ORGANOCHLORINE RESIDUES, OECD PRELIMINARY STUDY 1966-67

METHODS OF ANALYSIS, AND EXAMPLES OF CHROMATOGRAMS

The method of analysis used in the Swedish programme is described in brief below.

1. Extraction

Homogenates: The homogenate (5 to 10 grams) was mixed with sodium sulphate and then extracted with n-hexane in a Soxhlet extractor. After evaporation, the fatty residue was weighed.

Individual muscles: A piece of muscle (sample size one gram) was placed in a test tube (8 ml) together with one ml of methanol and four mls of n-hexane. By means of a left-handed bore fixed on to a dental boring machine, the whole mixture was homogenized and centrifuged. The solvent was pipetted off and the procedure repeated. The hexane was evaporated in a stream of nitrogen at 40°C.

(All solvents used were checked to give acceptable blanks on EC).

2. Clean-up

The fatty residue was dissolved in methylene chloride so that 100 ul contained 10 mg of fat. 100 ul of this solution was applied as a band on a small thin layer plate (7,5 x 3,5 cm, 1 mm layer of silicagel). Methylene chloride was used as mobile phase and the layer above the fat front was scraped off and extracted in a short glass column with a mixture of n-hexane and diethyl ether 60-40 (v/v). Exactly one ml eluate was collected.

3. Gas-Liquid Chromatography

For quantitative analysis, 5 to 10 ul of the eluate was injected. Silanized glass columns were used throughout; length 180 cm, inner diameter 1,8 mm. Packing a) Gaschrom P AW/DMCS 100-120 mesh; sedimented in metanol in order to remove dust followed by application of the stationary phase, 4 % SF 96 (methyl silicone)

b) As above but stationary phase 8 % QF-1 (fluoro silicone).

4. Operating conditions for GLC

Instrument: Varian Aerograph 204

Temperatures: Injector: 207°C

Columns: (a) 201°C, (b) 182°C

Detector: 210°C

Gas flow: Nitrogen adjusted to give pp-DDT a retention time of exactly 20,0 min.

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Recorder: Speedomax H 1 mv, chart speed 15"/h
Detector: Electron-capture, tritium
Confirmations: After injection of two samples was injected a standard containing lindane, BHC, aldrin pp-DDE, dieldrin, pp-DDD and pp-DDT. The checking should give correct peak composition and rf values on both column (a) and column (b). Also a PCB standard was injected twice a day.

Further confirmation

Nitration method: A test tube containing an aliquot of the evaporated eluate, as above, was treated with a mixture of conc. sulphuric acid and conc. nitric acid (1:1, v/v) for 5 minutes at 0°C. After addition of crushed ice the mixture was extracted with n-hexane. 5 to 10 ul of the extract was injected into the GLC. - This method will leave the PCB, lindane and BHC unaffected.

Sulphuric acid method: The n-hexane extract of the one gram sample of tissue was concentrated to one ml, to which was added an equal volume of 90 % sulphuric acid. After shaking for several minutes and centrifuging at high speed the sulphuric acid phase was frozen, and the hexane layer decanted off and washed with water. After repeated freezing and decanting, the solution was injected into the GLC.

The sulphuric acid method is quantitative and rapid, but removes oxygen-containing compounds, including dieldrin, endrin and heptachlorepoxide. The method is especially suitable for the detection of small amounts of the volatile compounds, e.g. aldrin, lindane and BHC, since no steps of evaporation are applied. The sensitivity is as high as 0,001 ppm.

Discussion

It is usually assumed that pp-DDD and dieldrin are not present in Swedish samples. However, it is very difficult to state the absence of these compounds due to the interference of some of the PCB peaks. On SF-96 columns, the peaks of pp-DDD and dieldrin are covered by those of PCB no 7 and DDE, and in the case of QF-1 the interference is caused by the PCB-peaks nos 8 and 10.

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Relative retention:
(DDE, most often present in the samples, is taken as 1,0).

QF-1	SF 96	
0,35	0,22	Lindan
0,46	0,53	Aldrin
1,0	1,0	pp-DDE
1,48	1	Dieldrin
1,83	1,31	pp-DDD
2,0	1,79	pp-DDT
0,36	0,21	1
0,65	0,68	2
0,79	0,82	3
0,98	0,97	4
1,07	1,04	5
1,14	1,18	6
1,32	1,27	7
1,40	1,53	8
1,55	1,67	9
1,77	1,83	10
2,15	2,18	11
2,78	2,68	12
3,01	3,14	13
3,50	3,72	14

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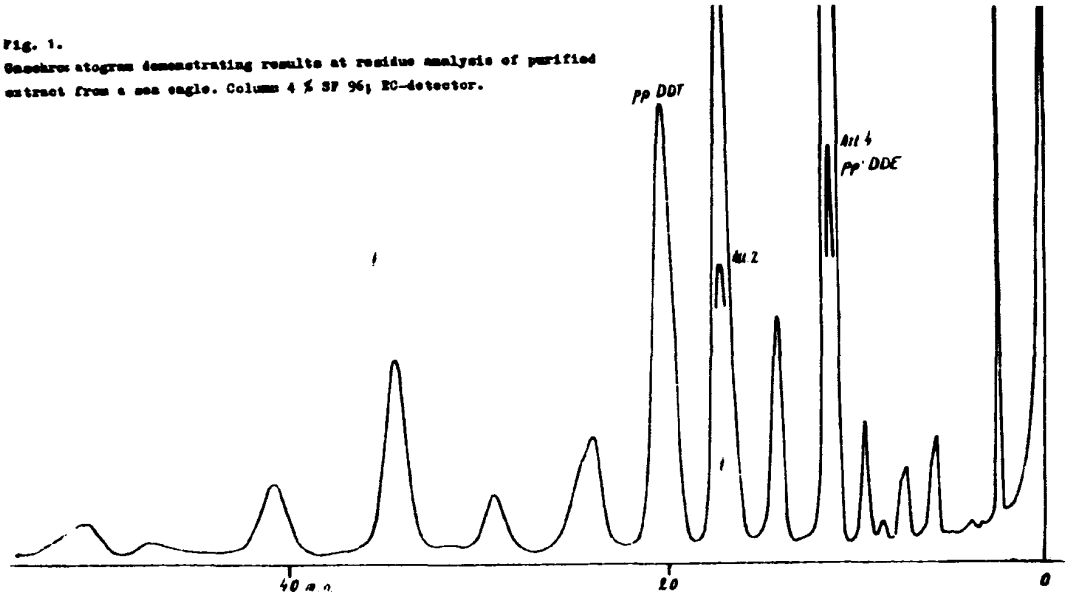
Fig. 1.
Gaschromatogram demonstrating results at residue analysis of purified extract from a sea eagle. Column 4 % SF 96; EC-detector.



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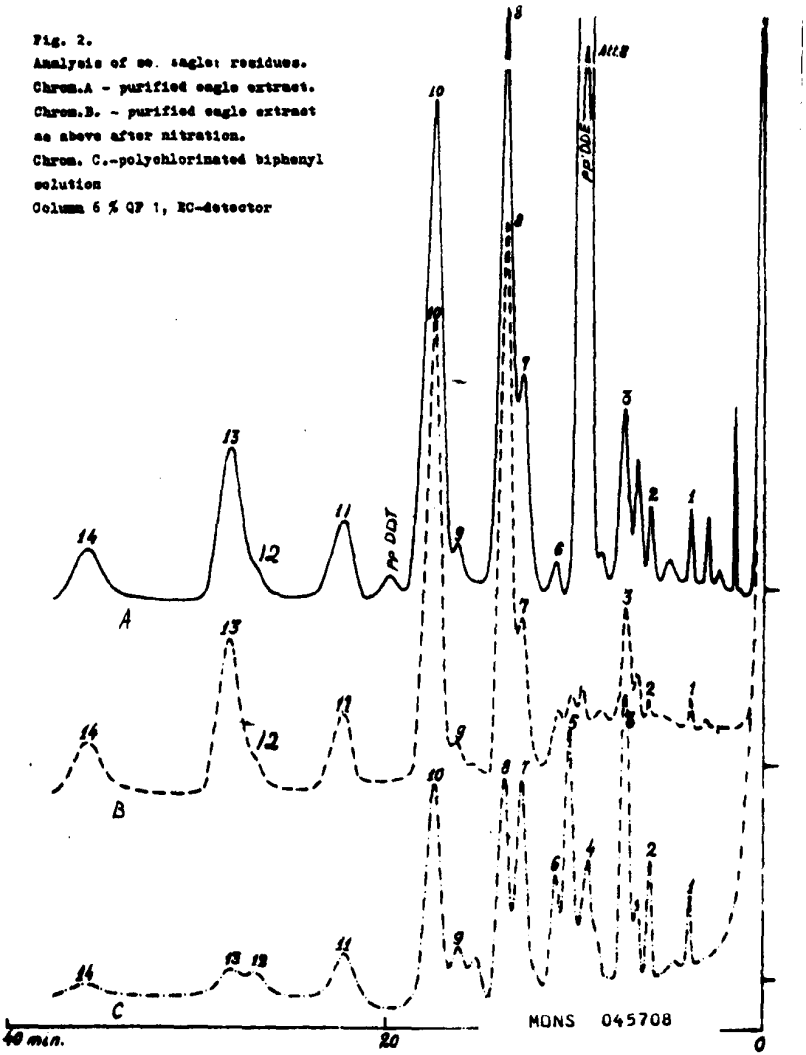

Fig. 2.
Analysis of se. eagle: residues.
Chrom.A - purified eagle extract.
Chrom.B. - purified eagle extract as above after nitration.
Chrom. C.-polychlorinated biphenyl solution
Column 6 % QF 1, EC-detector

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Fig. 3.
Analysis of standard solutions on 4 % SF 96 column, EC-detector.
Chrom.A. - Mixture of chlorinated insecticides and polychlorinated biphenyls
Chrom.B. - The same mixture as in A, except lindane, after nitration.
Chrom.C. - Pure polychlorinated biphenyl standard.
Chrom.D. - Mixture of the same chlorinated insecticides as shown in chrom.A. after nitration.

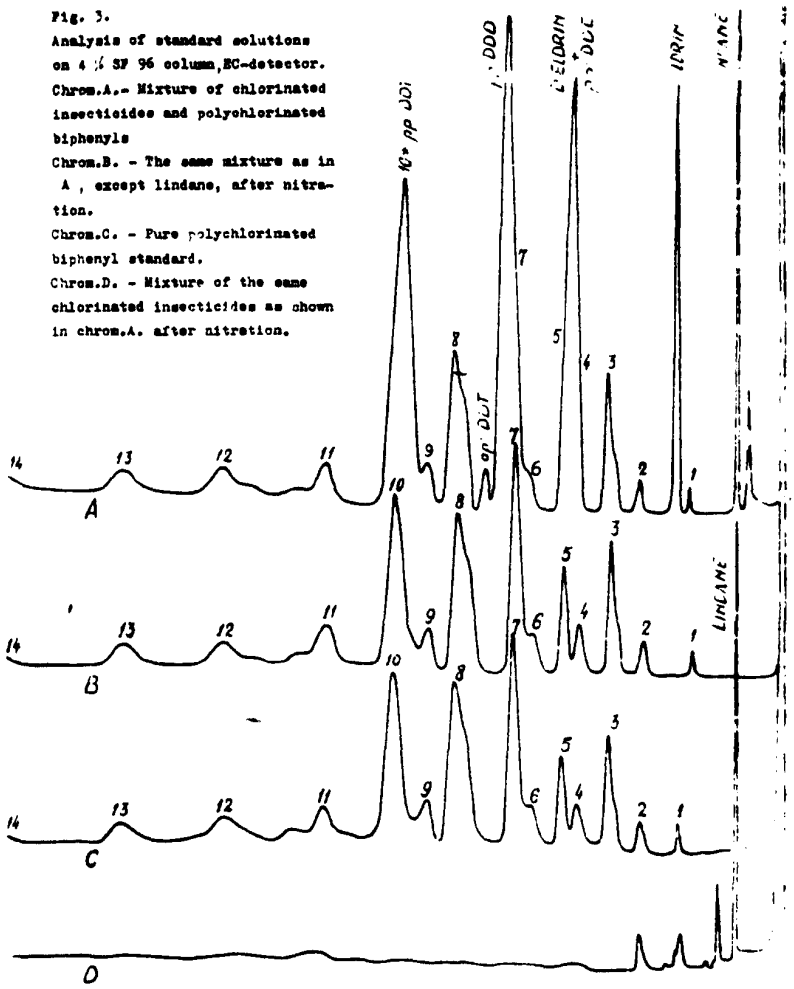

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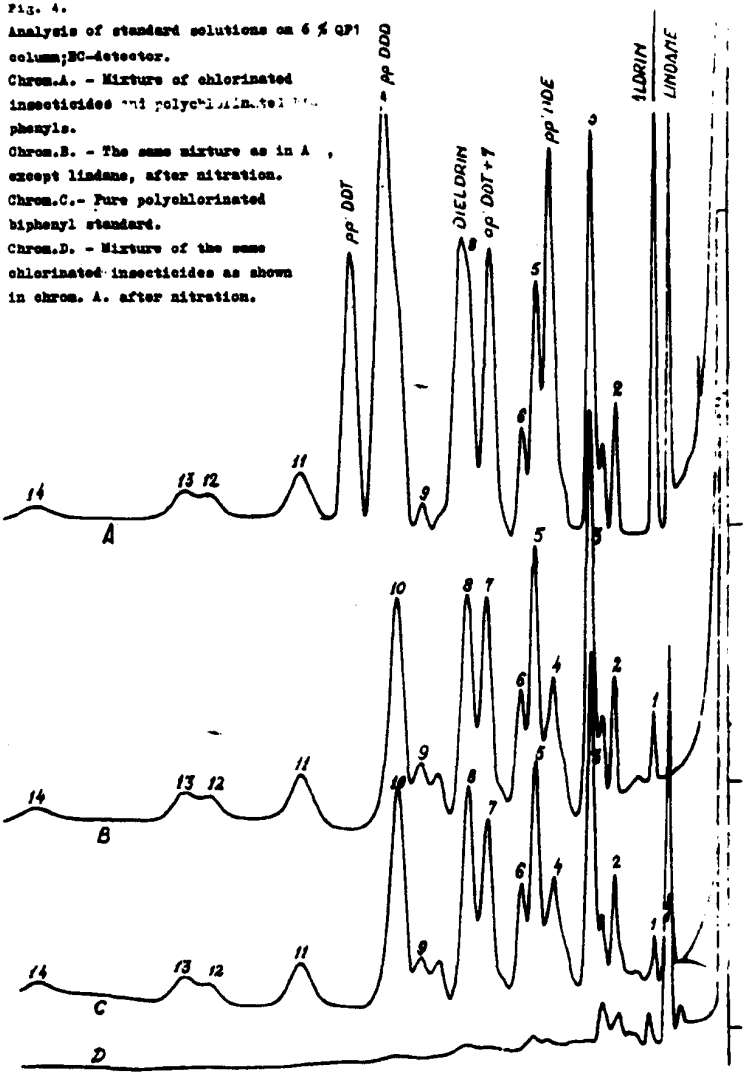
Fig. 4.
Analysis of standard solutions on 6 % QF1 column; EC-detector.
Chrom.A. - Mixture of chlorinated insecticides and polychlorinated biphenyls.
Chrom.B. - The same mixture as in A, except lindane, after nitration.
Chrom.C.- Pure polychlorinated biphenyl standard.
Chrom.D. - Mixture of the same chlorinated insecticides as shown in chrom. A. after nitration.



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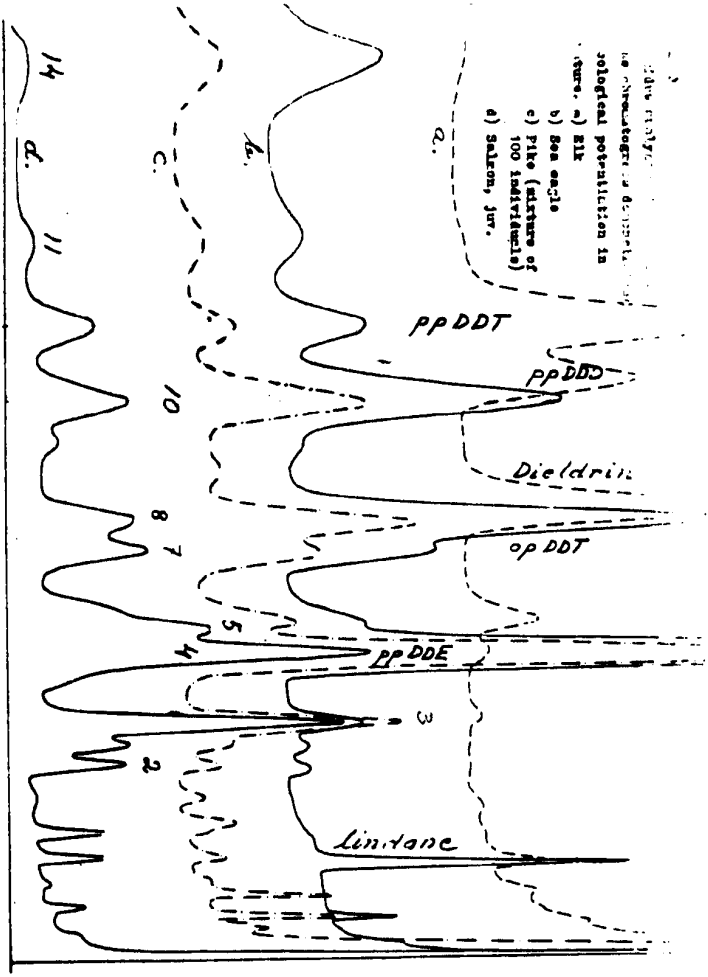

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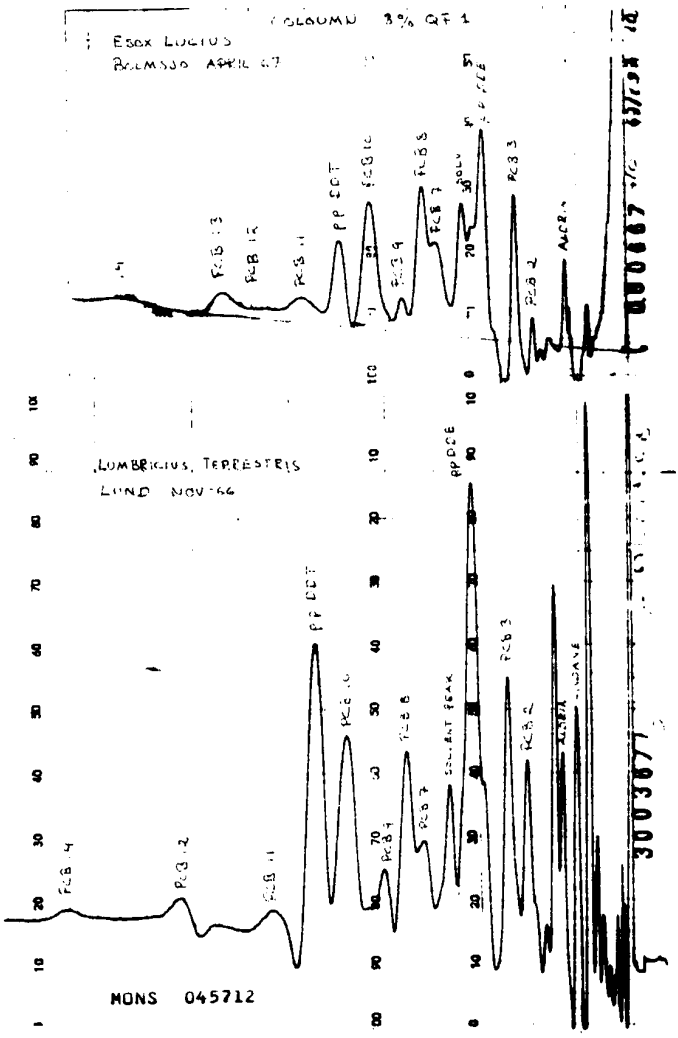

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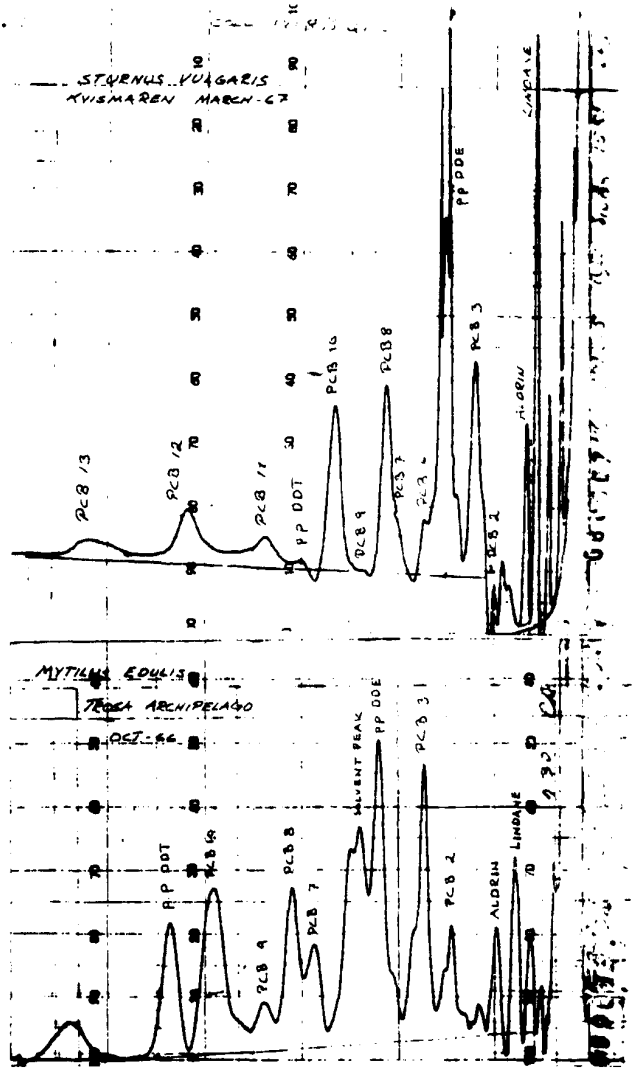

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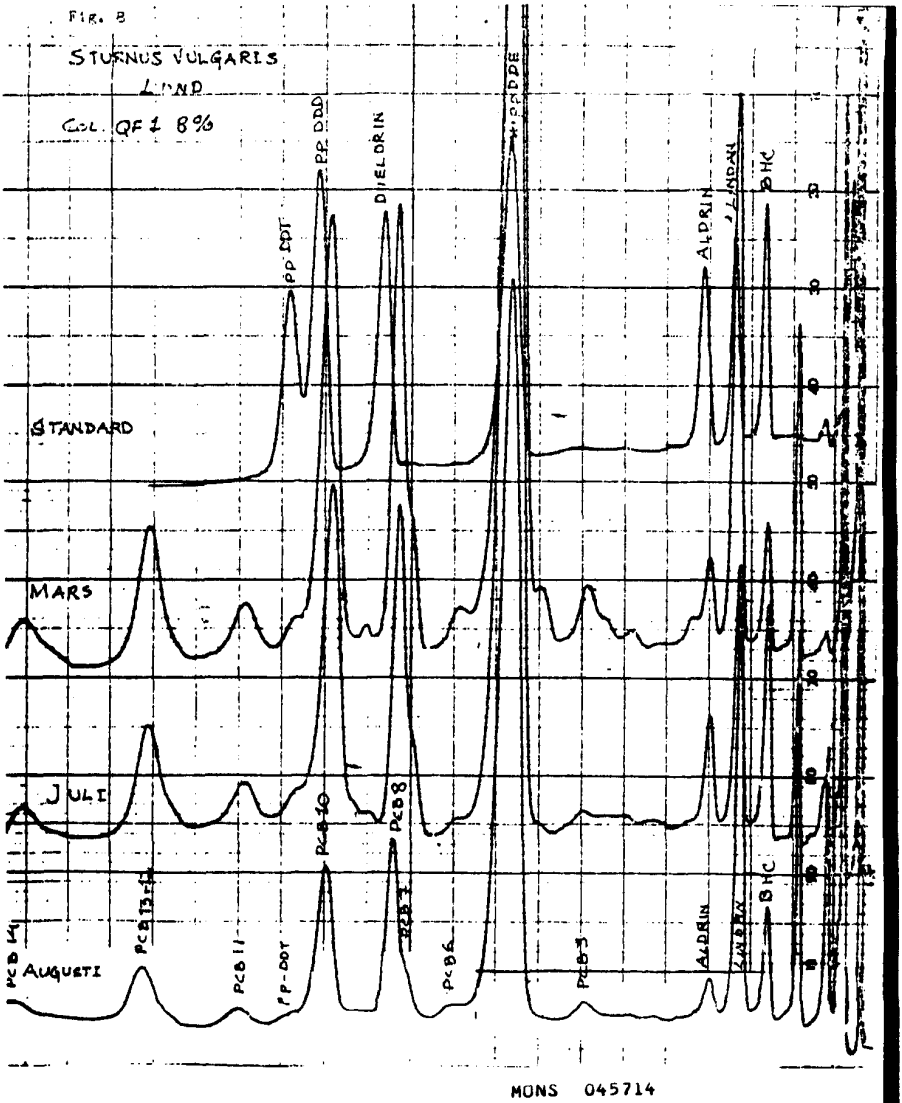

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