Monsanto

(FROM (NAME & LOCATION) R.A. Lidgett - Ruabon

DATE : 27th October, 1969.

SUBJECT : BIODEGRADATION OF AROCLORS

REFERENCE : RAL/meh

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We are at present writing a detailed report on the work carried out to date on Aroclor 1242 biodegradation studies. This cannot be completed for a few more days so I have attempted to put the main observations and conclusions drawn so far into this memo. We will send the details later in the week.

Experimental Method

The samples of Aroclor 1242 have been prepared at a standard 5 ppm level and submitted to biodegradation by J.H. Mainprize in the Effluent Research Group. This has involved a survey to determine the most effective means of dosing aqueous samples with both Aroclor 1242 and active bacteria and of methods for extracting the aqueous samples after degradation to obtain the Aroclor 1242 in a form suitable for electron capture gas chromatography.

The following method has been established :-

A standard solution of 100 ppm Aroclor 1242 in ether is prepared. This is diluted to give 5, 2 and 1 ppm solutions for gas chromatography. Aqueous samples are prepared by taking a 5 ml aliquot of the standard solution, evaporating to dryness in the sample flask by evaporation at ambient temperature in a low air flow, then making up to 100 mls with nutrient salt solution, inoculum etc. The inoculum used had previously been shown to be capable of complete degradation of biphenyl. These solutions are then used for the biodegradation tests.

The aqueous samples are shaken in closed vessels in the dark for specific periods, the flasks being vented every 3 days to ensure presence of sufficient oxygen, and are then extracted in a separating funnel with heptane (4 x 25 ml portions) to provide a 100 ml heptane solution for gas chromatography.

Gas Chromatography

Conditions

| | | |
|---|---|---|
| Instrument | - | H P 402 equipped with $Ni^{63}$ high temperature electron capture detector. |
| Column | - | 4' 3% Silicone Oil OV101 on 80-100 mesh Chromosorb W H.P. (ex Supelco) |
| Flow | - | 40 mls/min. through column plus 60 mls/min. purge flow in detector. Both of Argon/Methane 95/5. |



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| | | |
|---|---|---|
| Oven Temp. | - | 170°C |
| Flash Heater | - | 250°C |
| Detector | - | 250°C |
| Sample Size | - | 4 µl. |
| Pulser | - | 150 m. sec. |

A calibration is prepared using a standard solution of Aroclor in heptane of 5, 2 and 1 ppm Aroclor 1242 plus an internal standard solution of 3.3 ppm 2 chlorobiphenyl (previously shown to be absent from the 1242 traces).

Results and Discussion

In this work each series of experiments has led to a modification of the programme and this has meant that there has been little opportunity to carry out repeats or duplicates of critical experiments. As the pattern emerges, this will be done, but at present it should be understood that interpretation of results may be based on a single series of experiments.

Results of the individual experiments are given in bar graph form on sheets attached to this memo and the following observations can be made for each series.

Figure No.1

From the bar chart it can be seen that :-

(a) Peak No.1 in the chromatogram is very easily lost, being reduced by 90% in three days and completely lost in the 8 day and all subsequent samples.

(b) Samples 2 and 3 (3 and 8 days) appear to have undergone more severe degradation than 4, 5 and 6 (13 and 25 days). This may be an experimental factor with the former samples losing 1242 by a non degradative path or by physical loss. A further explanation could be that samples 4,5 and 6 have not been as efficiently biodegraded as samples 2 and 3. At the present time we have taken results on samples 4,5 and 6 as being more probably correct.

(c) Results for sample (1) are within experimental error and the dosing/extraction/gas chromatographic methods are acceptable. The reduction in peak (1) is probably caused by evaporation loss in the dosing step.

From these results it is possible to infer that :-

(a) Loss of 1242 at the 5 ppm level can reach 40% of initial concentration for the majority of the measured peaks after 13 days. Further loss appears to be inhibited: this may be caused by poisoning of the bacteria by 1242 or its degradation products at the relatively high concentration of 1242 used.

(b) The first peak in the chromatogram is rapidly lost under the conditions of the experiment. Results given later with sterile samples indicate that this loss is mainly caused by biodegradation.

Figure No.2

To ensure that the observed disappearance of 1242 was caused by biodegradation and not other factors J.H.M. also ran a series of control samples which contained copper sulphate solution at a level known to sterilise the media. These samples gave the surprising result that the 1242 was still lost in significant amounts and this can be seen in the bar chart. This can be interpreted as follows :-

(a) The dosing/extraction/gas chromatographic methods have an acceptable accuracy (however, see discussion later concerning electron capture detection of p.c.b's.)

(b) In the presence of $CuSO_4$ (known to be a bacteriostat) Aroclor 1242 is lost by a chemical or catalytic process and this process is time dependent. It is thought to be non hydrolytic as a similar situation does not occur when the $CuSO_4$ is replaced by $HgCl_2$ (see later).

Figure No.3

The two 25 day samples have been compared here with a similar sample in which 10 ppm $CuSO_4$ has been used as a bacteriostat.

It is interesting to see that peak (1) is lost with the inoculated samples but in the presence of $CuSO_4$ the rate of loss is much less. This is almost certainly loss through chemical reaction in the sterile sample.

Figure No.4

As there appeared to be a possible chemical reaction between 1242 and $CuSO_4$ a series of flasks was prepared in which the sterilising agent was $HgCl_2$ and not $CuSO_4$.

From the bar chart it can be seen that :-

(a) When 5 ppm of 1242 (without additives and with ether removed) is shaken for 14 days there is a loss of approximately 20-30% of the original concentration added to the flask. Or, if the concentration present before shaking but after ether evaporation is used, there is a loss of approx. 10-15% caused by the shaking procedure.

(b) Samples 4 and 5 show that shaking 1242 (5 ppm) in a sterile aqueous medium for 14 days causes a loss of approximately 20% of the 1242. It is noticeable that in these samples, peak No.1 behaves in an equivalent manner to the later peaks, i.e. biodegradation is not occurring.

(c) Samples 5, 6 and 7 give equivalent results.

The results can be interpreted as follows :-

(a) Shaking Aroclor 1242 in a sterile aqueous medium causes a loss of 1242, but this is relatively small. The loss may be due to adsorption of the Aroclor on glass surfaces or from other as yet unknown causes.

(b) The $HgCl_2$ is acting as a bacteriostat at all levels used. This is important for future work where sterile controls must be used.

From the foregoing results, the following tentative conclusions may be made :-

(a) Loss of Aroclor 1242 on shaking for 14 days in a sterile medium ($HgCl_2$) is approximately 20 - 30% based on measurement of the major peaks in the chromatogram.

(b) Loss of Aroclor 1242 on shaking for 13 days (or longer) in an active medium causes a loss of approximately 40% for later peaks and 100% for an early peak which may or may not be composed of more than one p.c.b. (this is being examined).

(c) Control samples containing $CuSO_4$ (10 ppm or greater) as a bacteriostat lose Aroclor 1242 at a significant rate via an at present unknown process (possibly catalytic or direct chemical). This appears to cause an overall loss of material but there is evidence that production of other compounds also electron capturing may be occurring.

The above conclusions are based on the assumption that the 60 - 65% loss of Aroclor 1242 (samples 2 and 3 Figure 1) is an erroneous result, but this will be re-examined.

While the above tentative interpretations of the results can be made, there is an increasing need to treat the quantitative measurement of Aroclor chromatograms obtained with the electron capture detector with more caution. This was also expressed by Widmark at his last lecture and at the present time there is no experimental evidence to suggest that all the Aroclor components have the same or similar electron capturing powers (it is not known even whether p.c.b. isomers have similar responses to the e.c. detector). Thus the loss of one peak or the appearance of a second peak in the 1242 chromatograms discussed above, leads us to believe that this behaviour could well be occurring beneath the envelope of later peaks which we know to be composed of many components. The complexity has been shown by comparison of a normal packed column electron capture detector chromatogram of 1242, and a wide bore tubular column chromatogram of the same product obtained with a flame ionisation detector and copies of these chromatograms are included with this memo.

For this reason our future work on degradation will proceed along the following lines :-

(1) Submission of individual p.c.b.'s (supplied earlier by Applied Sciences Section) to biodegradation procedures and quantitative measurement of component loss.

(2) Development of a wide bore tubular column for quantitative analysis of Aroclor 1242. Such a column can be used without an injection splitter, it will provide high resolution chromatograms suitable for quantitative measurement.

R.A. LIDGETT

RAL/meh
28th October, 1969.

MONS 036036

# K E Y

Figure No.1

1. 5 ppm 1242 in ether added to flask. Ether removed by low air flow Inoculum plus water (10 ml) and minerals (90 mls.) added, total 100 mls. Immediately extracted with heptane (4 x 25 mls).

2. As 1 - extracted after 3 days.

3. " " " " 8 days.

4. " " " " 13 days.

5. " " " " 25 days.

6. Duplicate of 5.

Figure No.2

1. 5 ppm of 1242 in heptane.

2. 5 ppm 1242 in ether added to flask. Ether removed by low air flow. 100 mls. heptane added.

3. 5 ppm 1242 in ether added to flask. Ether removed by low air flow. Mineral soln. (90 mls), water (10 mls) plus $CuSO_4$ (10 ppm) added. Extracted immediately with 4 x 25 ml portions heptane.

4. As 3 extracted after 3 days.

5. " " " " 13 days.

Note: Peak No.4, sample No.5 was not measured because it was off scale under the attenuation conditions used in this particular g.c. run.

MONS 036037

KEY (Continued)

Figure No.3

1. 5 ppm 1242 in ether added to flask. Ether removed by low air flow then mineral solution (90 mls.), water (10 mls) and inoculum added. Flask shaken 25 days before extraction with heptane (4 x 25 ml).

2. As 1.

3. As 1 but with addition of $CuSO_4$ (10 ppm).

Samples 1 and 2 in this chart are the same as 5 and 6 in Figure 1.

Figure No.4

1. 5 ppm 1242 in 100 mls heptane.

2. 5 ppm 1242 in ether added to flask. Ether removed by low air flow. Made up to 100 mls. with heptane.

3. 5 ppm 1242 in ether added to flask. Ether removed by low air flow. Flask shaken (1242 only in flask) for 14 days. Extracted with heptane 100 mls.

4. 5 ppm 1242 in ether added to flask. Ether removed by low air flow. Minerals (90 ml), water (10 ml) and $HgCl_2$ (50 ppm) added. Solution extracted immediately with 4 x 25 ml. heptane.

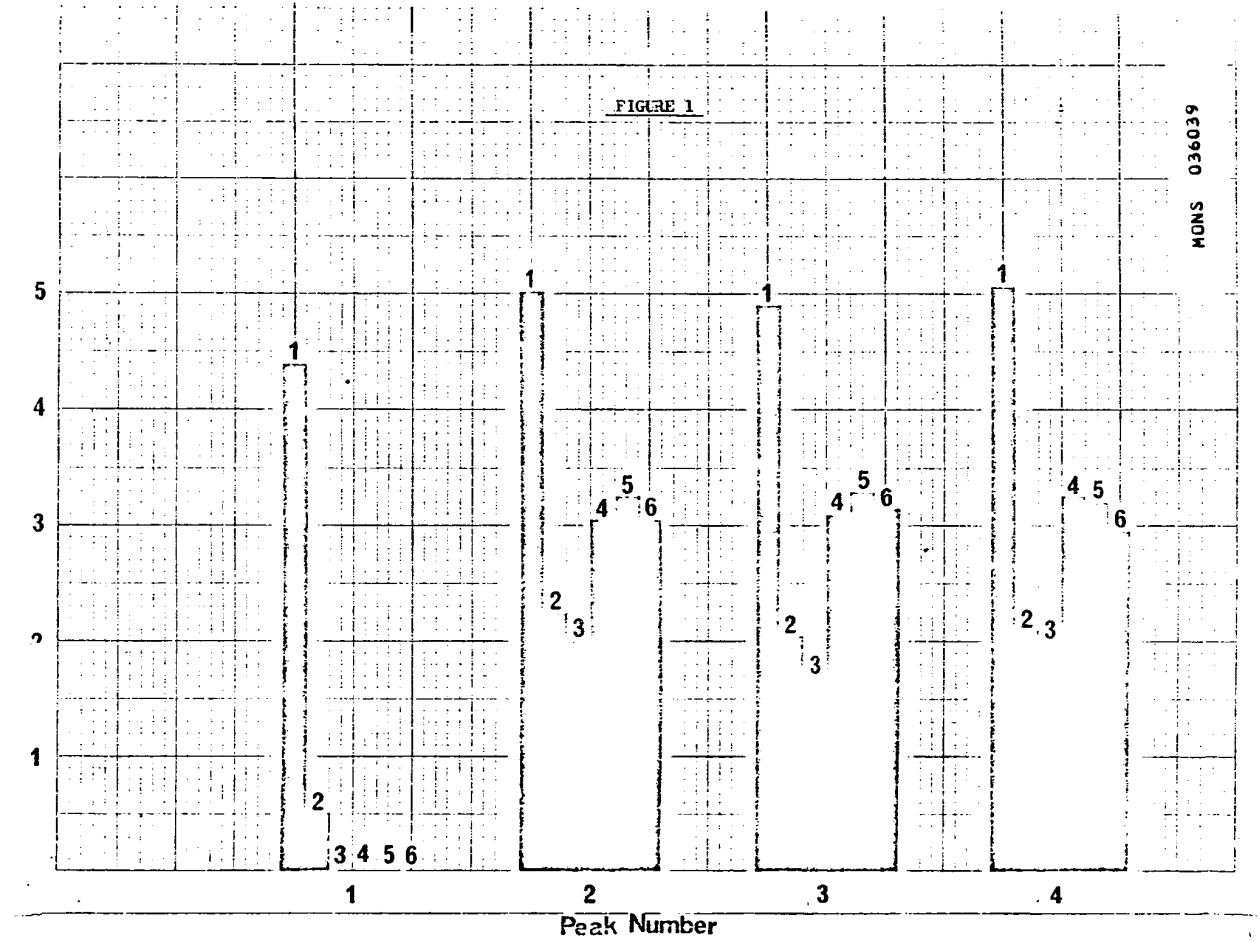
5. As 4 but shaken for 14 days before extraction with heptane (4 x 25 ml).

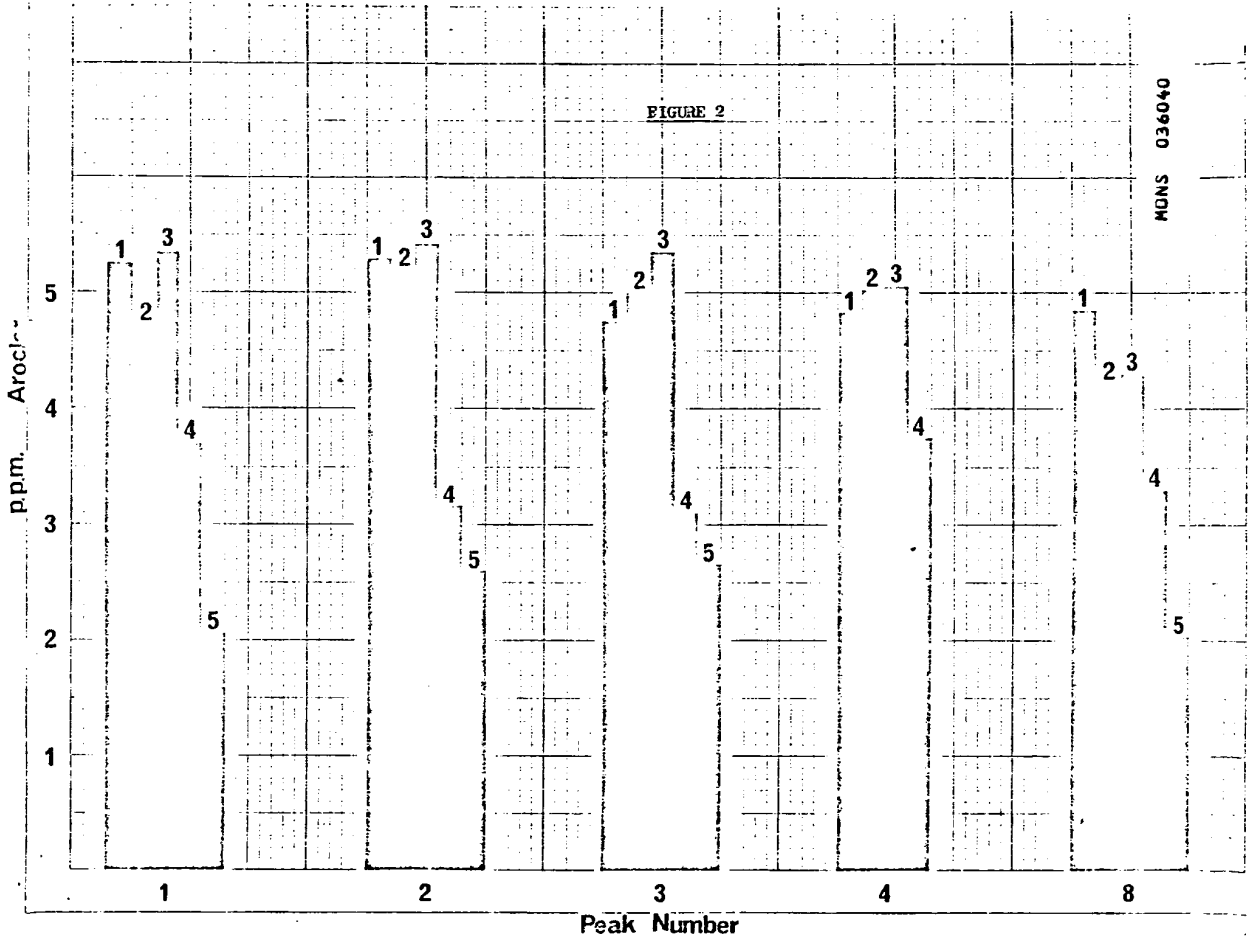
6. As 5 but 10 ppm $HgCl_2$ added in place of 50 ppm.

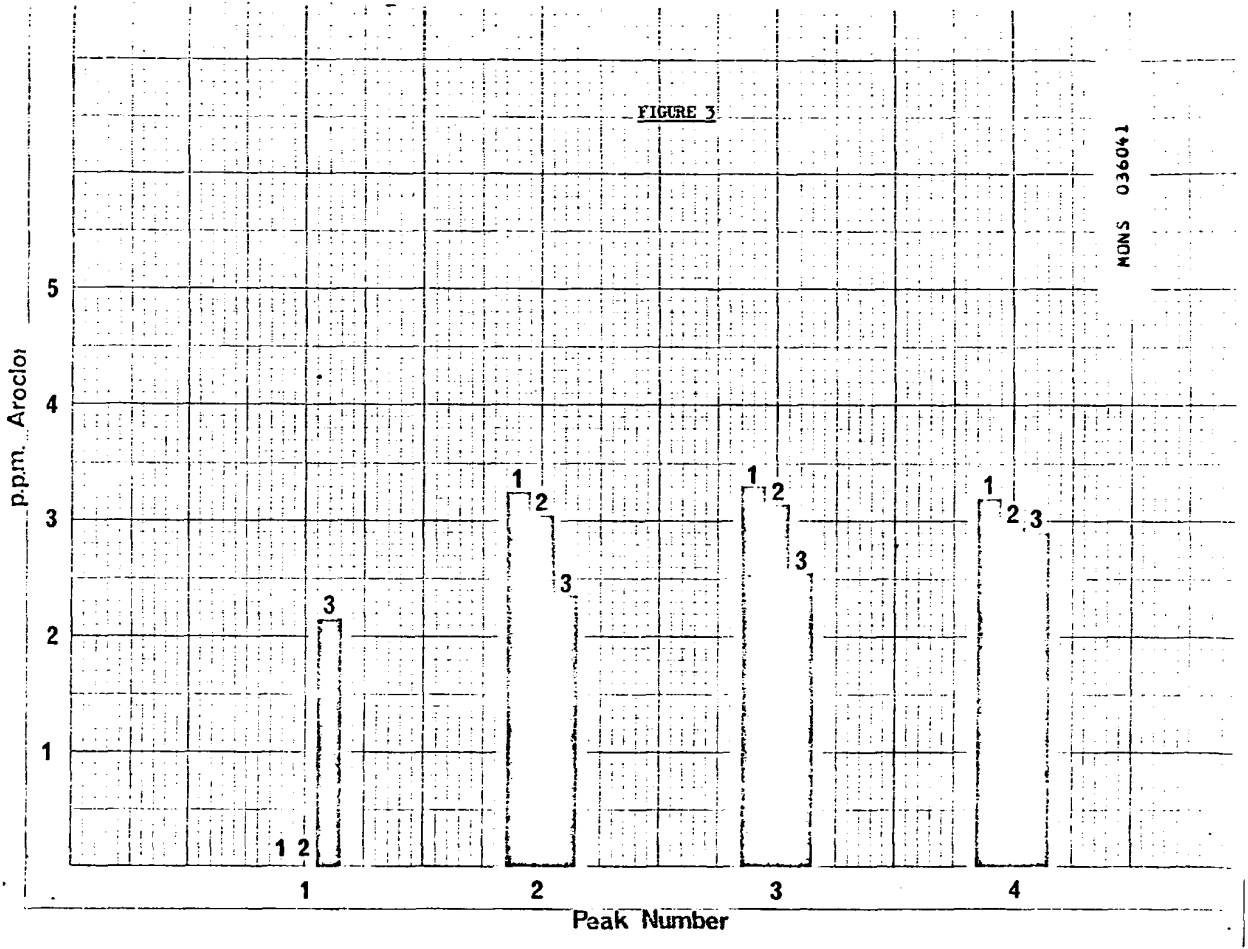
7. As 5 but 25 ppm " " " " " " "

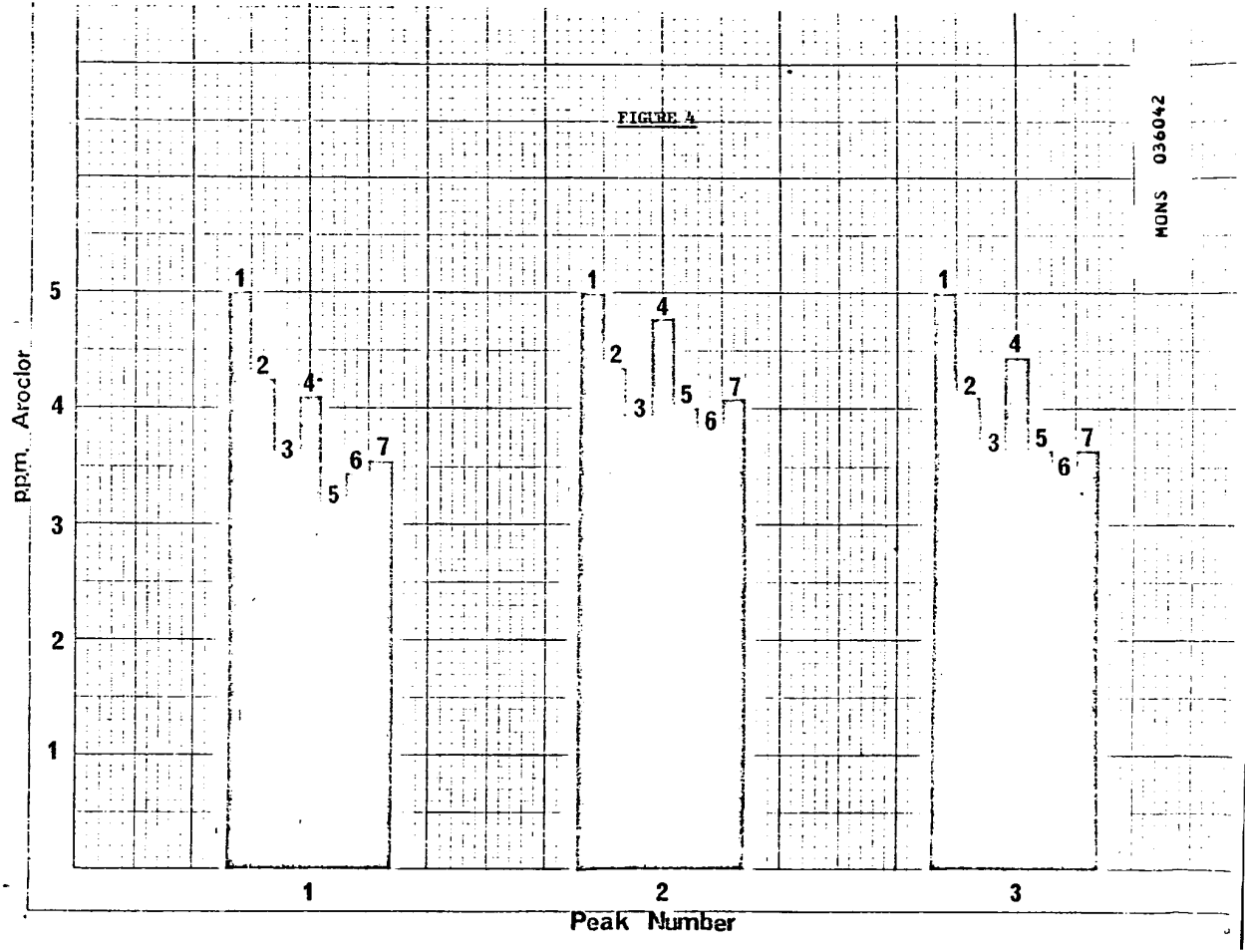
MONS 036038

FIGURE 1


MONS 036039
5
4
3
1
1
2
3 4 5 6
1
2
3
4 5 6
1
2
3
4 5 6
1
2 3
4 5 6
1
2
3
4
Peak Number


FIGURE 2
MONS 036040
p.p.m. Aroclor
5
4
3
2
1
1
2
3
4
8
Peak Number


FIGURE 3
MONS 036041
p.p.m. Aroclor
5
4
3
2
1
1 2
3
1
2
3
1
2
3
1
2
3
1
2
3
4
Peak Number


FIGURE 4
MONS 036042
ppm. Aroclor
5
4
3
2
1
1
2
3
4
5
6
7
1
2
3
4
5
6
7
1
2
3
4
5
6
7
1
2
3
Peak Number