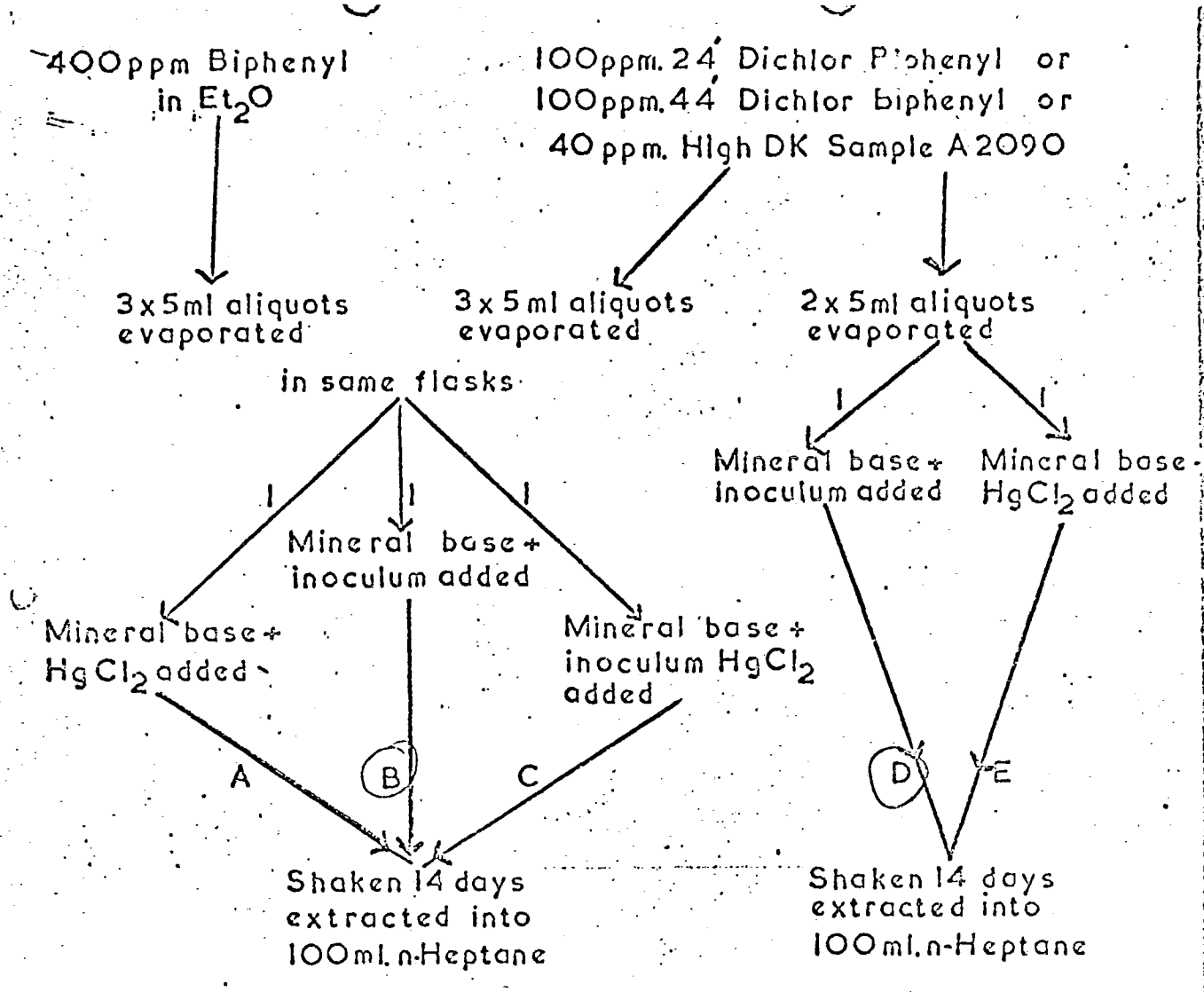

Reference solutions prepared by mixing 5ml of ether solutions of Biphenyl and relevant PCB and diluting to 100ml in n-Heptane, giving concentrations of 5 ppm for 2,4' & 4,4' Dichlor-Biphenyl, 2 ppm for sample A 2090, and 20 ppm for Biphenyl

Sample	2,4' Dichlor-Biphenyl		4,4' Dichlor—Biphenyl		High DK Sample A 2090		
	ppm recovered		ppm recovered		% recovered		ppm recovered
	$2,4'Cl_2BP$	BP	$4,4'Cl_2BP$	BP	Peak 1	Peak 2	BP
A	5·1	15	4·9	16	95	95	16
B	0·3	ND	4·7	N.D.	90	80	ND
C	2·6	ND	5·0	ND	90	80	ND
D	4·3	—	4·7	—	80	80	—
E	5·0	—	4·8	—	90	95	—

DSW 346719

FIG 7 EFFECT OF PRESENCE OF BIPHENYL ON BIODEGRADATION OF SELECTED SINGLE DICHLOR-BIPHENYLS AND A BINARY MIXTURE OF TRICHLOR-BIPHENYLS

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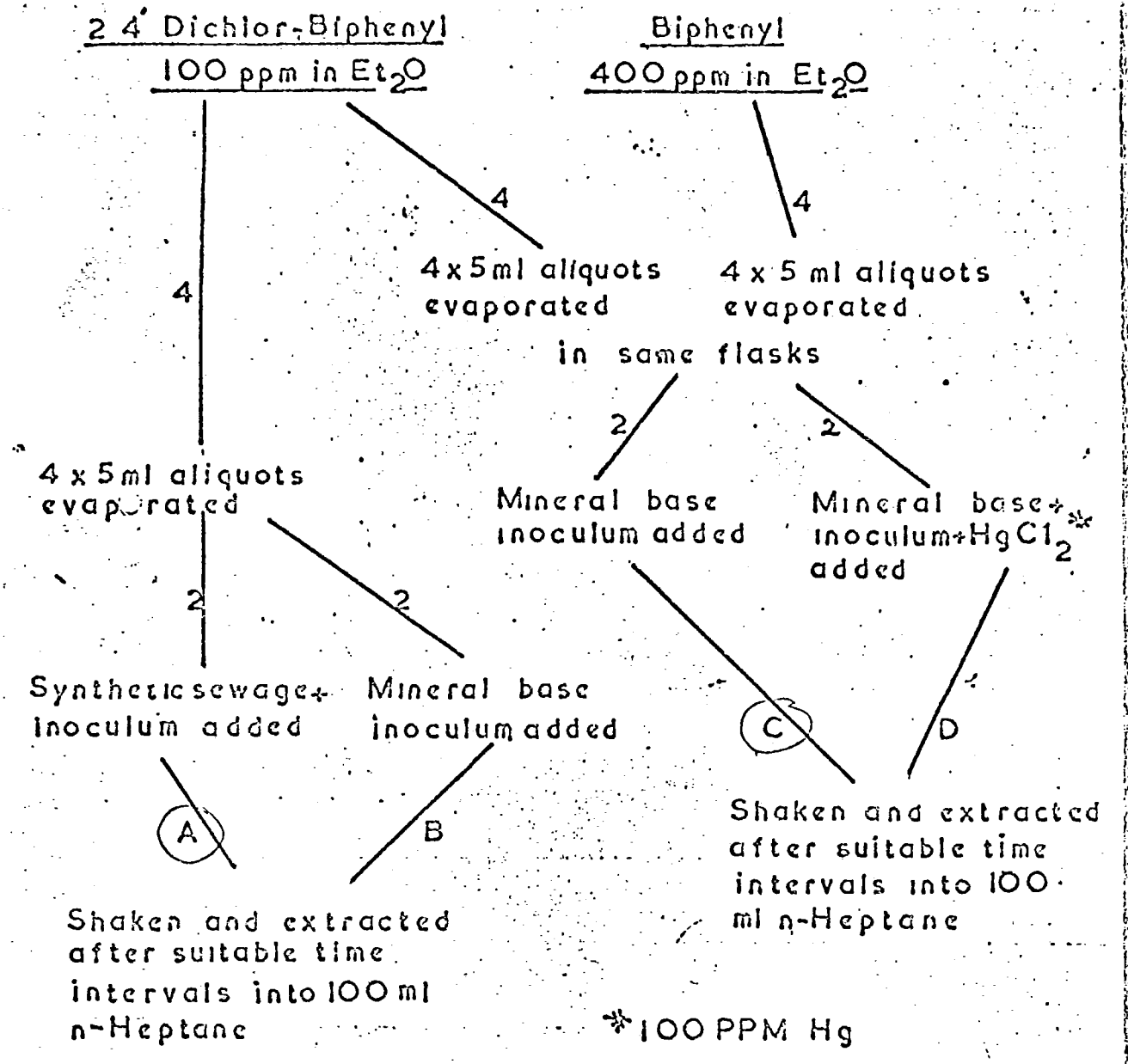

olution	Time shaken days	2,4'Dichlor-Biphenyl ppm Recovered	Biphenyl	
A	7	4·5	—	50 ppm Biphenyl to C after 14 days also synthetic sewage to A
	19	4·7	—	
B	7	4·9	—	
	19	4·9	—	
C	7	1·1	ND	
	19	0·3	ND	
D	7	4·7	16	
	19	4·7	16	

FIG 8 COMPARISON OF EFFECT OF SYNTHETIC SEWAGE AND BIPHENYL ON DEGRADATION

DSW 346720

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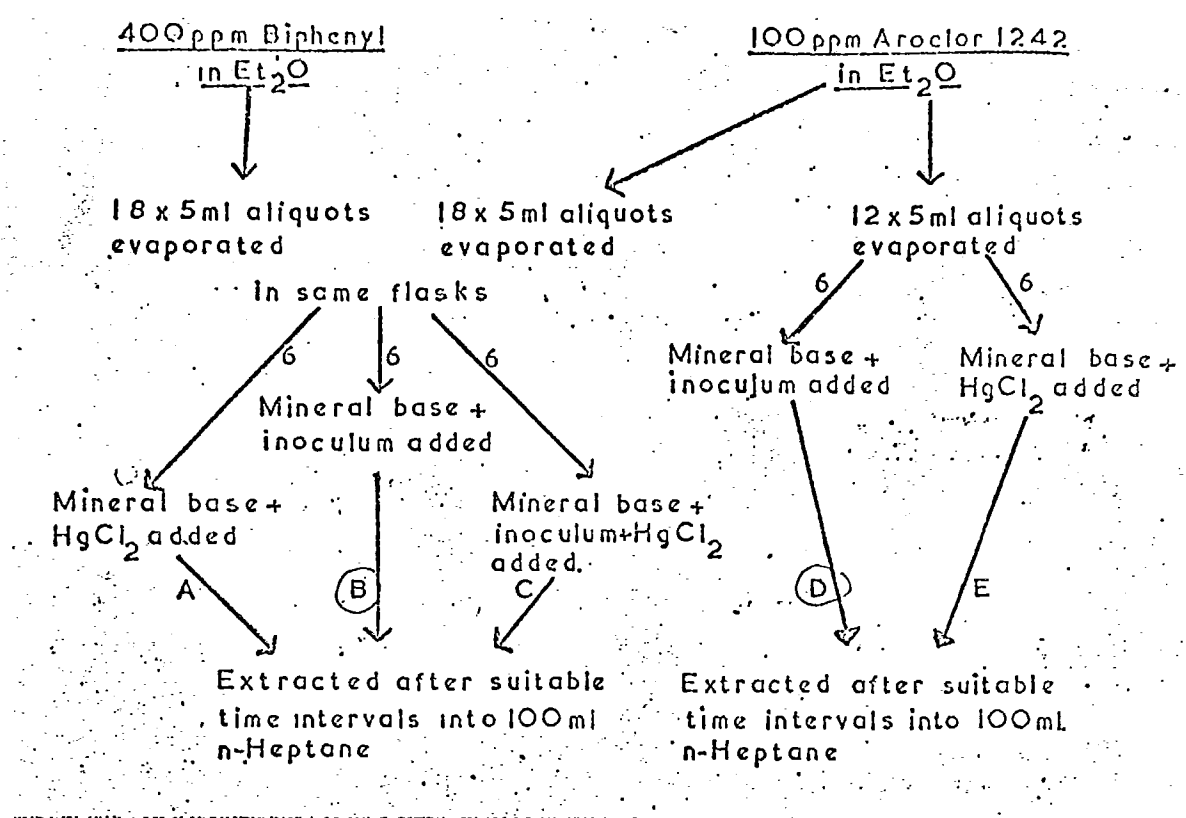

Solution	Time shaken days	% Recovered Peak 1	Peak 2	Peak 3	Peak 4	Biphenyl	Remarks
A	7	92	80	78	78	85	Complete recovery of BC & D ensured by further extraction Ref solution for analysis: 5ppm 1242 + 20ppm Biphenyl
	16	92	94	88	92	—	
B	7	ND	74	70	30	ND	
	16	ND	82	96	36	ND	
C	7	14	74	70	32	ND	
	16	ND	74	82	32	ND	
D	7	42	84	80	74	—	
	16	50	84	82	82	—	
E	7	78	64	62	62	—	
	16	100	96	94	94	—	

FIG 9 EFFECT OF PRESENCE OF BIPHENYL ON BIODEGRADATION OF AROCLOR 1242

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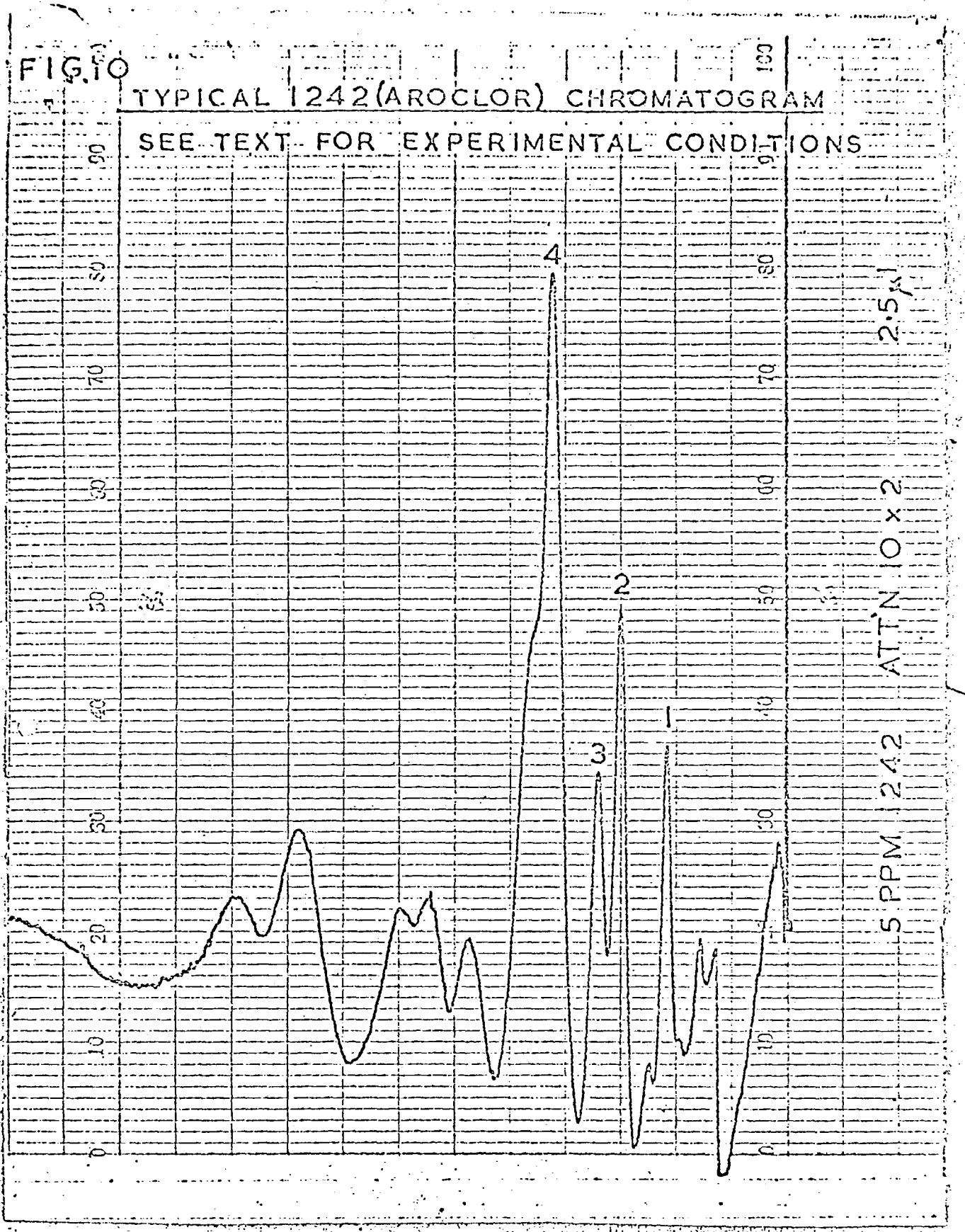
FIG. 10



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