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Type Review Report

TITLE: BIODEGRADABILITY OF POLYCHLORINATED BIPHENYLS

AUTHOR: J.H. Mainprize

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ABSTRACT

The degradation of single P.C.B's and complex mixtures was studied using a biphenyl degrading organism - C_2. From the results a theory was developed relating degradability with P.C.B. structure.

The relative degradabilities of Aroclor 1242 and potential alternative products was established.

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INTRODUCTION

A review report[1] issued earlier this year gave details of the development and application of laboratory techniques for the study of P.C.B. biodegradation. Using these techniques, little evidence was obtained of the microbial breakdown of P.C.B's when present as sole carbon sources. It was shown, however, that if unchlorinated biphenyl was included in the shake flask substrates then degradation of certain single isomers could be triggered or accelerated. A similar effect was observed in the case of Aroclor 1242 where a reduction in concentration occurred of P.C.B's associated with two predominant peaks in the gas chromatogram. The mechanism by which biphenyl influences P.C.B. breakdown is not known but it has been suggested that it may be analogous to the phenomenon of co-oxidation which has been shown by Raymond[2] to occur in the degradation of hydrocarbons.

Whilst there is no evidence to support the suggestion that P.C.B. degradation proceeds by a similar mechanism in the natural environment, it was felt that the inclusion of biphenyl in culture media would form the basis of a sorting test and thus assist in correlating P.C.B. structure with potential degradability.

For all the earlier work[1] substrates were inoculated with a suspension of activated sludge taken from the Ruabon biological treatment plant. Before use in the P.C.B. studies the inocula were normally preadapted to biphenyl degradation.

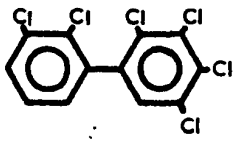
In February of this year a letter was received from Prof. W.C. Evans, Head of the Department of Biochemistry and Soil Science at Bangor University, N. Wales, in which he stated that they had isolated, in pure culture, a biphenyl degrading organism, C_2, from a soil source. In view of the then considerable publicity being given to environmental pollution by P.C.B's, Prof. Evans was keen to examine the action of the biphenyl organism on chlorinated derivatives, and asked whether we could assist by providing suitable compounds. A meeting was arranged with Prof. Evans at which we were given a sample of the culture for our own trials. A stock of active inoculum was grown on biphenyl in the laboratory and used exclusively for the work described in this report.

SUMMARY OF RESULTS

1. The biodegradation of single P.C.B. isomers and complex mixtures has been studied using a standardised shake flask procedure.

2. Results for single compounds and simple binary mixtures have indicated a correlation between biodegradability and P.C.B. structure. Theory based

on this correlation suggested that the most highly chlorinated degradable P.C.B. isomer would have the following structure –



This isomer was synthesised and shown to be degradable only when present in the substrate with lower chlorinated isomers, and with biphenyl also present.

3. Long term work with Aroclor 1242 and related products has shown a high degree of degradation when biphenyl is included in the substrate. For the M.C. products the indicated order of degradability is –

MCS 1016 > MCS 717 > MCS 762 > Aroclor 1242

4. Little degradation of Aroclor 1242 was achieved in river water die away tests over a period of 180 days. Breakdown was again improved by inclusion of biphenyl initially in the substrate.

5. H.B.40 is degraded by C_2 but again the extent of degradation is increased by initial addition of biphenyl.

DETAILS

1. Experimental Procedure

1.1 Preparation and maintenance of stock culture of biphenyl-degrading organism

A stock of active organisms was prepared and maintained using a procedure recommended by Prof. Evans. Basically this consisted of initially inoculating a prepared growth media (see Table 1) with the culture provided. The total mixture was aerated continuously in the dark at a temperature of 25°C using scrubbed and filtered air. When inocula were subsequently required for experimental substrates the air flow to the stock culture was stopped and after a brief time to allow excess biphenyl to settle, the required volume of supernatant was decanted into a separate vessel. Some difficulty was experienced in the initial work in avoiding carry-over of suspended biphenyl. Experience showed, however, that this could be overcome by careful manipulation of the stock culture during transfer.

When only relatively small volumes of the stock culture were required for experimental work (i.e. one or two litres) the bottle was replenished by addition of an equal volume of a solution containing the inorganic constituents of the growth medium. Where larger volumes were used, the practice was adopted of starting a fresh culture using new media which was inoculated with organisms reserved from the previous stock.

TABLE 1. MEDIA FOR CULTURE OF BIPHENYL-DEGRADING ORGANISM

Constituent	Wt. per litre gm.
$(NH_4)_2SO_4$	1.0
KH_2PO_4	1.0
$MgSO_4.7H_2O$	0.5
$FeSO_4$	0.05
Biphenyl	1.0

The media was prepared in 15 litre quantities. On the advice of Prof. Evans, no trace elements were added as he considered that these were adequately supplied by impurities in the major chemical constituents. The pH of the media as prepared was about 5.8 and was brought to 7 by addition of NaOH solution before introducing the inoculum.

1.2 Basic procedure for studying P.C.B. degradation

The basic procedure was essentially as described in the earlier report[1] and is shown in diagrammatic form in Fig. 1. The degradation of each compound of interest was studied alone and in the presence of added biphenyl and control flasks were included in which biological action was prevented by the addition of mercuric chloride (100 ppm. Hg^{++}). For the longer term work where several three flask sets of the same P.C.B's were set up, further additions of biphenyl were made to those substrates initially containing that material.

Residual P.C.B's were recovered, as before, by extraction into n-heptane, the extracts being submitted to Physical Methods Group for analysis. Details of analytical procedures are given in the Appendix.

2. Results and Conclusions

2.1 Degradation of pure P.C.B. isomers and simple binary mixtures

The overall objective of the biodegradation studies has been to obtain evidence of the potential breakdown of commercially produced Aroclors and also to assist in the assessment of possible alternative fluids. It was felt, however, that fundamental knowledge of the influence of P.C.B. structure on biodegradability was also required. Since, at the time, it was not possible because of analytical problems, to resolve P.C.B. chromatograms into individual identifiable peaks, it was decided that this type of fundamental information was best obtained by the study of single, or simple mixtures of, known compounds. Samples representing a range of possible P.C.B. structures were therefore assembled from U.S. and U.K. sources. Later, when results indicated that certain structural requirements were necessary for biodegradation to proceed, more highly chlorinated key compounds were synthesised in the Ruabon Research Laboratory. Detailed results for each isomer or mixture of isomers are given in the following sub-sections.

2.1.1 Degradation of 2,4'-dichlor biphenyl

Initial Concentration - 5 ppm Flasks shaken 7 days

Substrate	% Recovered
P.C.B. + culture	30
P.C.B. + B.P. culture	Nil
P.C.B. + B.P. + Hg^{++} culture	82

Conclusion Degradable

2.1.2 Degradation of 4,4'-dichlor biphenyl

Initial Concentration - 5 ppm Flasks shaken 12 days

Substrate	% Recovered
P.C.B. + culture	70
P.C.B. + B.P. + culture	66
P.C.B. + B.P. + Hg^{++} + culture	72

Conclusion Non-degradable

2.1.3 Degradation of mixture of 2,3- and 3,4-dichlor biphenyls

Initial Concentration - 5 ppm Shaken 8 days

Substrate	% Recovered	
	2,3-	3,4-
P.C.B. + culture	33	20
P.C.B. + B.P. + culture	36	Nil
P.C.B. + B.P. + Hg^{++} + culture	82	82

Conclusion Both degradable 3,4 more readily than 2,3

2.1.4 Degradation of mixture of 2,3,2' and 2,3',4' trichlor biphenyls

Initial Concentration - 2 ppm Shaken 7 days

Substrate	% Recovered	
	2,3,2'	2,3',4'
P.C.B. + culture	50	6
P.C.B. + B.P. + culture	5	Nil
P.C.B. + B.P. + Hg^{++} + culture	90	95

Conclusion Both degradable 2,3',4' more readily than 2,3,2'

2.1.5 Degradation of 3,3',4-trichlor biphenyl

Initial Concentration - 5 ppm Shaken 12 days

Substrate	% Recovered
P.C.B. + culture	24
P.C.B. + B.P. + culture	30
P.C.B. + B.P. + Hg^{++} + culture	98

Conclusion Degradable

2.1.6. Degradation of 2,4,6-trichlor biphenyl

Initial Concentration - 5 ppm Shaken 12 days

Substrate	% Recovered
P.C.B. + culture	100
P.C.B. + B.P. + culture	100
P.C.B. + B.P. + Hg^{++} + culture	100

Conclusion Non-degradable

2.1.7. Degradation of 2,2',4,4'-tetrachlor biphenyl

Initial Concentration - 5 ppm Shaken 9 days

Substrate	% Recovered
P.C.B. + culture	100
P.C.B. + B.P. + culture	100
P.C.B. + B.P. + Hg^{++} + culture	100

Conclusion Non-degradable

2.1.8. Degradation of 2,2',4,6-tetrachlor biphenyl

Initial Concentration - 5 ppm Shaken 9 days

Substrate	% Recovered
P.C.B. + culture	94
P.C.B. + B.P. + culture	92
P.C.B. + B.P. + Hg^{++} + culture	94

Conclusion Non-degradable

2.1.9. Summary of results obtained and suggested correlation between biodegradability and structure

The results given in Sections 2.1.1. - 2.1.8. are summarised in Fig. 2.

Microbiological and analytical work carried out by Prof. Evans at Bangor University[3] has shown that metabolism of biphenyl follows the pathway shown in Fig. 3.

The reasonable assumption that breakdown of degradable P.C.B's follows the same initial steps indicates ways in which microbial attack could be prevented or hindered by chlorine substitution at specific sites in the biphenyl molecule. With the supporting evidence of the results summarised in Table 1 it was theorised that the following basic structural requirements must be fulfilled if, at least, primary degradation of P.C.B's was to occur -

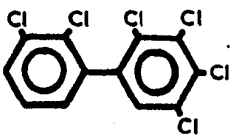
(a) Chlorine substitution at neither or only one of the two para (4 or 4') positions. Supported by failure to degrade 4,4' dichlor biphenyl and 2,2',4,4' tetra chlor biphenyl. Substitution at both 4-positions presumably prevents hydroxylation at the adjacent 2,3 positions and would also interfere with the ring opening step III.

(b) At least two of the four adjacent 2,3 positions must be unsubstituted.

(c) No chlorine substitution at the 2 and 6 positions in the same ring. Supported by failure to degrade 2,4,6-trichlor biphenyl. It is suggested that degradation is prevented by the steric effects of chlorine at the 2 or 6 positions which blocks hydroxylation at the 2' and 6' positions on the unchlorinated ring.

In the absence of other possible factors which could limit biodegradability,

e.g. toxicity, insolubility, the above theorising suggested that the most highly chlorinated biphenyl which could still be subjected to at least primary degradation would have the following structure -



Also the structure of all other degradable P.C.B's can be arrived at by progressively reducing the chlorine content of the above isomer.

To obtain further support for the proposed correlation between structure and degradability, two isomers were synthesised in the Ruabon Research Laboratory, (i) 2,3,3',4,5-penta chlor biphenyl, and (ii) 2,2',3,3',4',5'-hexa chlor biphenyl, i.e. as above.

These were tested for degradability under the standard shake flask conditions. Results after 14 days shaking showed no degradation to have occurred. In the case of the penta substituted isomer, a second set of shake flask substrates were analysed after 33 days agitation, again with negative results.

In discussion of the above results, it was suggested that failure to degrade these key compounds might be associated with their very low solubility and high melting points which prevented effective contact between P.C.B. and organism active centres. Also the gentle agitation given by the oscillating motion of the flask shaker may not have been sufficient to ensure satisfactory dispersion of the evaporated P.C.B. deposit in the culture flask. To overcome these possible limitations, a further run was carried out using the hexa chlor biphenyl mixed in the ratio of 1:1 by weight with the isomeric mixture of 2,3',4' and 2,3,2'-trichlor biphenyls (see Section 2.1.4.). Substrates were prepared in the usual way with a total P.C.B. content of 5 ppm, i.e. 2.5 ppm of hexa substituted isomer but flasks were agitated in a vibratory bench shaker. After 15 days agitation the flask contents were extracted into n-heptane and the abstracts submitted for analysis. Results showed that a 50% loss of the hexa chlor biphenyl was achieved when biphenyl was also included in the substrate; no degradation was observed when biphenyl was excluded. The significant degradation of this previously refractory P.C.B. could have been attributable to either of the changed experimental conditions,

i.e. improved agitation or solubilisation by mixing with other lower chlorinated isomers. Unfortunately when the same technique was applied to the study of the degradation of the penta compound, completely negative results were again obtained.

To confirm the observed degradation of the hexa compound, the earlier run was repeated but with the effect of agitation also being compared. Results of this run, which are given in Table 2, confirm that significant degradation can be achieved in the presence of the lower chlorinated isomers with agitation apparently not, under the experimental conditions, being a controlling factor. In the light of these results, the failure to degrade the penta chlor isomer cannot be explained and the run is again being repeated. Also the earlier theorising on the influence of P.C.B. structure on degradation cannot be considered proven until it has been demonstrated that degradation of other hitherto intractable isomers, e.g. 4,4'-dichlor- cannot also be induced by mixing with lower melting point derivatives.

TABLE 2. DEGRADATION OF 2',3',4',5',2,6-HEXA CHLOR BIPHENYL IN THE PRESENCE OF MIXTURE OF 2,3,2' and 2,3',4'-TRICHLOR BIPHENYLS

Concentrations 2',3',4',5',2,6, hexa chlor biphenyl 2.5 ppm
Mixed trichlor biphenyls 2.5 ppm

		Substrate	% Recovered
Shaker 1	1.	Hexa chlor biphenyl + C_2	100
	2.	Hexa chlor biphenyl + C_2 + mixt. of 2,3',4'and 2,3,2'	100
	3.	As 2 but with 50 ppm B.P. added initially and after 3 and 11 days	52
	4.	As 2 but with 50 ppm B.P. and 100 ppm Hg^{++} added initially	100
Shaker 2	1.	Hexa chlor biphenyl + C_2	100
	2.	Hexa chlor biphenyl + C_2 + mixt. of 2,3',4' and 2,3,2' trichlor biphenyls	92
	3.	As 2 but with 50 ppm B.P. added initially and after 3 and 11 days	36
	4.	As 2 but with 50 ppm B.P. and 100 ppm Hg^{++} added initially	100

Shaker 1 High frequency bench vibratory instrument
Shaker 2 Oscillating shaker, as used for all other work.

3. Degradation of complex P.C.B. mixtures (Aroclors and related products)

The degradation of a range of P.C.B. mixtures was studied using the standardised technique with culture C_2 as the active inoculum. Also by setting up several three-flask sets which were removed after increasing time intervals, it was possible to obtain some measure of the rate of degradation and to produce base chromatograms representing residual non-degradable P.C.B's. Results are given in Figs. 4 - 21. For convenience of visual comparison, chromatograms were traced on to a single page by hand. Whilst the general forms of the chromatograms are faithfull copies of the originals, excessive levels of recorded 'noise' were deliberately 'cleaned up' for ease of comparison. Brief notes on the significance of the results are given in the following sections under the relevant headings. No attempt has been made to quantify the degree of degradation although it is hoped that this will be possible in the near future with the continuing improvement which is being achieved in higher resolution chromatography.

3.1. Degradation of Aroclor 1242 - Figs. 4 and 5

Degradation of 1242 compared after agitation for 37 and 83 days respectively. Results demonstrate accelerating effect which biphenyl (B.P.) has on the rate of P.C.B. degradation.

3.2. Degradation of Aroclor 1242 - Figs. 6, 7 and 8

Three-flask systems set up in quadruplicate. Extracted after 13, 38 and 73 days respectively. Biphenyl additions made to relevant flasks after increasing time intervals. Because of instrument problems, the chromatograms of the 38 day samples were recorded at half the attenuation used for the 13 and 73 day extracts. The results indicate that no further degradation occurred after 38 days.

3.3. Degradation of Pyralene 1500 - Figs. 9 and 10

Pyralene 1500 is a competitive blend of polychlorinated biphenyls manufactured by Prodelec of France. In this run, provision was made to demonstrate the need for the continuing presence of biphenyl if maximum breakdown of P.C.B's is to be avhieved within reasonable contact times. The results unfortunately are anomalous in that greater degradation occurred in the flask containing no added biphenyl than in that to which biphenyl was initially added (Fig. 10, Traces 2 and 3). This anomaly could be explained by accidental interchange of samples at some stage between substrate preparation and extraction but there is no evidence to show that this occurred. However, comparison of chromatograms 2 or 3 in Fig. 9 with chromatogram 4 indicate that degradation was enhanced in the latter by the second addition of biphenyl made after 34 days.

3.4. Degradation of Aroclor 1242 - Effect of Agitation - Fig. 10

When visiting Prof. Evans' laboratory at Bangor University, it was noted that, for degradation work culture flasks were agitated in a high frequency vibratory shaker. It was suggested that this type of agitation might cause better dispersion of liquid P.C.B's and Aroclors and hence more effective contact with the organisms. As reported earlier in Section 3.4 it was shown that for simple mixtures the degree of agitation had no effect on the rate or extent of degradation. This was confirmed for Aroclor 1242 by the results shown in Fig. 11.

3.5. Degradation of MCS 717, MCS 762 and MCS 1016 (1242 B) - Figs. 12 to 14, 15 to 17 and 18 to 20.

This run was set up simultaneously with that for 1242 described in Section all flasks being treated similarly with respect to agitation time and frequency of biphenyl addition. Visual comparison of the chromatograms therefore gives some indication of the relative biodegradability of each P.C.B. mixture. For convenience of comparison, chromatograms representing the highest degree of degradation for each product are shown in Fig. 20.

Assuming that peak areas can be related quantitatively to the concentration of residual P.C.B's, comparison of the chromatograms shown in Fig. 21 indicate the approximate order of degradability to be MCS 1016 > MCS 717 > MCS 762 > 1242. It is hoped that with the continuing progress in higher resolution chromatography and identification of P.C.B's associated with individual peaks, more detailed information will shortly be generated on the nature of non-degradable residues.

One unexplained aspect of this work is the appearance of an additional peak in the chromatograms of non-degraded residues for MCS 762, MCS 717 and MCS 1016. This peak presumably represents the accumulation of some non-degradable breakdown product of PCB's originally present.

4. Other biodegradation work

4.1. Degradation of HB 40

This material has been shown to be a suitable alternative to Aroclor 1242 for use in the manufacture of no-carbon duplicating paper. Degradation studies were undertaken using the same basic procedure as for P.C.B's and also using the culture C_2. Unfortunately at a level of 5 ppm the concentrations of extracts were too low for satisfactory measurement using a flame-ionisation detector. The chromatograms shown in Fig. 22 were obtained by preparing a 10-fold concentrate of the extracts before analysis.

4.2. River water die away tests

To determine whether micro-organisms populating normal surface waters were capable of long term acclimatisation to P.C.B. degradation, a run was carried out in which the basic procedure was used but flasks were inoculated with fresh samples of water taken from the River Dee. Results in Fig. 23 show that after 180 days contact measurable losses occurred only in that substrate to which biphenyl was initially added.

5. Discussion of Results

Work to date has shown that considerable degradation of P.C.B's can be achieved using specific organisms under laboratory conditions. Although these conditions are probably more favourable than those which exist in the natural environment, when results are considered in conjunction with the failure to detect significant levels of lower chlorinated residues in marine life, they lend strong support to the statement that degradation is probably occurring.

The apparent correlation between P.C.B. structure and biodegradability, although not yet proven, should assist in the formation of suitable products although the difficulty of excluding non-degradable isomers may be insurmountable, at least on a commercial basis.

It is now considered that priority should be given to improved analytical evaluation of the nature of non-degraded residues. Considerable progress is being made in improving chromatogram resolution and in identifying individual peaks. Typical recent chromatograms of degraded and non-degraded 1242 are shown in Fig. 24.

Further work

Prof. Evans has recently isolated, from a soil source, another biphenyl degrading organism with a different metabolic pathway. A stock of this culture is being grown at Ruabon and used to repeat some of the work described in this report.

References

1. Review Report - Biodegradability of polychlorinated biphenyls. Acc. No. AP32412.
2. Process Biochemistry, September 1969, pp. 71-74.
3. Private communication, Lunt D. and Evans W.C., 1970.

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J.H. MAINPRIZE

APPENDIX

Gas Chromatographic Analyses

Analyses of extracts of single components, simple mixtures and complex mixtures represented by Figures 4 to 23 were made using a Hewlett Packard 402 chromatograph equipped with a Ni^{63} high temperature electron capture detector. The column consisted of 4' glass tubing packed with 4% Silicone Oil OV101 on 60 - 80 mesh Gas Chrom Q.

Operating conditions were -

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

Oven temperature	170°C
Flash Heater	250C
Detector	250°C
Pulser	150 m. sec.

For the more highly resolved chromatographs shown in Fig. 24 and 25, the column consisted of 150' S.S. Capillary coated with Apiezon L.

Operating conditions with E.C. detector -

Temperature	200°C isothermal	
Flow	5 psi through column 2 psi purge	95% Argon/5% Methane

FIG 1 BASIC PROCEDURE FOR THE STUDY OF P.C.B. BIODEGRADATION

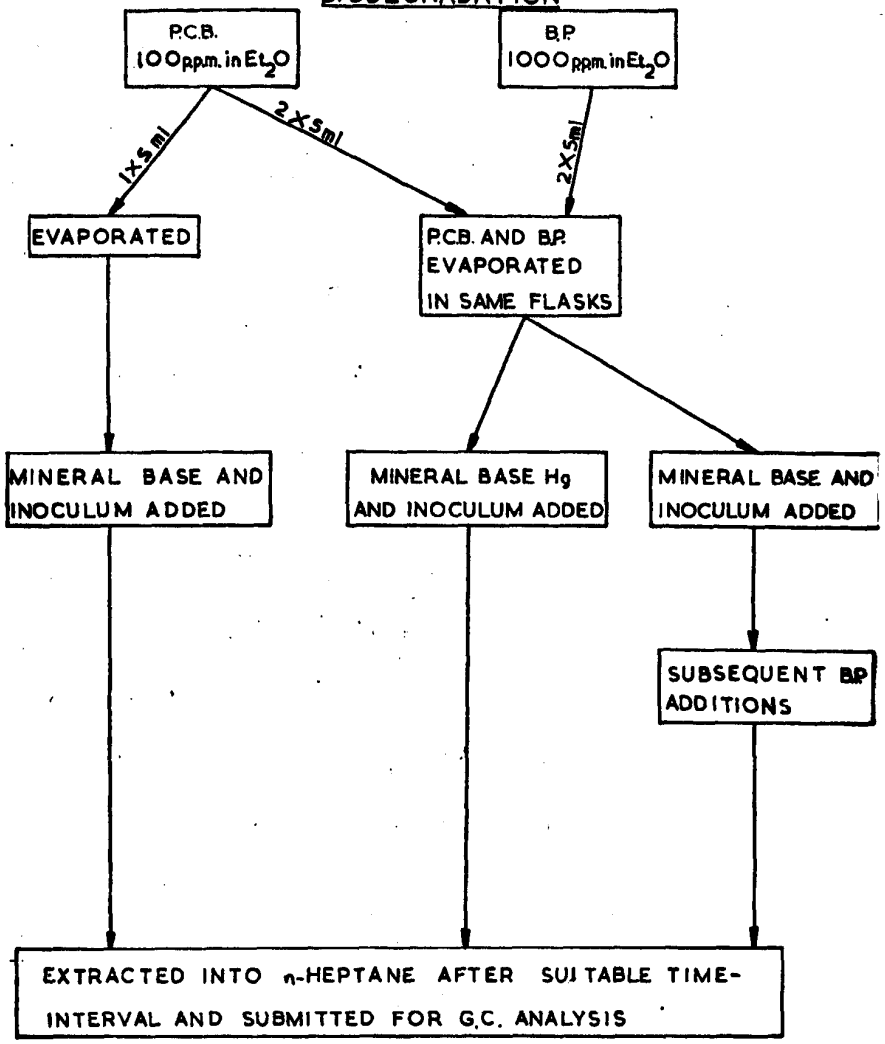

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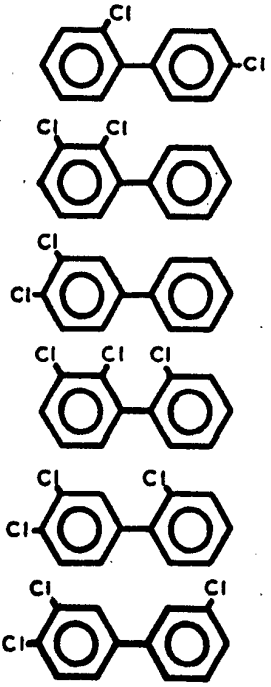
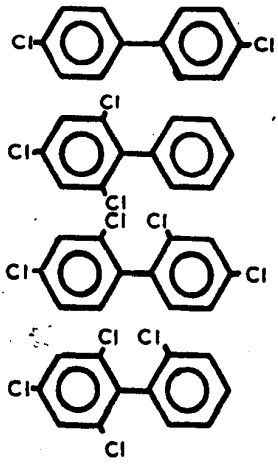
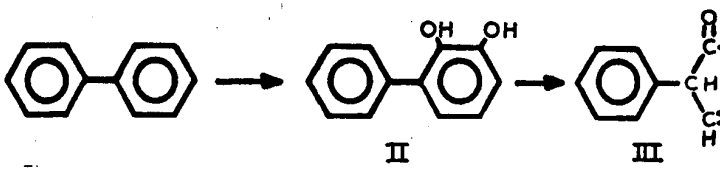
FIG 2 DEGRADABILITY OF SINGLE P.C.B. ISOMERS

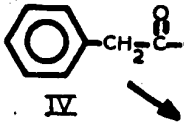
DEGRADABLE



NON-DEGRADABLE







KNOWN PATHWAYS

FIG 3 PATHWAY OF BIPHENYL METABOLISM BY CULTURE C_2

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FIG4 DEGRADATION OF AROCLOR 1242 BY CULTURE C_2

1. CONTROL Ie AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. AROCLOR 1242 + C_2 SHAKEN 37 DAYS
3. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 9 DAYS

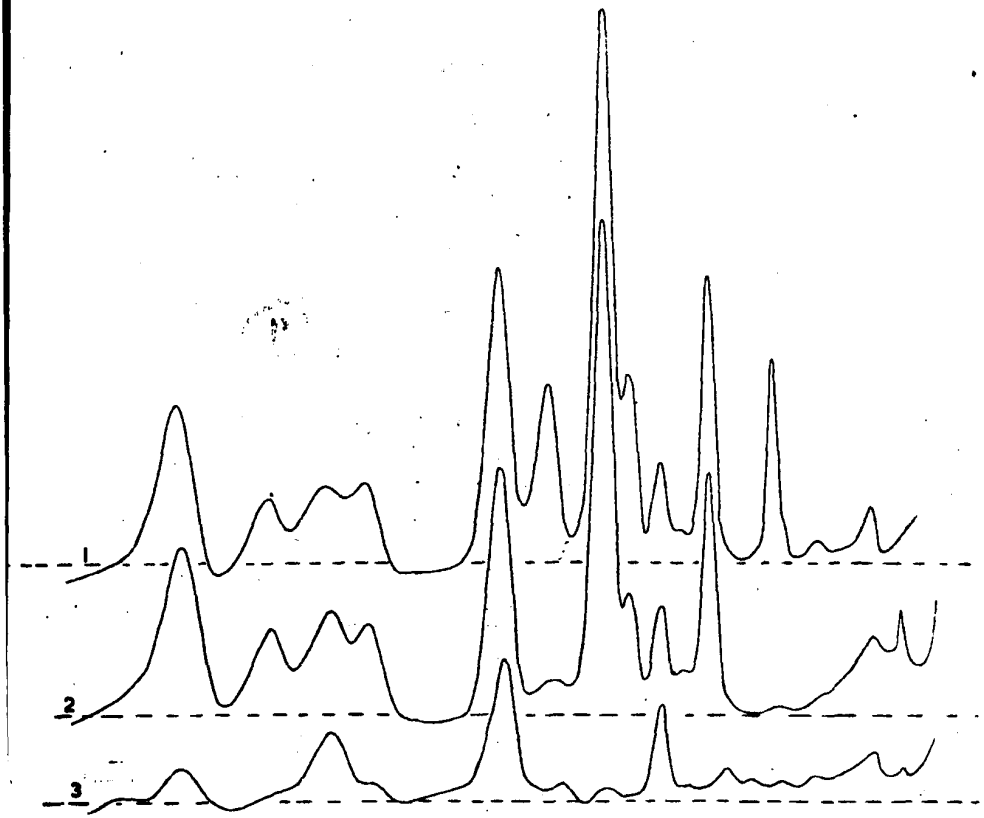

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FIG 5 DEGRADATION OF AROCLOR 1242 BY CULTURE C_2

1. CONTROL 1e AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. AROCLOR 1242 + C_2 SHAKEN 83 DAYS
3. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 9, 36, & 49 DAYS

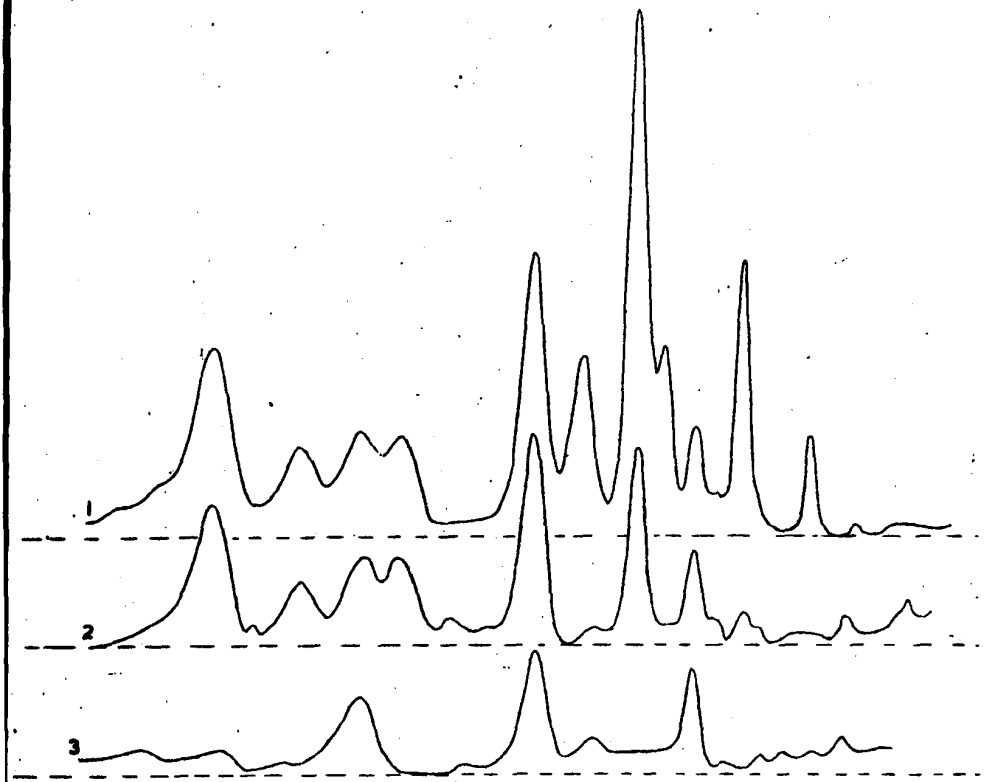

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FIG 6 DEGRADATION OF AROCLOR 1242 BY CULTURE C_2

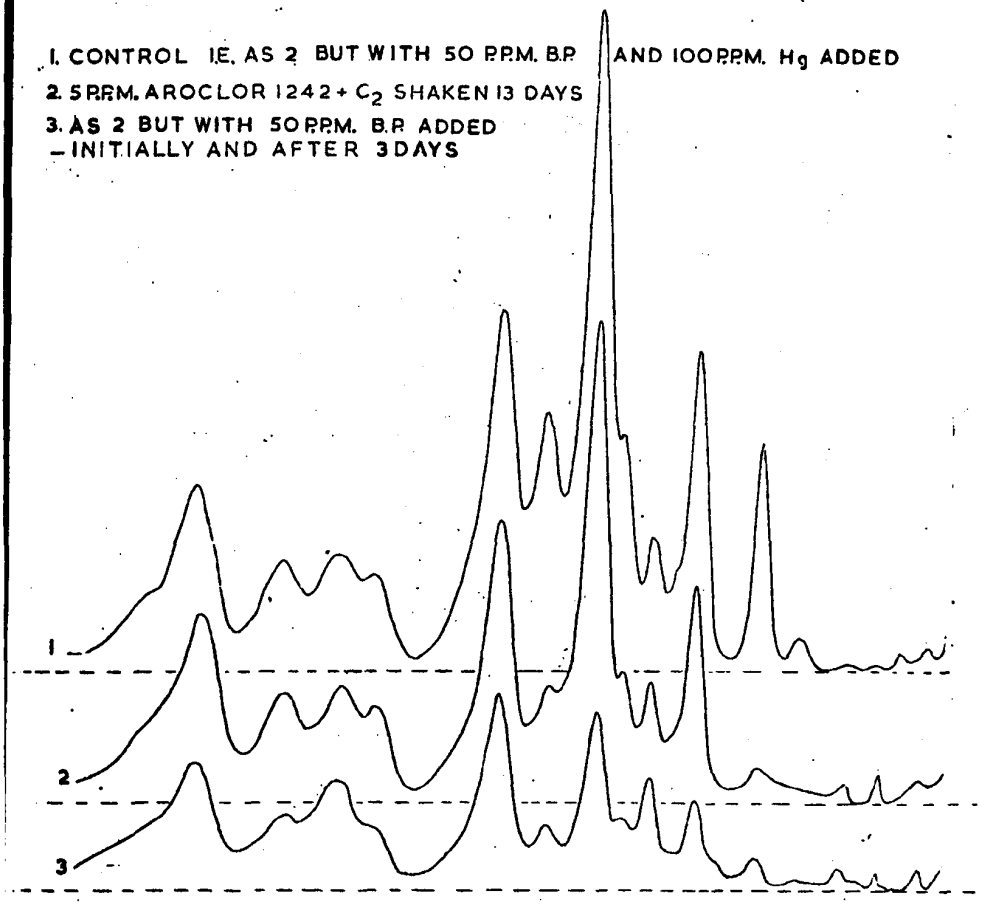

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FIG 7 DEGRADATION OF AROCLOR 1242 BY CULTURE C_2

1. CONTROL ie. AS 2 BUT WITH 50 P.P.M. BP AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. AROCLOR 1242 C_2 SHAKEN 38 DAYS
3. AS 2 BUT WITH 50 P.P.M. BP ADDED INITIALLY AND AFTER 3, 8 AND 18 DAYS

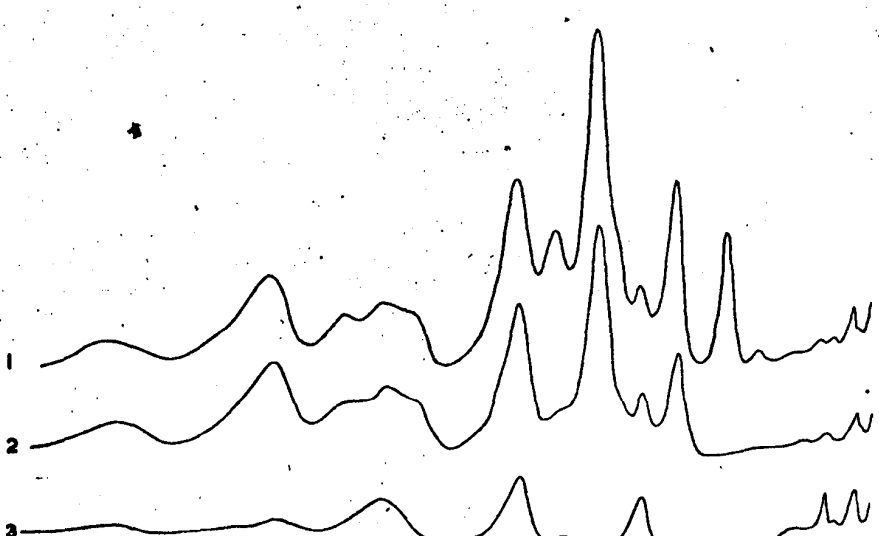

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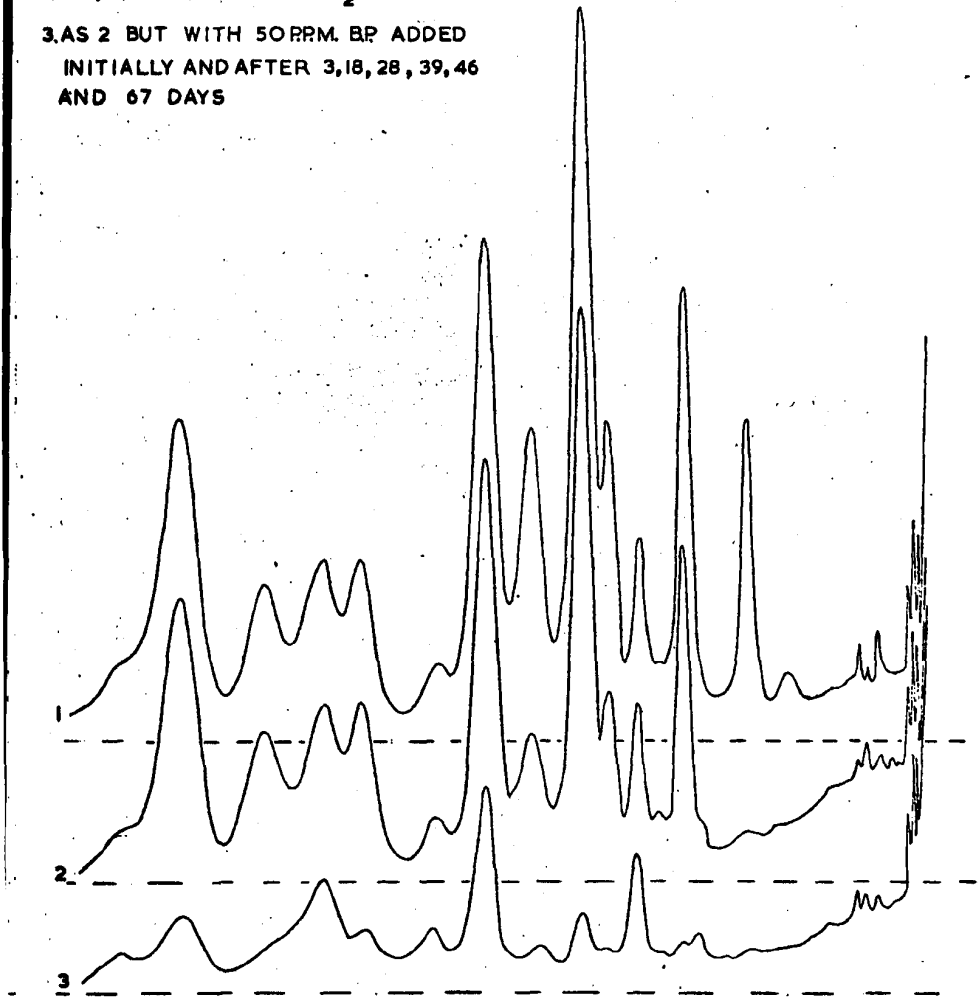
FIG 8 DEGRADATION OF AROCLOR 1242 BY CULTURE C_2

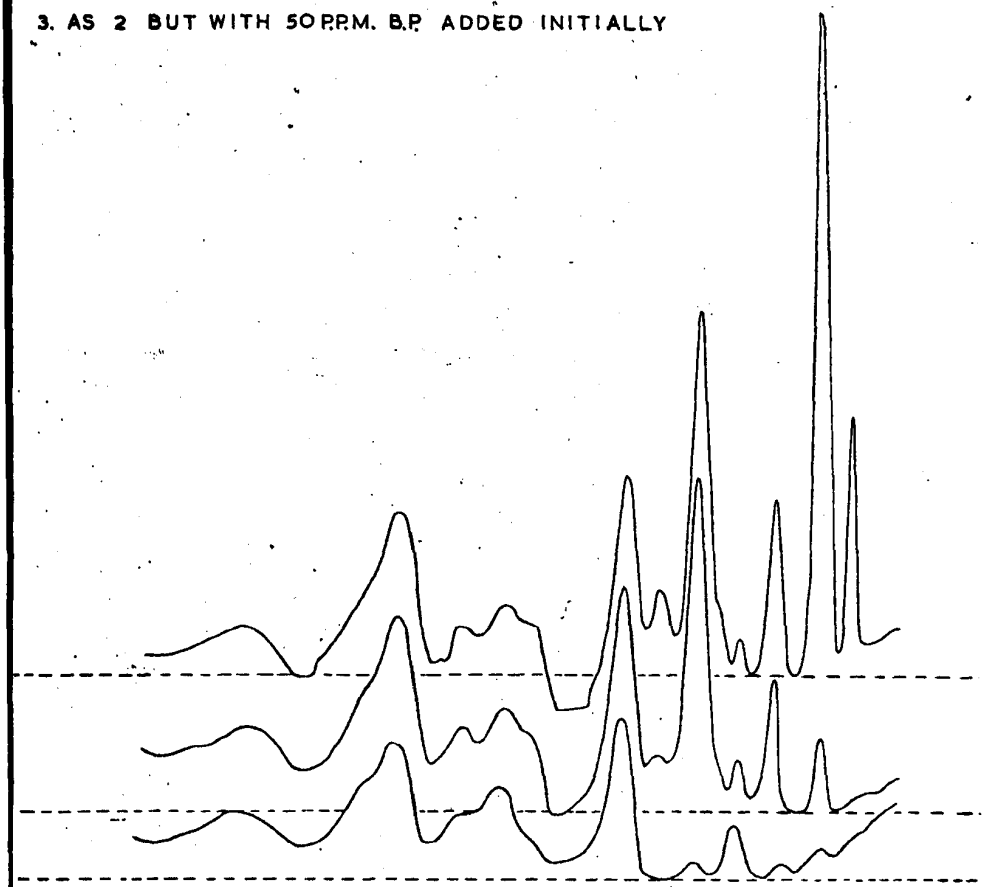
1. CONTROL i.e. AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. AROCLOR 1242 + C_2 SHAKEN 73 DAYS
3. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 3, 18, 28, 39, 46 AND 67 DAYS



MONS 067758

FIG.9 DEGRADATION OF PYRALENE BY CULTURE C_2

1. CONTROL Ie AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. PYRALENE + C_2 SHAKEN 18 DAYS
3. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY



MCNS 067759

FIG 10 DEGRADATION OF PYRALENE BY CULTURE C_2

1. CONTROL i.e. AS 3 BUT WITH 100 P.P.M. Hg ADDED
2. 5 P.P.M. PYRALENE C_2 SHAKEN 59 DAYS
3. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY
4. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 34 DAYS

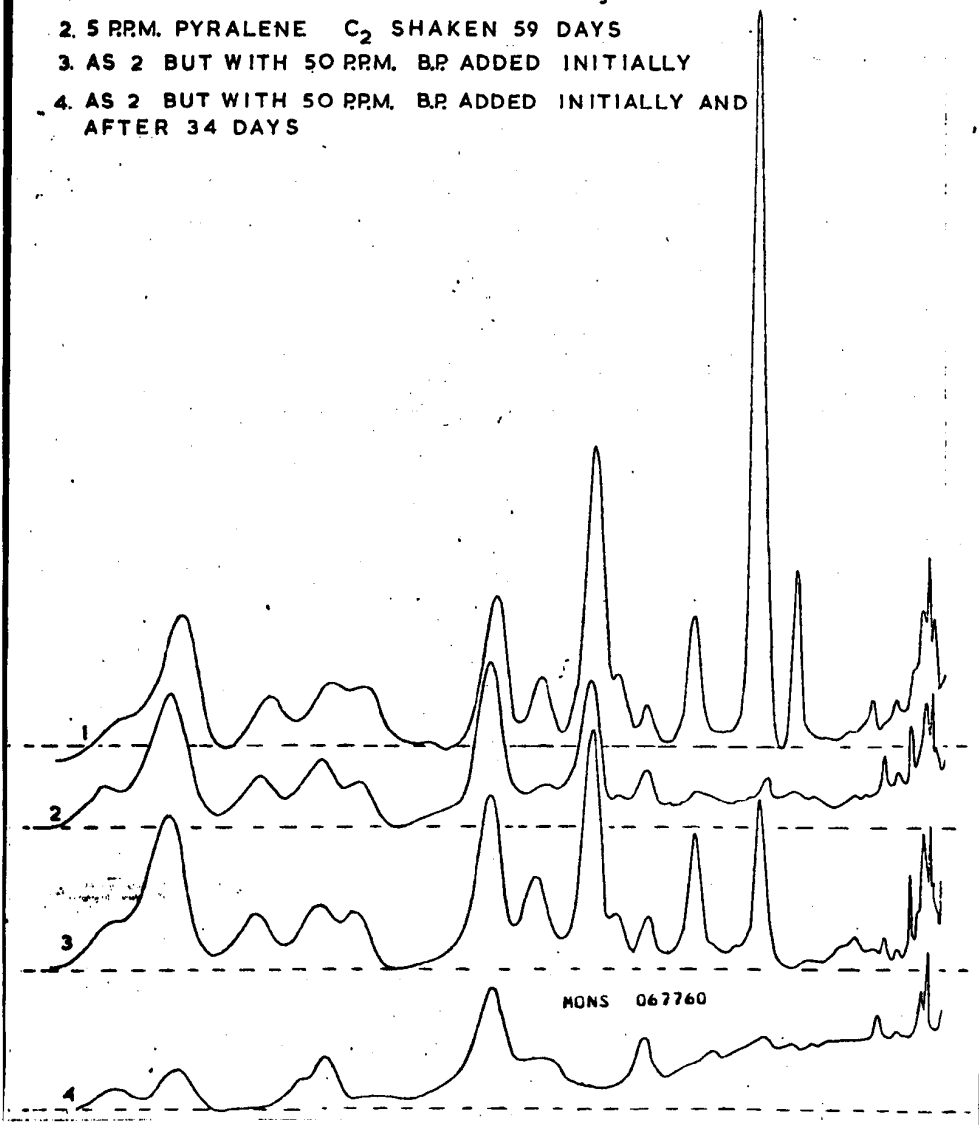
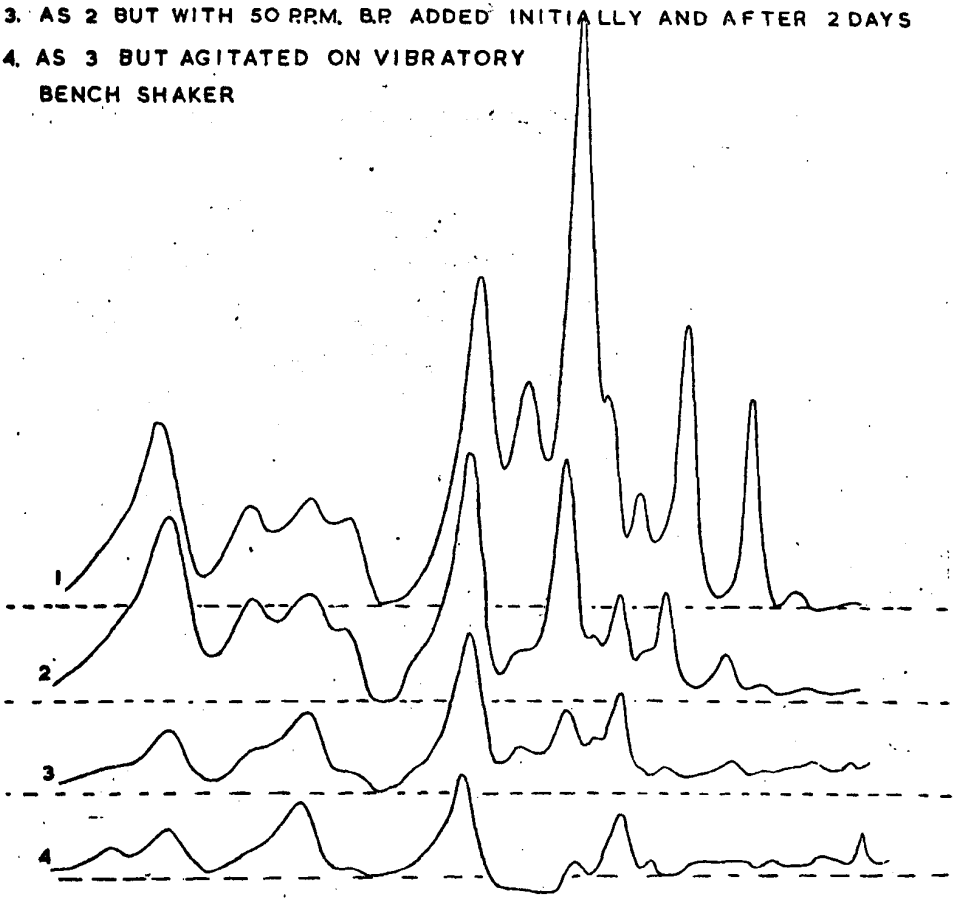


FIG II DEGRADATION OF AROCLOR 1242 BY C_2 (EFFECT OF AGITATION)

1. CONTROL Ie AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. AROCLOR 1242 + C_2 SHAKEN 8 DAYS
3. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 2 DAYS
4. AS 3 BUT AGITATED ON VIBRATORY BENCH SHAKER



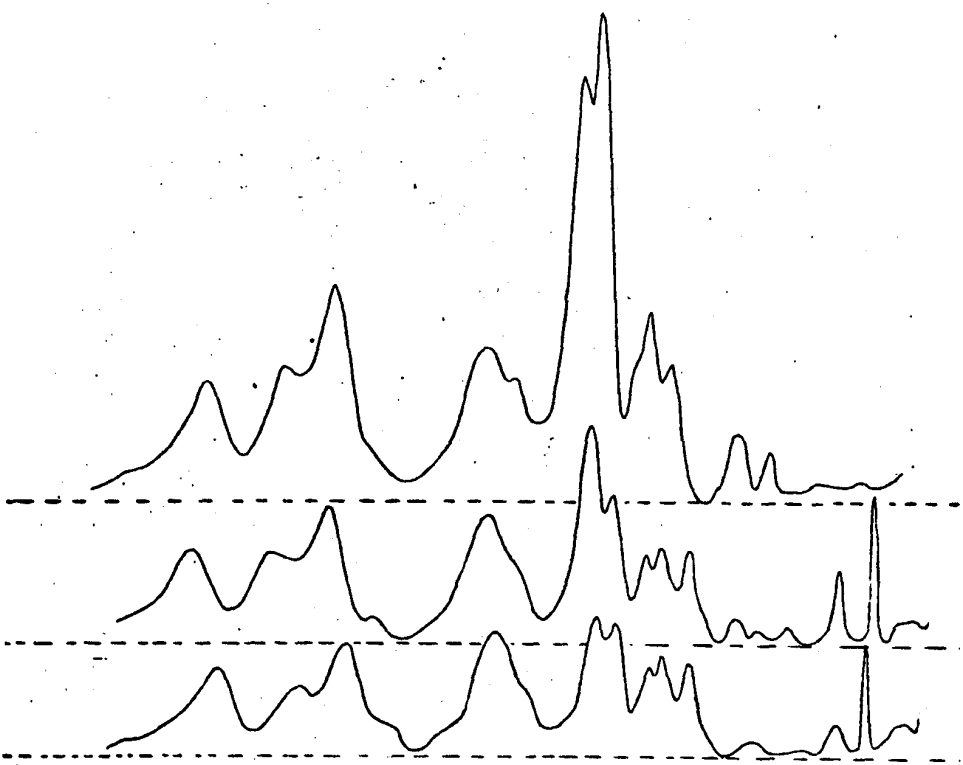
MONS 067761

FIG 12 DEGRADATION OF MCS 717 BY CULTURE C_2

1 CONTROL ie AS 2 BUT WITH 50 P.P.M. B.P. AND 100 PPM Hg ADDED

2, 5 P.P.M. MCS 717 + C_2 SHAKEN 13 DAYS

3 AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 3 DAYS



MONS 067762

FIG13 DEGRADATION OF MCS 717 BY CULTURE C_2

1. CONTROL i.e. AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. MCS 717 + C_2 SHAKEN 38 DAYS
3. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 3, 18, AND 28 DAYS

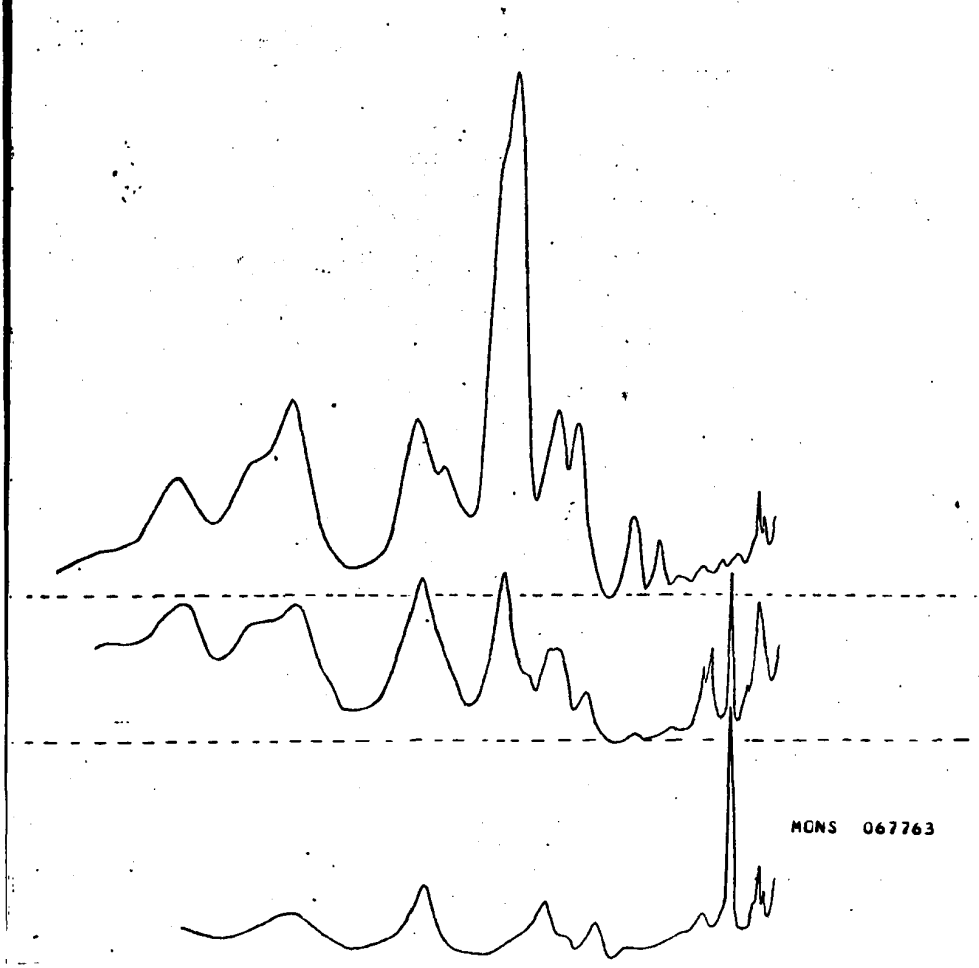
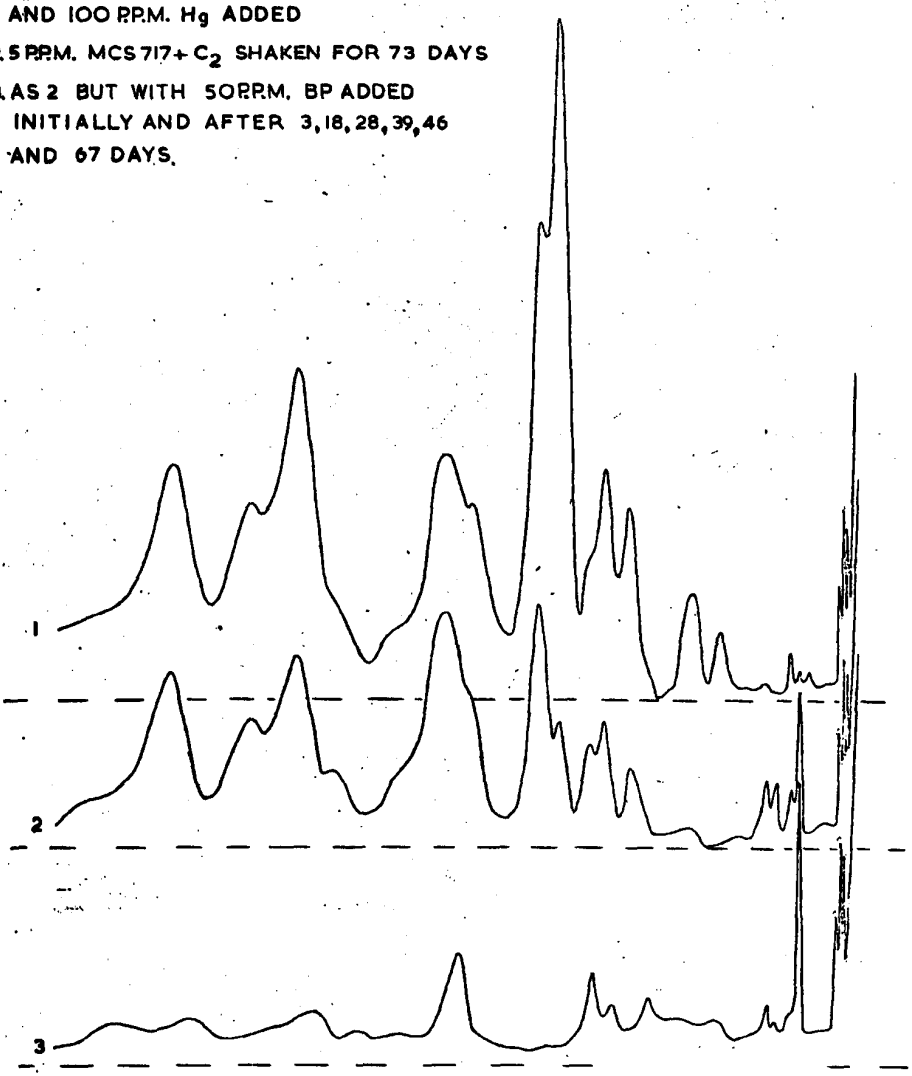


FIG 14 DEGRADATION OF MCS 717 BY CULTURE C_2

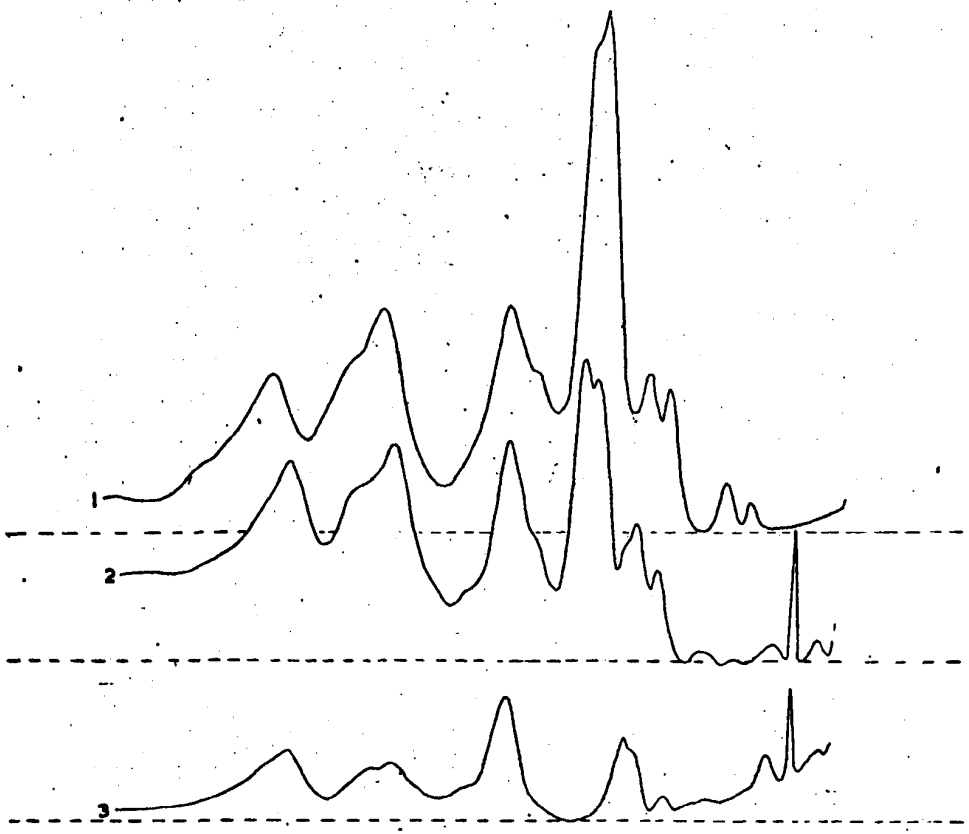
1. CONTROL i.e. AS 2 BUT WITH 50 P.P.M. BP AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. MCS 717 + C_2 SHAKEN FOR 73 DAYS
3. AS 2 BUT WITH 50 P.P.M. BP ADDED INITIALLY AND AFTER 3, 18, 28, 39, 46 AND 67 DAYS.



MONS 067764

FIG 15 DEGRADATION OF MCS 762 BY CULTURE C_2

1. CONTROL Ie AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. MCS. 762 + C_2 SHAKEN 13 DAYS
3. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 3 DAYS



MGNS 067765

FIG 16 DEGRADATION OF MCS 762 BY CULTURE C_2

1. CONTROL IE AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. MCS 762 + C_2 SHAKEN 38 DAYS
3. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 3, 18 AND 28 DAYS

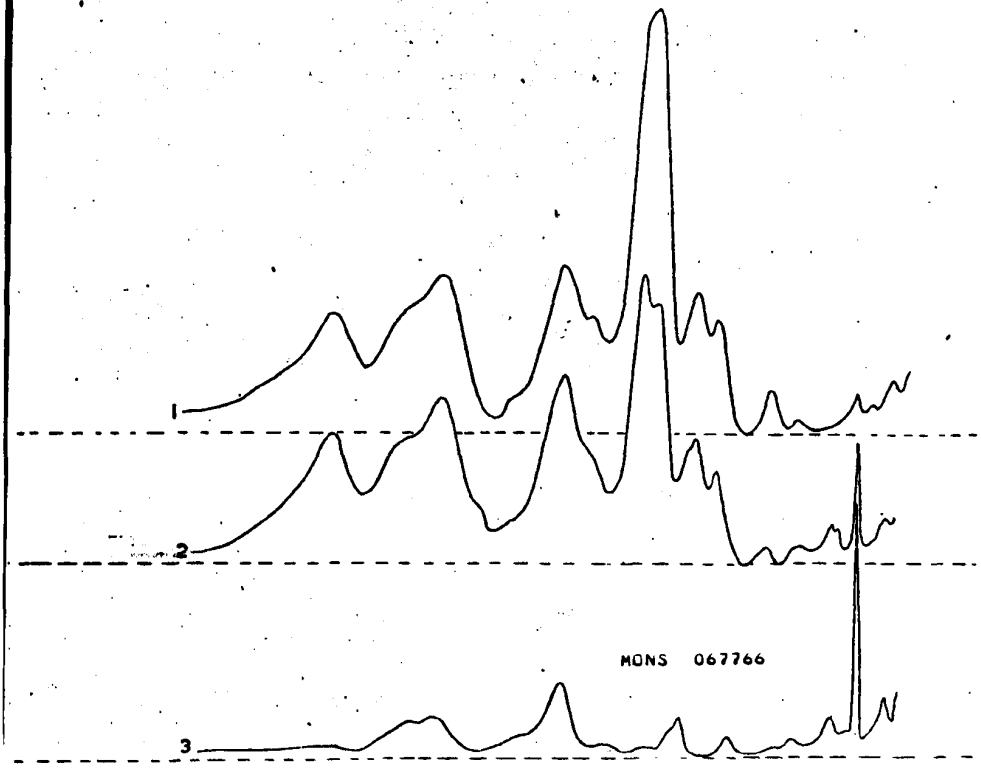
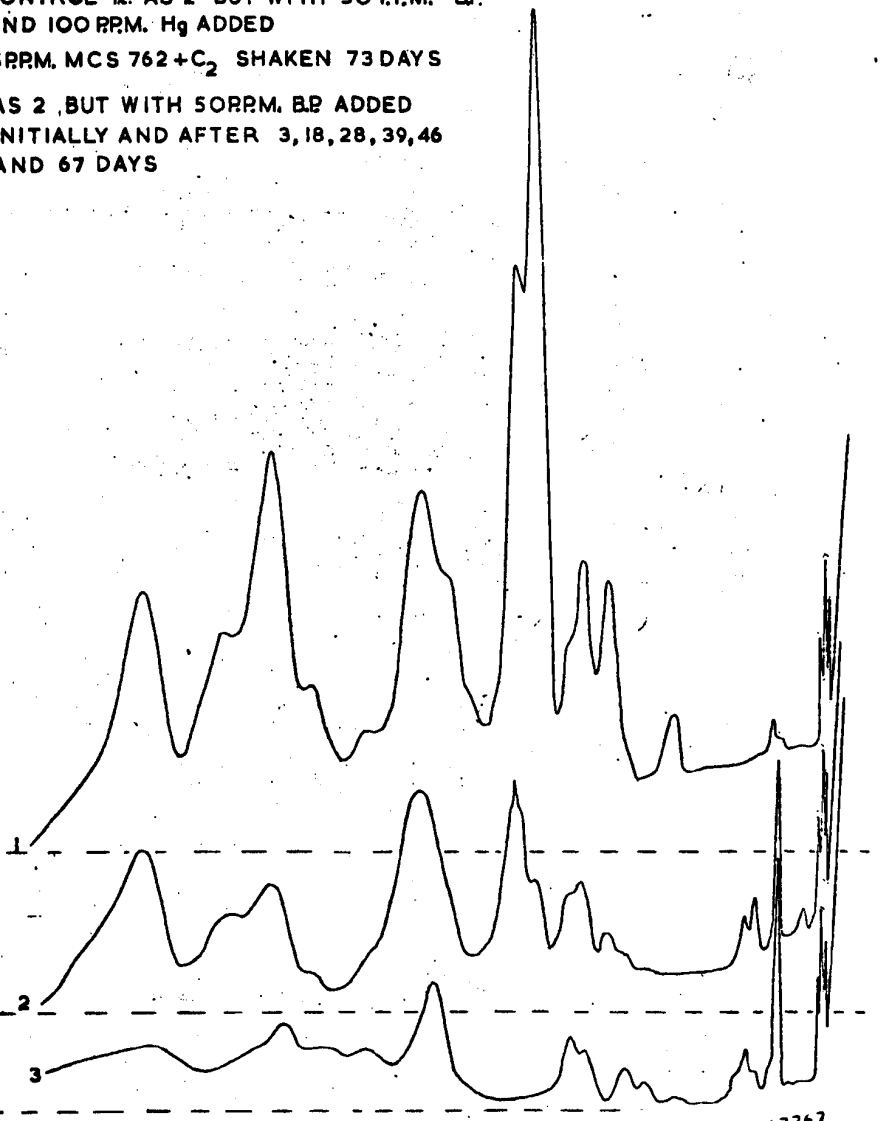


FIG 17 DEGRADATION OF MCS 762 BY CULTURE C_2

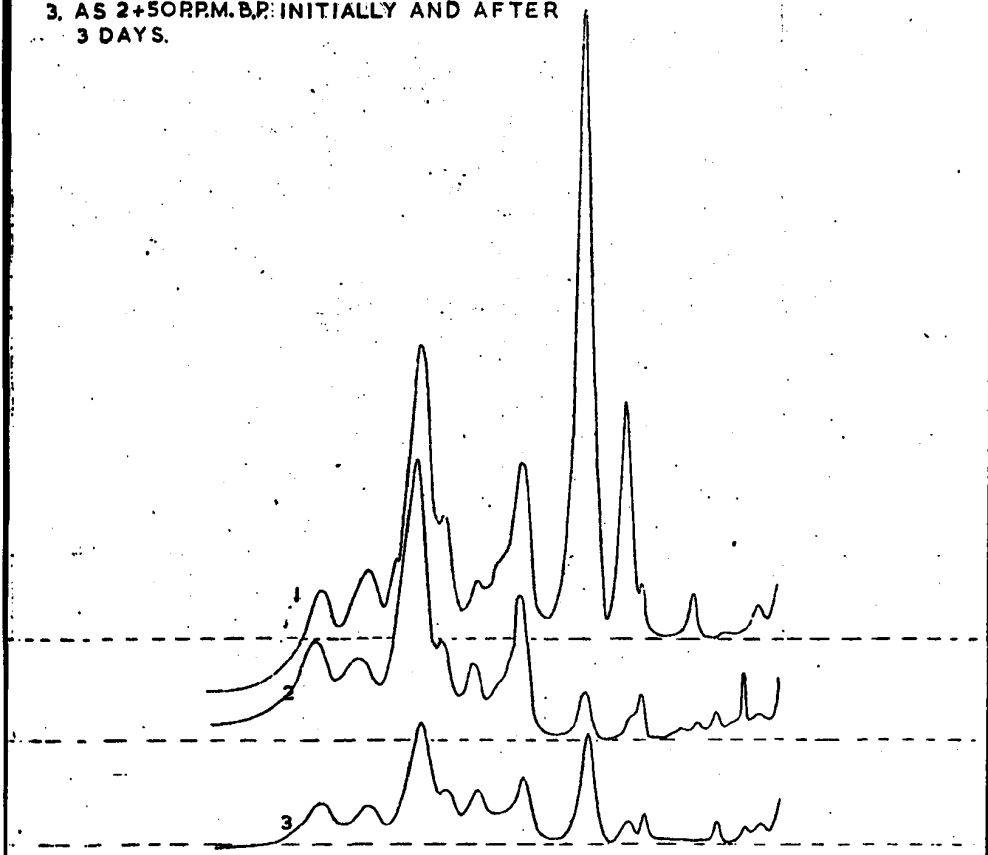
1. CONTROL i.e. AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. MCS 762 + C_2 SHAKEN 73 DAYS
3. AS 2, BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 3, 18, 28, 39, 46 AND 67 DAYS



MONS 067767

FIG 18 DEGRADATION OF AROCLOR 1242 B (MCS 1016) BY CULTURE C_2

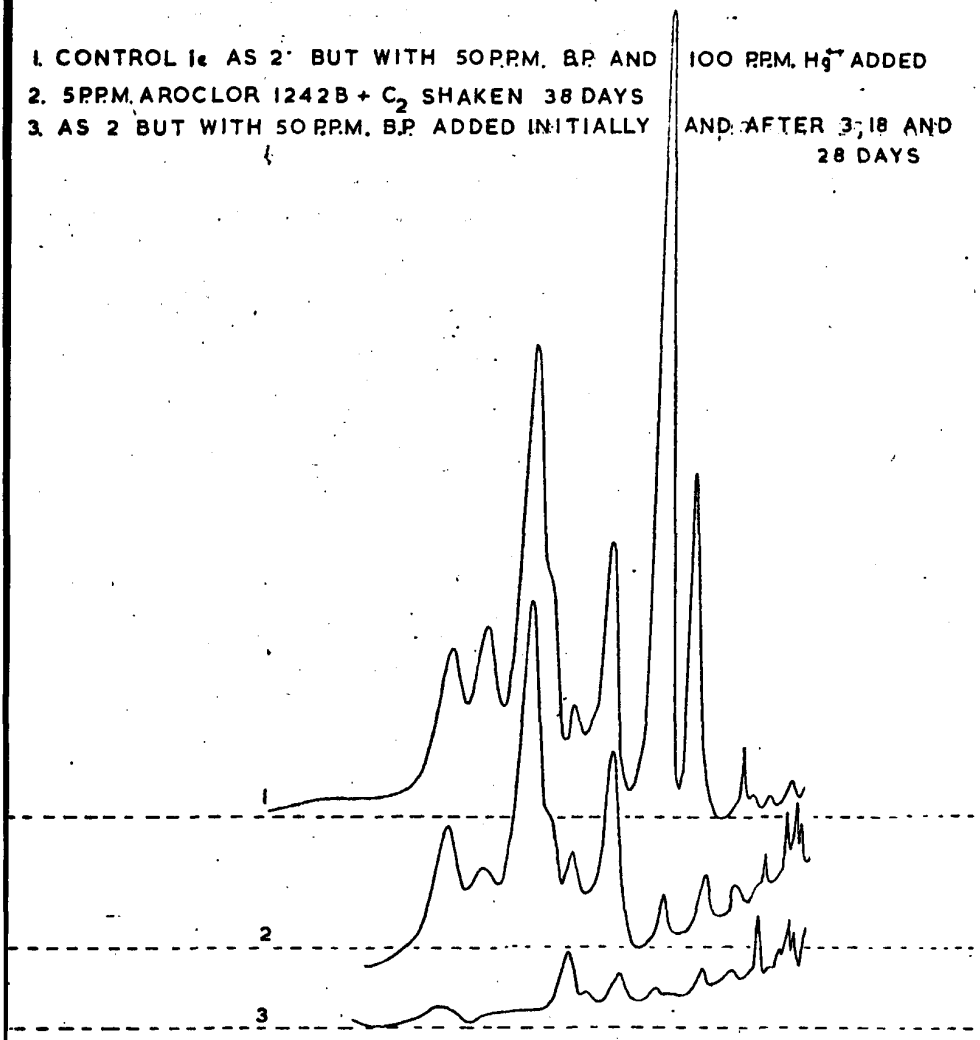
1. CONTROL AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. AROCLOR 1242 B + C_2 SHAKEN 13 DAYS
3. AS 2 + 50 P.P.M. B.P. INITIALLY AND AFTER 3 DAYS.



MONS 067768

FIG 19 DEGRADATION OF AROCLOR 1242 B(MCS 1016) BY CULTURE C_2

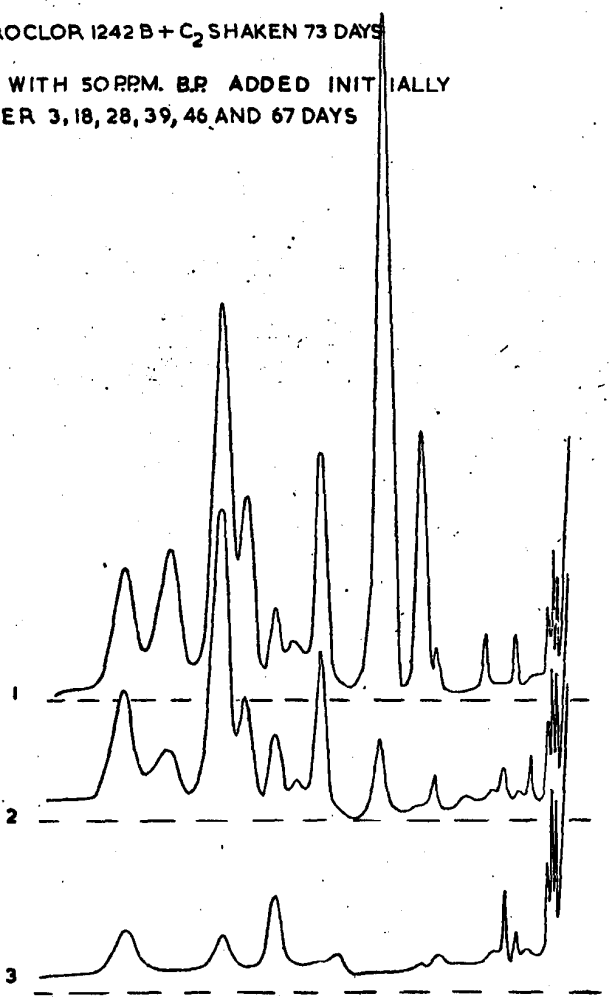
1. CONTROL Ic AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg^{++} ADDED
2. 5 P.P.M. AROCLOR 1242 B + C_2 SHAKEN 38 DAYS
3. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 3, 18 AND 28 DAYS



MONS 067769

FIG 20 DEGRADATION OF AROCLOR 1242 B (MCS 1016) BY CULTURE C_2

1. CONTROL ie. AS 2 BUT WITH 50 P.P.M. B.P. AND 100 P.P.M. Hg ADDED
2. 5 P.P.M. AROCLOR 1242 B + C_2 SHAKEN 73 DAYS
3. AS 2 BUT WITH 50 P.P.M. B.P. ADDED INITIALLY AND AFTER 3, 18, 28, 39, 46 AND 67 DAYS



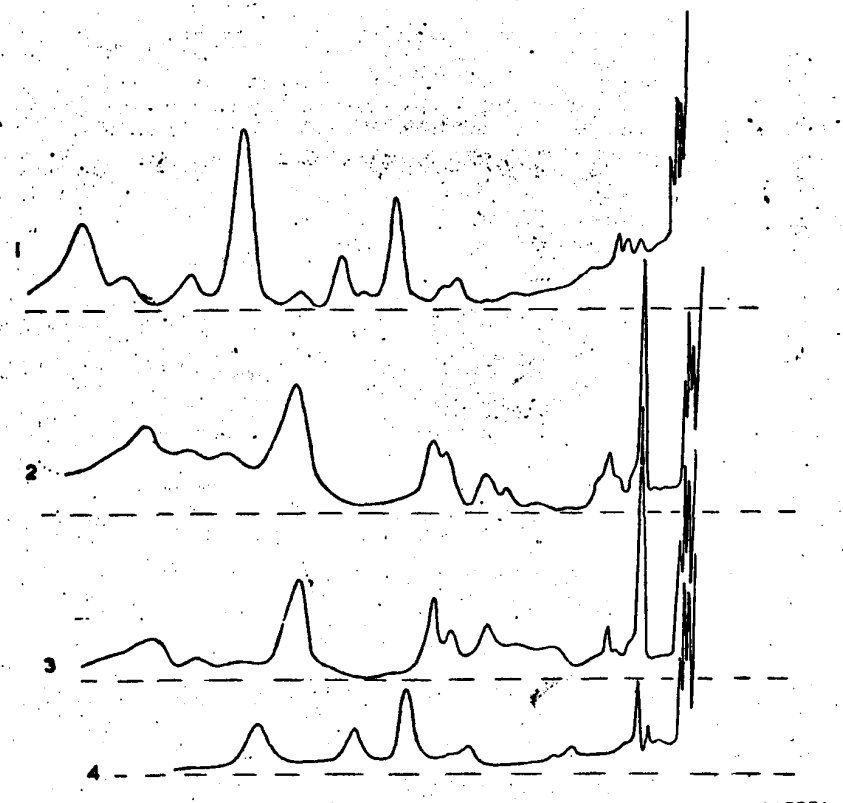
MONS 067770

FIG 21 COMPARISON OF THE DEGRADATION OF FOUR COMPLEX P.C.B. MIXTURES

1. AROCLOR 1242
2. MCS 762
3. MCS 717
4. AROCLOR 1242 B (MCS1016)

5 P.P.M. + C_2 SHAKEN 73 DAYS.

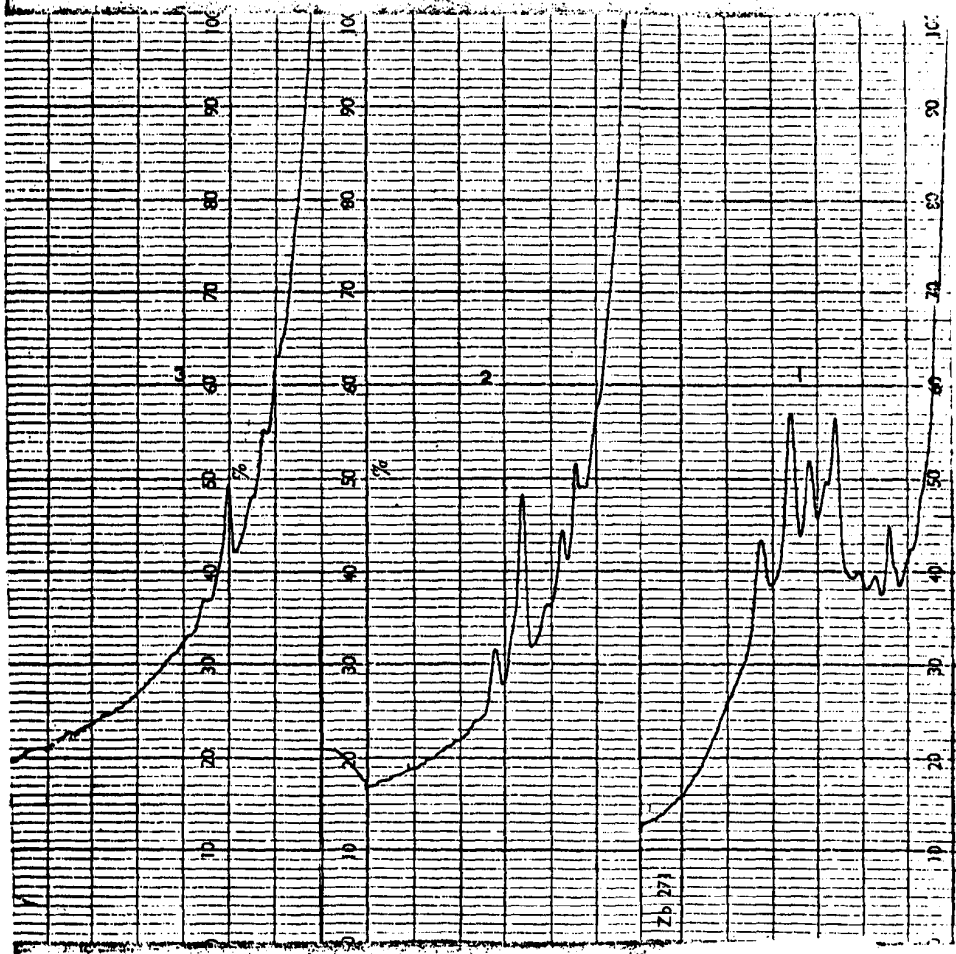
50 P.P.M. B.P ADDED INITIALLY AND AFTER 3, 8, 18, 28, 39 46 AND 67 DAYS



MONS 067771

FIG 22 DEGRADATION OF HB40 BY CULTURE C_2

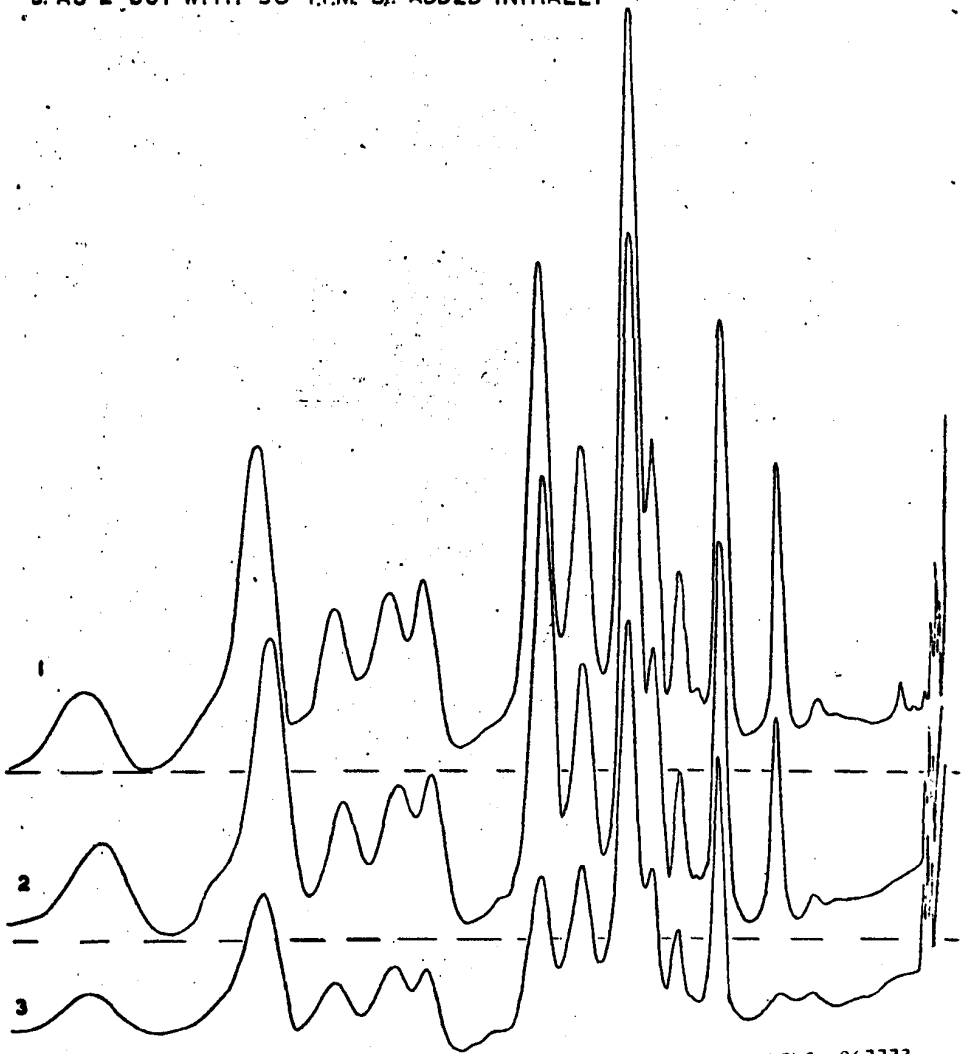
1. CONTROL i.e. AS 2 BUT WITH 50PPM BR AND 100PPM Hg ADDED.
2. 5PPM HB 40 + C_2 SHAKEN FOR 117 DAYS.
3. AS 2 BUT WITH 50 PPM BR ADDED INITIALLY.



MONS 067772

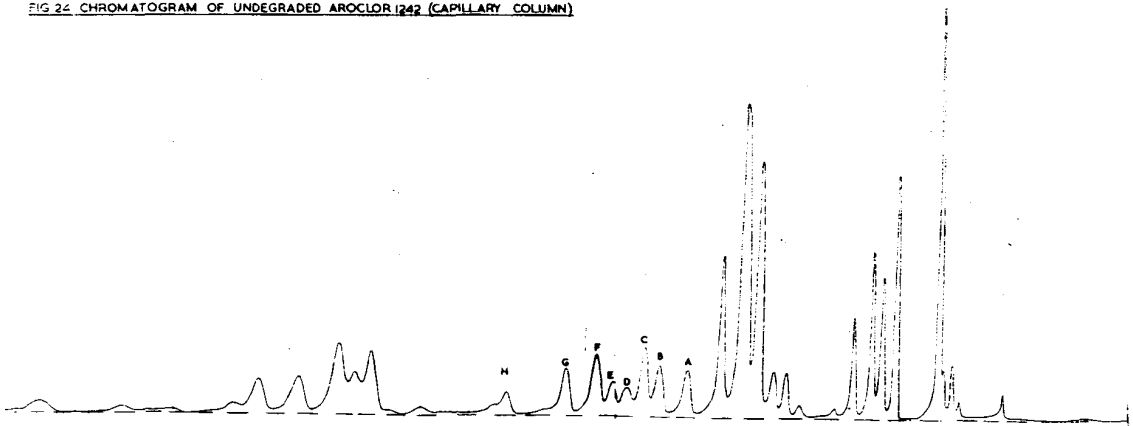
FIG 23 DEGRADATION OF AROCLOR 1242 BY CULTURE C_2

1. CONTROL ie. AS 2. BUT WITH 100 P.P.M. Hg ADDED
2. 5 P.P.M. AROCLOR 1242 + 100 ml RIVER WATER SHAKEN FOR 180 DAYS
3. AS 2 BUT WITH 50 P.P.M. BP ADDED INITIALLY



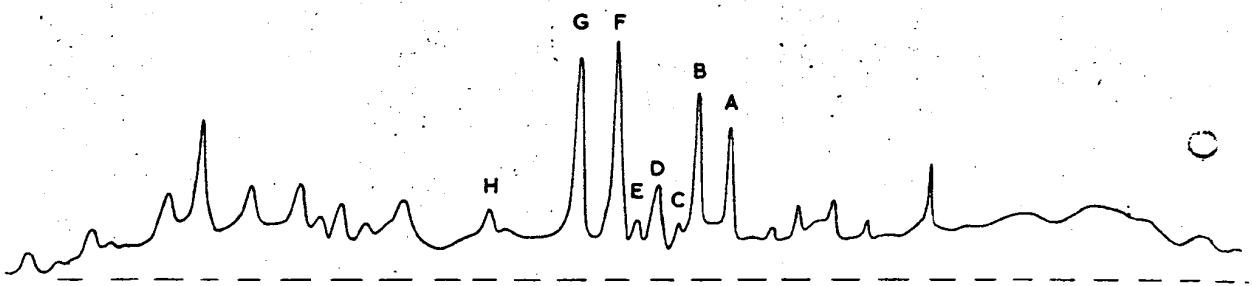
MONS 067773

FIG 24 CHROMATOGRAM OF UNDEGRADED AROCLOR 1242 (CAPILLARY COLUMN)



MONS 067774

FIG 25 CHROMATOGRAM OF DEGRADED AROCLOR 1242 (CAPILLARY COLUMN)



MONS 067775