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CANADIAN WILDLIFE SERVICE

MANUSCRIPT REPORTS

ANALYTICAL REFERENCE MATERIALS: ORGANOCHLORINE RESIDUES IN CWS-79-1, A HERRING GULL EGG POOL FROM LAKE ERIE, 1979

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Analytical Reference Materials: Organochlorine Residues in CWS-79-1, a Herring Gull Egg Pool from Lake Erie, 1979

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ABSTRACT

A 7.3 kg pool of Lake Erie Herring Gull Eggs was made and subdivided into 10 g portions in glass and plastic vials to serve as secondary reference samples for determination of organochlorine and heavy metal residues in Great Lakes samples. The levels of 11 organochlorine compounds in this material (CWS-79-1) were determined by glass capillary GC-EC and packed column GC-EC. Results from the two procedures were comparable except for cis-chlordane and cis-nonachlor, for which capillary GC was required. The relative SD of mean residue levels was <5% (n=12) for most residues, indicating that the material was homogeneous and suitable for use as a secondary reference material.

RESUME

Des oeufs de goéland argenté du lac Erié ont été regroupés en une masse commune de 7.3 kg, par la suite subdivisée en portions de 10 g dans des contenants de verre et de plastique. Elles serviront d'échantillons de référence secondaire pour la détermination des résidus d'organochlorés et de métaux lourds dans les échantillons des Grands Lacs. Les concentrations de 11 composés organochlorés ont été déterminées dans ce matériel (CWS-79-1) par GC-EC capillaire sur verre et par colonnes GC-EC. Les résultats obtenus par ces deux procédés étaient comparables, sauf pour le cis-chlordane et le cis-nonachlore, lesquels exigent une GC capillaire. L'écart-type relatif à la moyenne des concentrations de résidus était <5% (n=12) pour la plupart des résidus, indiquant que le matériel était homogène et convenable en vue de son utilisation comme matériel de référence secondaire.

INTRODUCTION

Analytical reference materials are an essential part of a good quality control program in chemical analysis (Amore 1979). They allow the analyst to check both precision (reproducibility) and accuracy of analytical data. If the substrate type is the same as that of the samples to be analyzed, and not easily distinguished from them, the reference material may also be used as a "blind" check on the quality of analysis.

Certified reference materials are available from the U.S. National Bureau of Standards for analysis of metals in a variety of (dry) substrates. Such primary reference materials do not currently exist for analysis of organic substances in environmental samples, although a fish oil standard is under development at the NRC Atlantic Regional Laboratory, Halifax, N.S. These materials could not, in any case, be used as reference materials for whole tissue substrates. It is therefore necessary to prepare a secondary reference material and analyze it rigorously to establish the homogeneity of the material and the concentration of the compounds of interest.

One of the major analytical programs in CWS is the analysis under contract of organochlorine, mercury and lead residues in Herring Gull eggs from the Great Lakes as part of the IJC Wildlife Surveillance Program. There existed an urgent need for a reference material for the Program. Ideally, the reference samples would serve as blind checks of both accuracy and precision of analysis for each batch of samples

submitted to the contractor(s). In the case of organochlorine compounds, spiked samples would not serve this purpose since the complex nature of known and unknown residue patterns (particularly for PCBs) in "real" samples is easily distinguished from spiked samples.

A Herring Gull colony in Lake Erie was being destroyed by an airport runway extension in 1979. This colony therefore provided the opportunity to collect sufficient naturally contaminated eggs to serve as a reference material. This report details the characterization of the organochlorine residues in 12 subsamples taken from various portions of a 7.3 kg homogenate of these eggs.

METHODS

Preparation and Pooling of Eggs

A total of 84 Herring Gull eggs obtained fresh from Big Chick Island and Middle Sister Islands in Lake Erie in 1979 were used for the egg pool. Four to five eggs were homogenized (without shells) in a Sunbeam blender. Each homogenate was poured into a five gallon glass jar. After addition of all the eggs (7.3 kg), the jar was rolled and shaken thoroughly to mix the contents, and frozen before the next step.

The egg pool in the glass jar was rehomogenized with the Sunbeam blender after thawing out at room temperature. Approximately

300-400 g of egg was homogenized in each batch and the homogenate poured into two smaller glass jars. When the original jar was empty, the homogenate in the two smaller jars was returned to it. The egg pool in the large glass jar was then mixed well by shaking. The egg pool was divided into 34 sub-pools in glass jars which were numbered and put into the freezer for storage prior to further subsampling. The contents in these jars were thawed out, rehomogenized with the Sunbeam blender and 10 g aliquots poured into 20 ml glass scintillation vials with aluminum foil lined lids and 20 ml plastic (linear polyethylene) scintillation vials. Approximately 3/4 of each pool was put into glass (organic analysis) vials and 1/4 into polyethylene (metals analysis) vials. All the vials from a sub-pool were identified by the sub-pool number. In total, 571 glass vials and 155 plastic vials were prepared. The vials were stored in the walk-in freezer at -40°C.

The glass scintillation vials, available from Fisher Scientific, Cat. No. 3-337-15, were washed with detergent, rinsed with hot water, three times with acetone, three times with hexane and air-dried before being used. The plastic (linear polyethylene) scintillation vials, available from Fisher Scientific, Cat. No. 3-337-12, were cleaned by filling with nitric acid (20%, v/v), installing caps, turning over and letting sit overnight. The caps and vials were rinsed with deionized, distilled water the next morning and air-dried before being used.

Sample Analysis

One vial from every third homogenate was taken for analysis. Approximately 6 g of egg homogenate was ground with excess sodium sulphate (30 g) and extracted with ca. 350 ml hexane. The extract was evaporated and made up to 30 ml in a graduated cylinder.

Two x 2.5 ml of the 30 ml final volume extract were pipetted for lipid determination. The percent water of the egg homogenate was not determined.

The remaining extract (25 ml) was concentrated to ca. 5 ml and placed on a glass column (2.1 cm I.D. x 30 cm long) containing 35 g of Florisil PR (deactivated with 1.2% water), topped with one cm of Na_2SO_4 and then prewet with hexane. The 35 g Florisil makes a 15 cm packing height on the 2.1 cm I.D. glass column. The extract was eluted with 150 ml hexane (fraction 1) followed by 150 ml of 15% CH_2Cl_2/hexane (v/v) (fraction 2) and 200 ml of 50% CH_2Cl_2/hexane (v/v) (fraction 3). The solvents in the fractions were rotary evaporated to a small volume (but never to dryness) and then made up to a suitable volume for analysis of OCs by EC/GC. A portion of the first fraction was nitrated in order to determine mirex and photomirex without interference of PCB (Norstrom et al. 1980).

All compounds were determined on a Hewlett-Packard 5840A Ni-63 EC/GC with a 30.5 m x 0.26 mm I.D. OV-17 WCOT glass capillary column (#79-185) from Canadian Capillary. This column had 70,000 plates at K=6. Volume of injection for all analyses was 3 µl.

Samples (in hexane) were injected in the splitless mode using a 7671A automatic liquid sampler. See Fig. 1 and Fig. 6 for the GC conditions employed.

In the capillary GC analyses, compounds in the 1st, nitrated 1st and 3rd fractions were calculated by using area response factors. Compounds in the 2nd fraction were calculated by using peak height response factors.

Three methods were used for quantitating PCBs in the capillary GC analyses. The first, indicated as Aroclor 1254 in Table 1, was calculated from area response factors with standard Aroclor 1254 using Peak I (Fig. 2-5). The second, indicated as Aroclor 1254/1260 (1:1) in Table 1, was calculated from area response factors with standard Aroclor 1254/1260 (1:1) using Peak II (Fig. 2-5). The third, indicated as Aroclor 1260 in Table 1, was calculated from area response factors with standard Aroclor 1254/1260 (1:1) using Peak III (Fig. 2-5). The response of this peak in the (1:1) standard was corrected for a small contribution (5% due to Aroclor 1254 and half the nominal weight of total Aroclors was used in the calculations.

Organochlorine residues in egg pool no. 10 were also analyzed by packed column GC on an HP-5730A Gas Chromatograph with EC detector. The 1st fraction was analyzed using a six foot glass column packed with 1% SP2100 coated onto Supelcoport 100/120 mesh. The carrier gas flow on this column was 40 ml/min of 5% methane, 95% argon. The 2nd, 3rd and nitrated 1st fraction were analyzed using a six foot glass column packed with 6 inches of 1% SP2100/2%

AN600 coated onto Supelcoport 100/120 mesh (in the exit end); the remaining portion of the column was packed with 1% SP2100/2% SP2401 coated onto Supelcoport 100/120 mesh. The carrier gas flow in this column was 30 ml/min of 5% methane, 95% argon. The GC conditions were:

Injection port temperature: 250°

Oven temperature: 180°

Detector temperature: 300°

The results of these analyses are given in parentheses in Table 2. Also, fraction 1 of all samples was analyzed for p,p'-DDE and PCB 1254/1260 (1:1) by packed column. The results for p,p'-DDE and PCB 1:1 are given in Table 1 in the last two columns.

Results and Discussion

Tables 1 and 2 summarize the analytical results as ppm residues on wet weight of egg homogenate basis.

In the capillary GC analyses, three values for PCBs are given: they are Aroclor 1254, Aroclor 1254/1260 (1:1) and Aroclor 1260 in the first three columns in Table 1. The results of the Aroclor 1254/1260 (1:1) are calculated by using only one peak instead of the average of two different peaks as used in the packed column analysis. The results for Aroclor 1254/1260 (1:1) in the packed column (1% SP2100) were calculated by the average of the 141 and 166 peaks (Reynolds and Cooper 1979) using factors from

the Aroclor 1254/1260 (1:1) standard. This is a standard ORF method and can be used for direct comparison with the results from this contract laboratory. The mean value for Aroclor 1254/1260 (1:1) of 95.9 ± 3.7 ppm by packed column chromatography compares well with the mean value of 94.4 ± 6.2 by capillary column chromatography. The higher SDs of the capillary column results in this instance are due to the measurement of one peak (II) in an incompletely separated group of peaks. The packed column results based on peak 166 (Reynolds and Cooper 1975) are a measure of this whole group of peaks, which are completely unresolved. The weight percentage of the peak(s) used for calculation is approximately the same in both Aroclor 1254 and Aroclor 1260, which can be seen by the constant height of Peak II in Fig. 3-5. These chromatograms represent equal amounts of Aroclor 1254, Aroclor 1260 and the 1:1 mixture of the two, respectively. Since Aroclors 1254 and 1260 are the only mixtures which contain significant amounts of Peak II, fairly large variations in the relative amounts of these two Aroclors can be tolerated without affecting the "weight of PCB" calculated from this peak (or peak 166 in the case of the packed column results). This value is therefore a relatively good representation of the combined weight of Aroclor 1254 + Aroclor 1260, regardless of the ratio.

The PCB results calculated as Aroclor 1260 are based on a major peak (III) in this mixture which is present in much smaller relative amounts in Aroclor 1254. The mean value of 37.0 ± 2.9 ppm Aroclor 1260 represents 39% of the combined 1254/1260 value of

94.4 ppm based on Peak II. Taken together, these results imply that the ratio of Aroclors 1254/1260 was 1.56:1. A similar calculation based on Peak I, which is present to a significant extent only in Aroclor 1254, gives an estimated level of 28.4 ± 1.1 ppm of this Aroclor. The sum of the two individual Aroclor calculations gives 65.4 ppm, or approximately 69% of the estimate of combined Aroclors based on Peak II. It is probable that Peak I is metabolized by the gull somewhat more rapidly than the other two peaks and is therefore an underestimate of the amount of Aroclor 1254.

None of the methods of calculating PCBs in organisms such as Herring Gulls is more than a crude estimate since the composition of the mixtures stored in the tissues, including eggs, has been altered considerably from that of the standard Aroclors by metabolism. It has been shown, however, that the composition of the PCBs in the fish which are eaten by the gulls in the Great Lakes is relatively unaltered from standard Aroclor 1254 and 1260 (Norstrom et al. 1978). The methods of calculation presented in Table 1 are therefore reasonably good representations of the exposure of the Herring Gull to these PCB mixtures. The actual "weight of PCB" present in Herring Gull eggs calculated as a mixture of Aroclors 1254 and 1260 is overestimated by at least 25%, and possibly 50%. This estimate is based on a study of total chlorine in Herring Gull eggs in Lake Ontario in 1977 by Hall Detector and Neutron Activation Analysis (absolute methods) compared to the sum of compounds estimated by EC/GC (Norstrom et al., in preparation).

Total PCB concentration can also be estimated by perchlorination of the sample and measurement of the resulting decachlorobiphenyl, however this number can represent anything from a single PCB isomer to the whole range of possible isomers. Such total PCB values have no intrinsic advantage in interpreting the data since toxicity, rates of metabolism, etc., are extremely variable within this family of compounds. Ideally, all major PCB compounds should be determined directly, but in the absence of standards and adequate methodology to enable this to be done, the methods based on Aroclor 1254/1260 mixtures have the advantage of giving consistent results and allowing some definition of the type and ratio of the PCB mixtures from whence the measured compounds arose.

From Table 1, the mean value for p,p'-DDE by packed column GC is 4.85 ± 0.19, compared to 3.99 ± 0.20 by capillary GC. The 18% higher result by packed column is probably due to interference from PCB peaks. Lake Erie samples represent the worst possible situation in this regard in the Great Lakes since the DDE/PCB ratio is lowest in this lake and potential interference from PCB is the highest.

Table 2 summarizes the results for the remaining organochlorine compounds. Typical capillary column chromatograms are shown in Fig. 2-11. Analysis of pool no. 10 was repeated using packed column GC. There is excellent agreement between the two methods for HCB, mirex, heptachlor epoxide and dieldrin. The results for oxychlordane and p,p'-DDD are 13-14% higher by packed column GC,

probably due to minor interferences. Photomirex values are lower by packed column GC, but not much beyond the SD for this compound, which has the highest relative SD of all compounds (11%). The cis-chlordane value determined on the mixed-phase packed column was 600% higher than the capillary column result due to interferences. Cis-nonachlor could not be determined at all by packed column GC. Fig. 9 indicates clearly that there is a major unidentified peak (RT 40.19) which probably co-elutes with cis-chlordane on the packed column.

The relative SD of replicate injections is approximately 2-3% for this set of analyses. The mean levels of the majority of compounds (PCBs by packed column, DDE, dieldrin, oxychlordane, cis-nonachlor and DDD) have relative standard deviations <5%. This degree of variability is expected for complete analysis in replicate of samples with identical concentrations of compounds. The Lake Erie Herring Gull egg pool therefore meets the criterion for homogeneity, i.e., that "fluctuations in the chemical composition determined in different areas of the sample are not significantly larger (in statistical terms) than the error of the analytical procedure" (Danzer and Marx 1979). For those compounds which have relative SD values significantly greater than 5%, there was no trend which would suggest lack of homogeneity. In all these cases there is some part of the procedure which could introduce greater variability: loss during solvent evaporation for HCB, the nitration step for

photomirex and inability of the integrator to set correct baselines for capillary PCB analysis. It can be concluded that the Lake Erie pool designated as CWS-79-1 is suitable as a secondary reference material for the analysis of organochlorine compounds in egg substrates.

ACKNOWLEDGMENTS

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Table 1. PCB and p,p'-DDE residues in Herring Gull egg pool from Lake Erie in 1979 (reference material CWS-79-1).

Pool No.	% Fat	Residues: ppm wet weight					
		capillary chromatography				packed chromatography[d]	
		p,p'-DDE	PCB 1254[a]	PCB 1:1 1254/1260[b]	PCB 1260[c]	p,p'-DDE	PCB 1:1 1254/1260
1	8.47	4.15	29.0	94.6	39.6	4.97	97.1
4	8.38	4.20	30.4	107.7	41.5	5.04	101.9
7	8.35	4.10	28.3	88.2	33.8	4.86	96.8
10	8.36	3.83	27.5	90.4	37.6	4.54	91.5
13	8.44	4.01	29.6	104.7	40.7	4.67	93.3
16	8.48	4.23	29.4	92.1	35.5	4.79	94.9
19	8.07	3.67	27.3	94.7	37.7	4.58	92.4
22	8.13	4.04	27.9	90.1	33.1	4.86	96.6
25	8.30	3.91	27.8	92.8	37.5	5.20	103.4
28	8.18	3.63	26.8	93.1	36.1	4.86	96.6
31	8.31	4.12	28.9	97.5	38.5	4.94	94.8
34	8.34	3.94	27.4	87.2	32.7	4.91	92.1
Mean	8.32	3.99	28.4	94.4	37.0	4.85	96.0
SD	0.13	0.20	1.1	6.2	2.9	0.19	3.7
SE	0.04	0.06	0.3	1.8	0.8	0.05	1.1
Rel. SD (%)	1.57	4.93	3.9	6.6	7.8	3.88	3.9

[a] The Aroclor 1254 results were calculated from area response factors with standard Aroclor 1254 using the peak at RT 50.43-50.56 min (Peak I, Fig. 2-5). This peak is contributed entirely from Aroclor 1254.

[b] The Aroclor 1254/1260 (1:1) results were calculated from area response factors with standard Aroclor 1254/1260 (1:1) using the peak at RT 57.48-57.63 min (Peak II, Fig. 2-5).

[c] The Aroclor 1260 results were calculated from area response factors with standard Aroclor 1254/1260 (1:1) using the peak at RT 66.39-66.56 min (Peak III, Fig. 2-5) as outlined in the methods section.

[d] These results were obtained using the 6 foot column packed with 1% SP2100 coated on 100/120 mesh Supelcoport. PCBs were calculated by the same method used by ORF, assuming the ratio of Aroclor 1254/1260 was 1:1 using the average values obtained from the heights of peak 141 and 166 (Reynolds and Cooper 1975).

Table 2. Organochlorine residues in Herring Gull egg pool from Lake Erie in 1979 (reference material CWS-79-1)[a].

Pool No.	Residues: ppm wet weight								
	1st Fraction			2nd Fraction				3rd Fraction	
	HCB	photomirex[c]	mirex[c]	oxy-chlordane	cis-chlordane	cis-nonachlor	p,p'-DDD	heptachlor epoxide	dieldrin
1	0.194	0.0291	0.0892	0.156	0.0225	0.0860	0.0469	0.116	0.247
4	0.204	0.0355	0.0892	0.156	0.0221	0.0885	0.0514	0.116	0.255
7	0.173	0.0389	0.0913	0.156	0.0213	0.0868	0.0514	0.122	0.257
10	0.166	0.0408	0.1012	0.155	0.0241	0.0860	0.0506	0.122	0.259
(10)	(0.168)	(0.0338)	(0.0968)	(0.178)	(0.148)	(NA)	(0.0572)	(0.122)	(0.261)
13	0.191	0.0459	0.1049	0.154	0.0201	0.0892	0.0506	0.100	0.235
16	0.169	0.0425	0.0936	0.155	0.0201	0.0845	0.0476	0.115	0.259
19	0.183	0.0446	0.0983	0.148	0.0252	0.0845	0.0476	0.121	0.260
22	0.165	0.0409	0.0924	0.147	0.0220	0.0837	0.0460	0.119	0.251
25	0.178	0.0438	0.0989	0.153	0.0227	0.0854	0.0524	0.128	0.268
28	0.182	0.0417	0.0945	0.154	0.0218	0.0846	0.0500	0.121	0.255
31	0.180	0.0402	0.0885	0.144	0.0223	0.0808	0.0485	0.121	0.244
34	0.160	0.0438	0.0969	0.151	0.0227	0.0838	0.0500	0.124	0.261
Mean[b]	0.179	0.0405	0.0949	0.152	0.0222	0.0853	0.0494	0.119	0.254
SD	0.013	0.0045	0.0052	0.004	0.0014	0.0022	0.0020	0.007	0.009
SE	0.004	0.0013	0.0015	0.001	0.0004	0.0006	0.0006	0.002	0.003
Rel. SD (%)	7.32	11.02	5.49	2.65	6.51	2.63	4.12	5.88	3.47

(footnotes on next page)

Footnotes for Table 2

[a] Residue results were obtained by capillary GC except those in parentheses, which were obtained by packed column for comparison. Column for first fraction was the 6 foot 1% SP2100 column. Column for second fraction, third fraction and nitrated first fraction was the 6 foot mixed-phase column. See Table 1 for PCB and DDE results.

[b] The mean values in this table do not include the values in parentheses for the calculation.

[c] Analyzed from nitrated first fraction.

Figure 1

Hewlett-Packard 5840A program used for chromatograms on Figures 2 to 6 inclusive.

```
TEMP1      400    80    80
TIME1        5.00
RATE         2.50
TEMP2      400   190
TIME2        7.00
INJ TEMP   400   210   210
FID TEMP   400   100   100
AUX TEMP   400   300   300

CHT SPD      0.25
ZERO        10.0
ATTN 2↑      5
AUX SGNL     A
SLP SENS     0.07
AREA REJ    9990000000
FLOW  A      0.0     0.0
FLOW  B     40.0    31.0

  1.00 IFN              9
  1.15 VLV/EXT          4
  2.00 VLV/EXT          0
  2.00 VLV/EXT          2
  5.00 AREA REJ              2000
 25.00 ATTN 2↑          7
 30.00 RATE             1.00
 78.00 TEMP2           60
```

Figure 2

Chromatogram of First Fraction, Pool No. 1

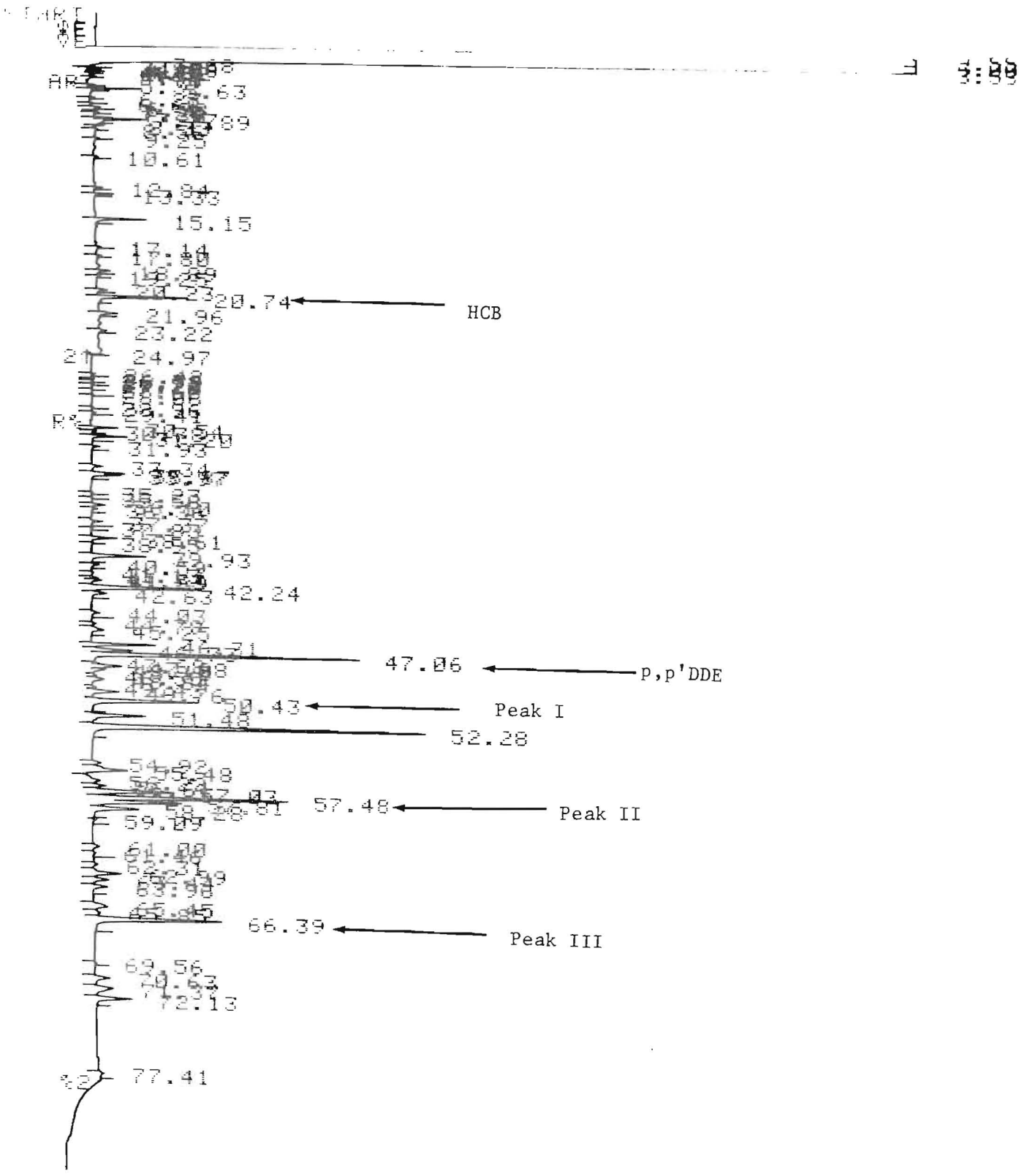

Figure 3

Chromatogram of Standard Aroclor 1254:1260 (1:1)
Conc. $5.0X10^{-10}$ g/ul

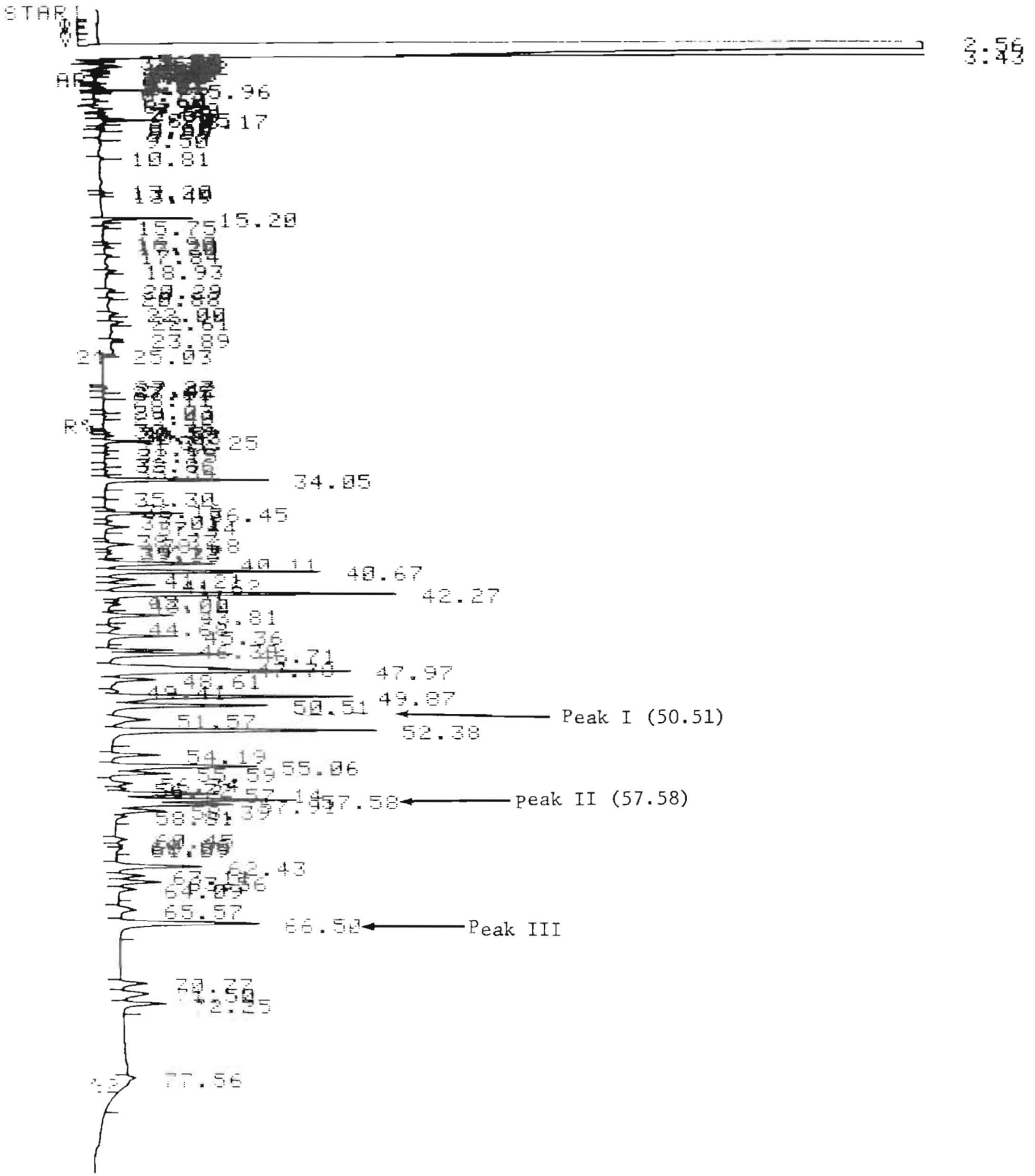

Figure 4

Chromatogram of Standard Aroclor 1254, Conc. $5.0X10^{-10}$ g/ul

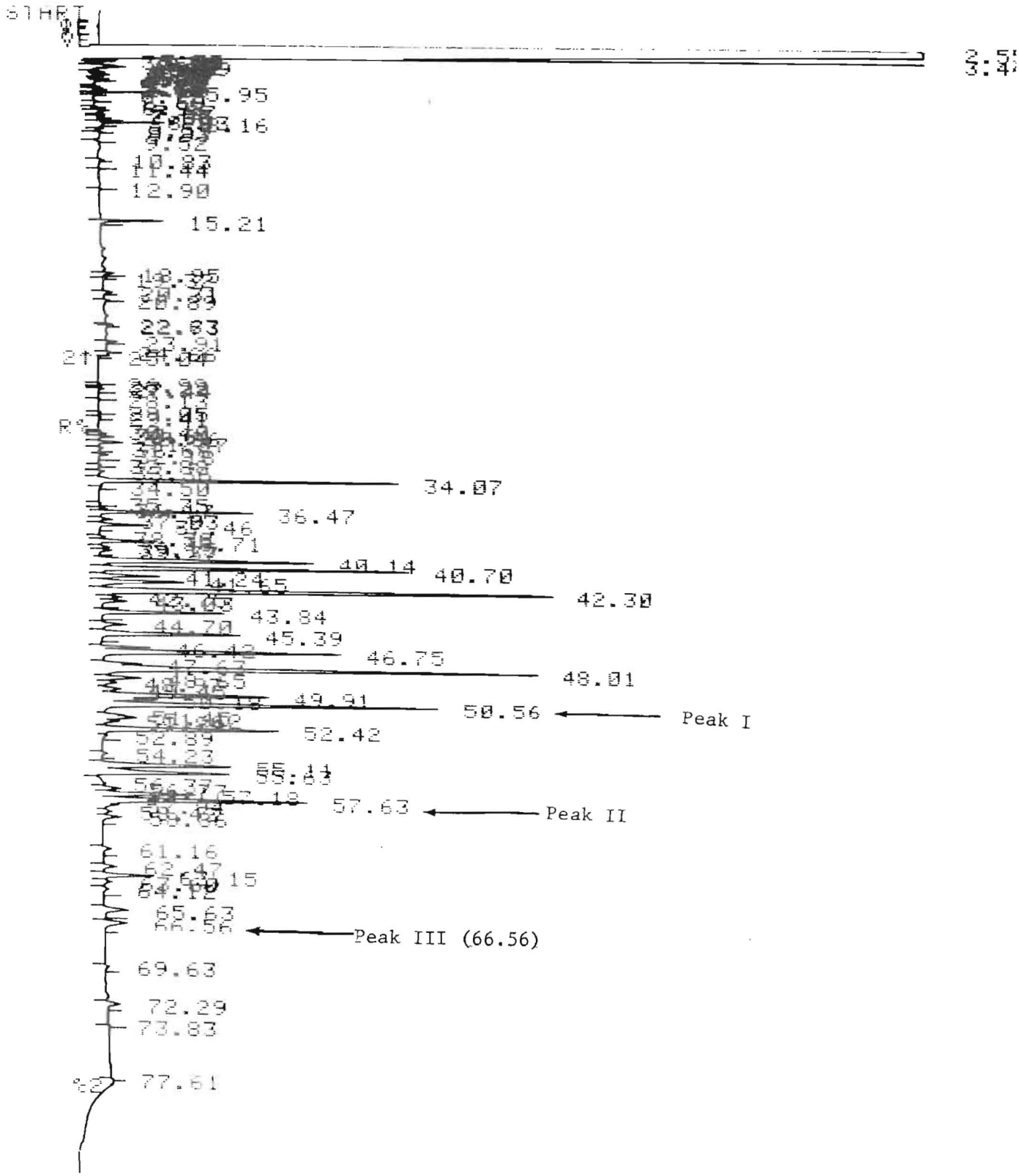

Figure 5

Chromatogram of Standard 1260, Conc. $5.0X10^{-10}$ g/ul

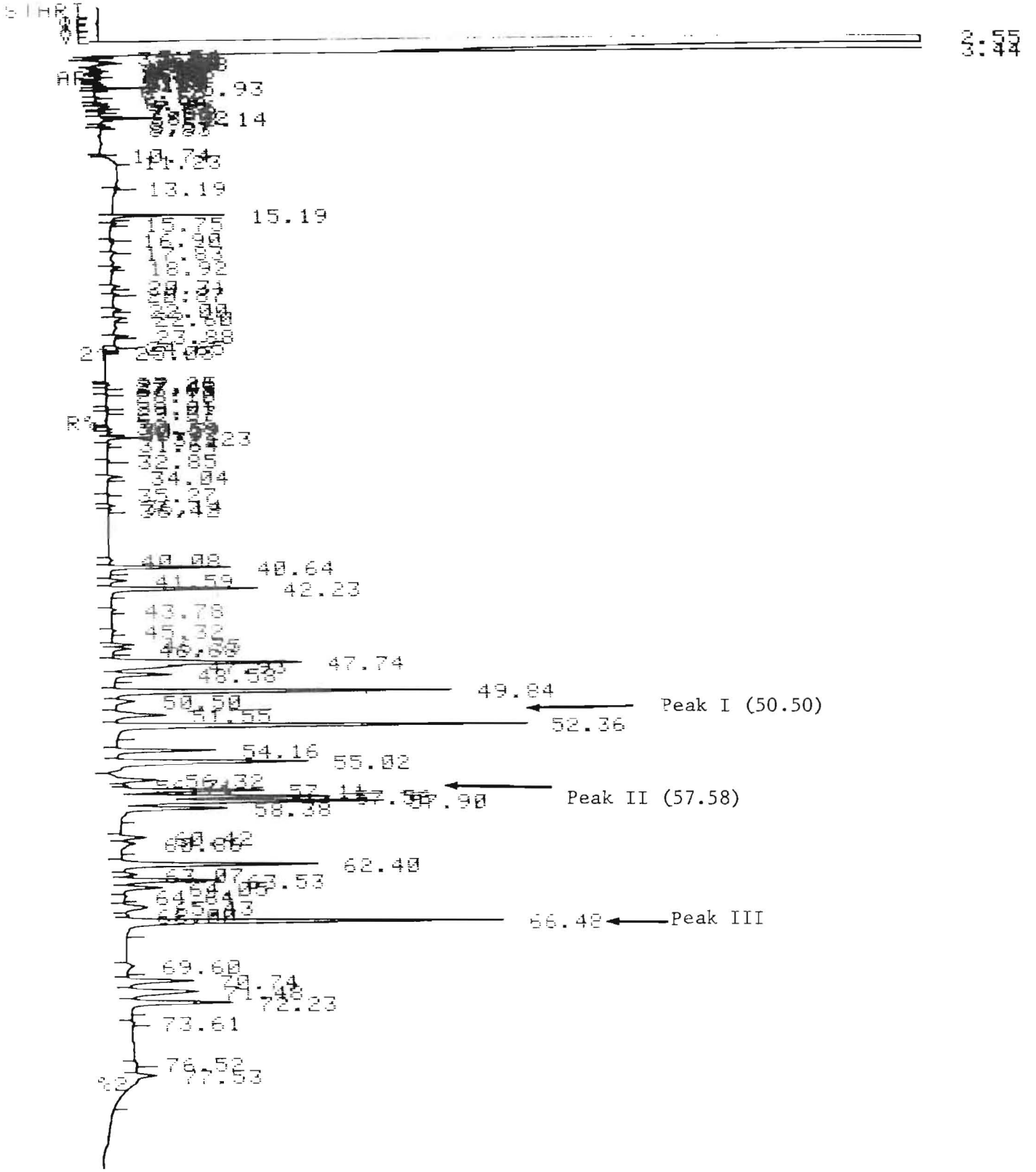

Figure 6

Chromatogram of Standard Mixture of Nineteen Compounds.
Conc. 30pg/ul Range (p,p'DDE)

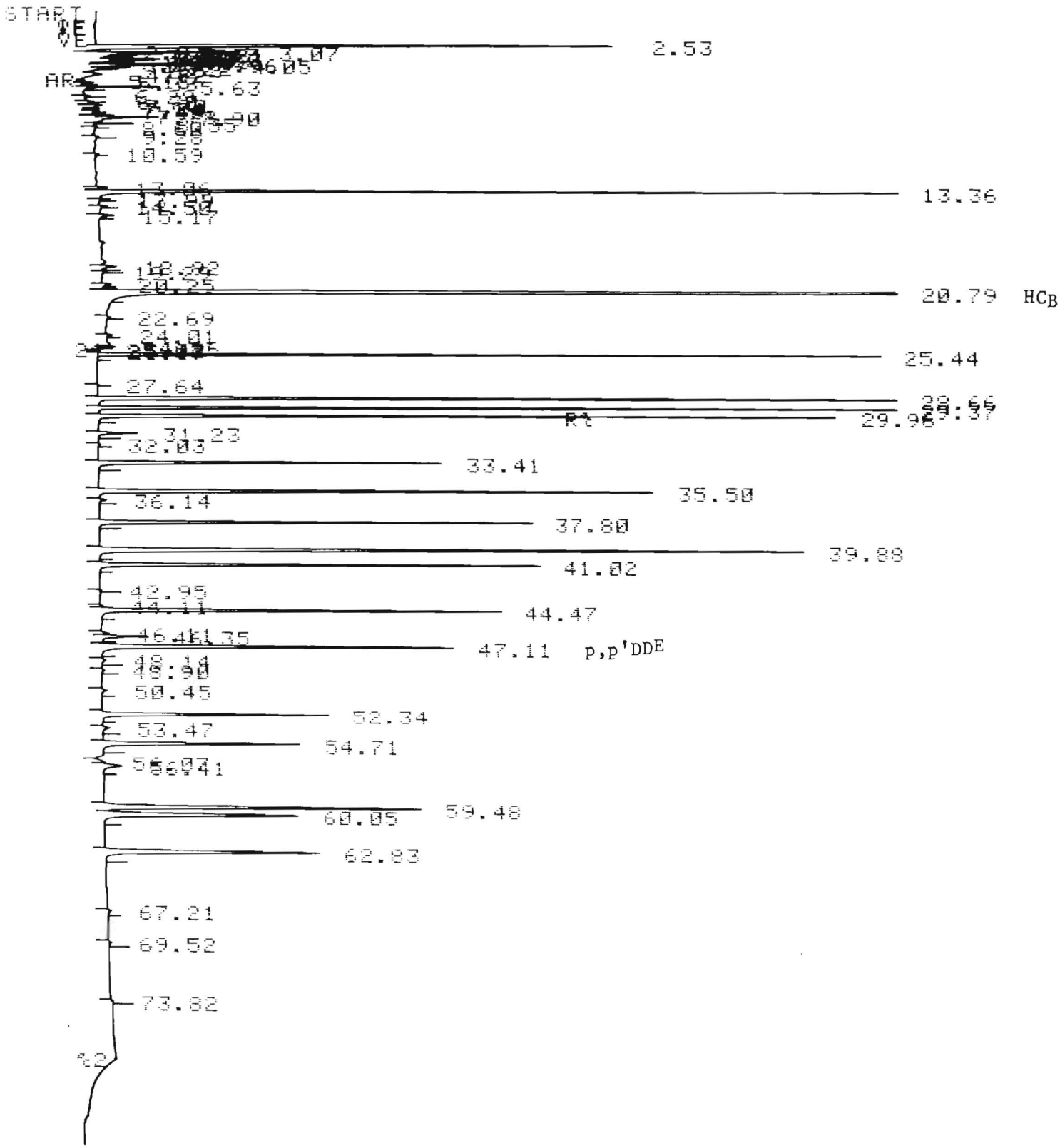

Figure 7

Hewlett-Packard 5840A program used for chromatograms on Figures 8 to 11 inclusive.

```
TEMP1       400    80    80
TIME1         5.00
RATE          2.50
TEMP2       400   210
TIME2        10.00
INJ TEMP    400   210   210
FID TEMP    400   100   100
AUX TEMP    400   300   300

CHT SPD       0.25
ZERO         10.0
ATTN 2↑      variable
AUX SGNL     A
SLP SENS      0.07
AREA REJ     9990000000
FLOW  A       0.0     0.0
FLOW  B      40.0    31.0

  1.00 IFN              9
  1.15 VLV/EXT          4
  2.00 VLV/EXT          0
  2.00 VLV/EXT          2
  8.00 AREA REJ                2000
 62.00 TEMP2           60
```

Figure 8

Chromatogram of Nitrated First Fraction, Pool No. 1
Att. 2↑8

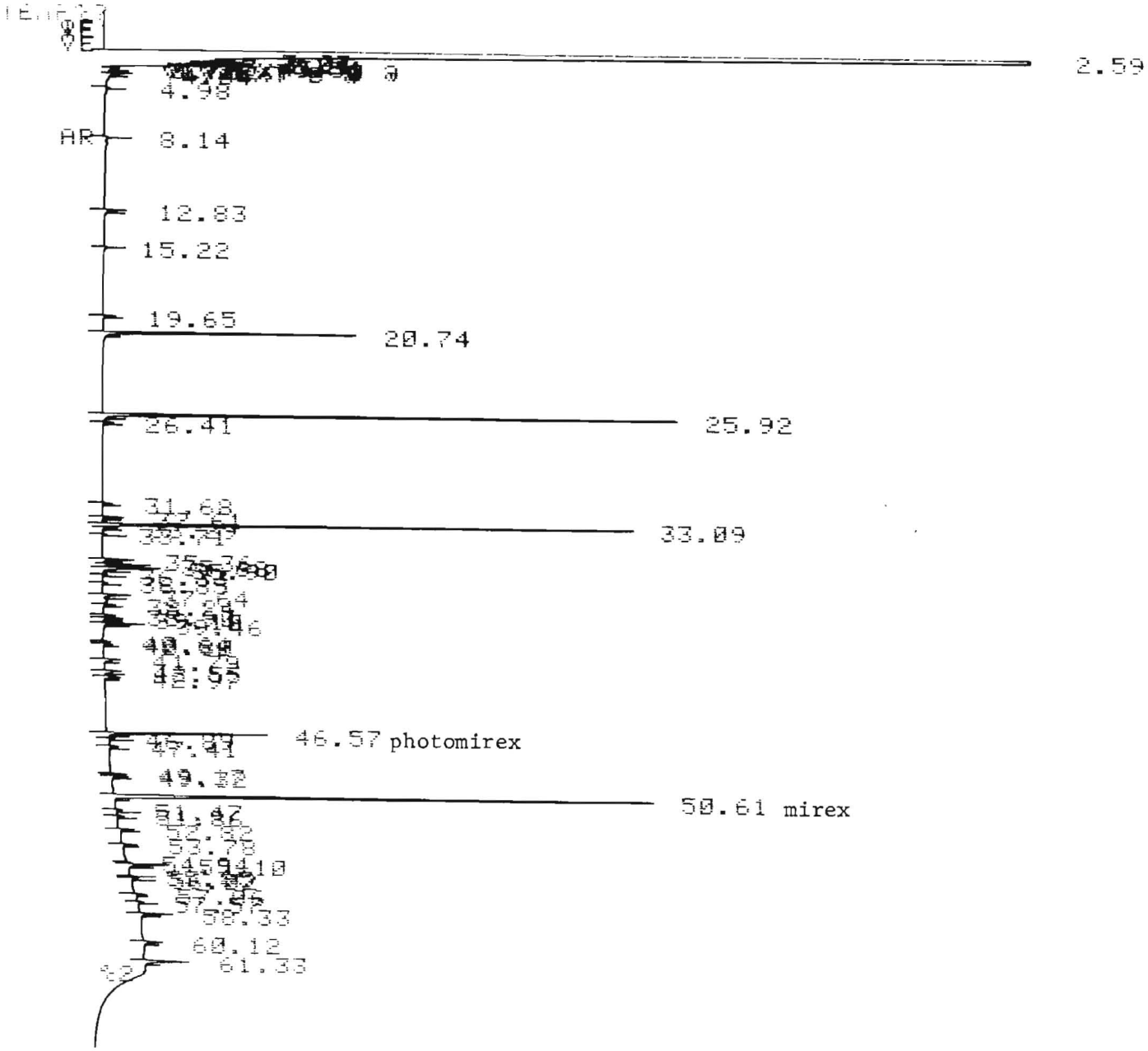

Figure 9

Chromatogram of Second Fraction, Pool No. 1
Att. 2↑9

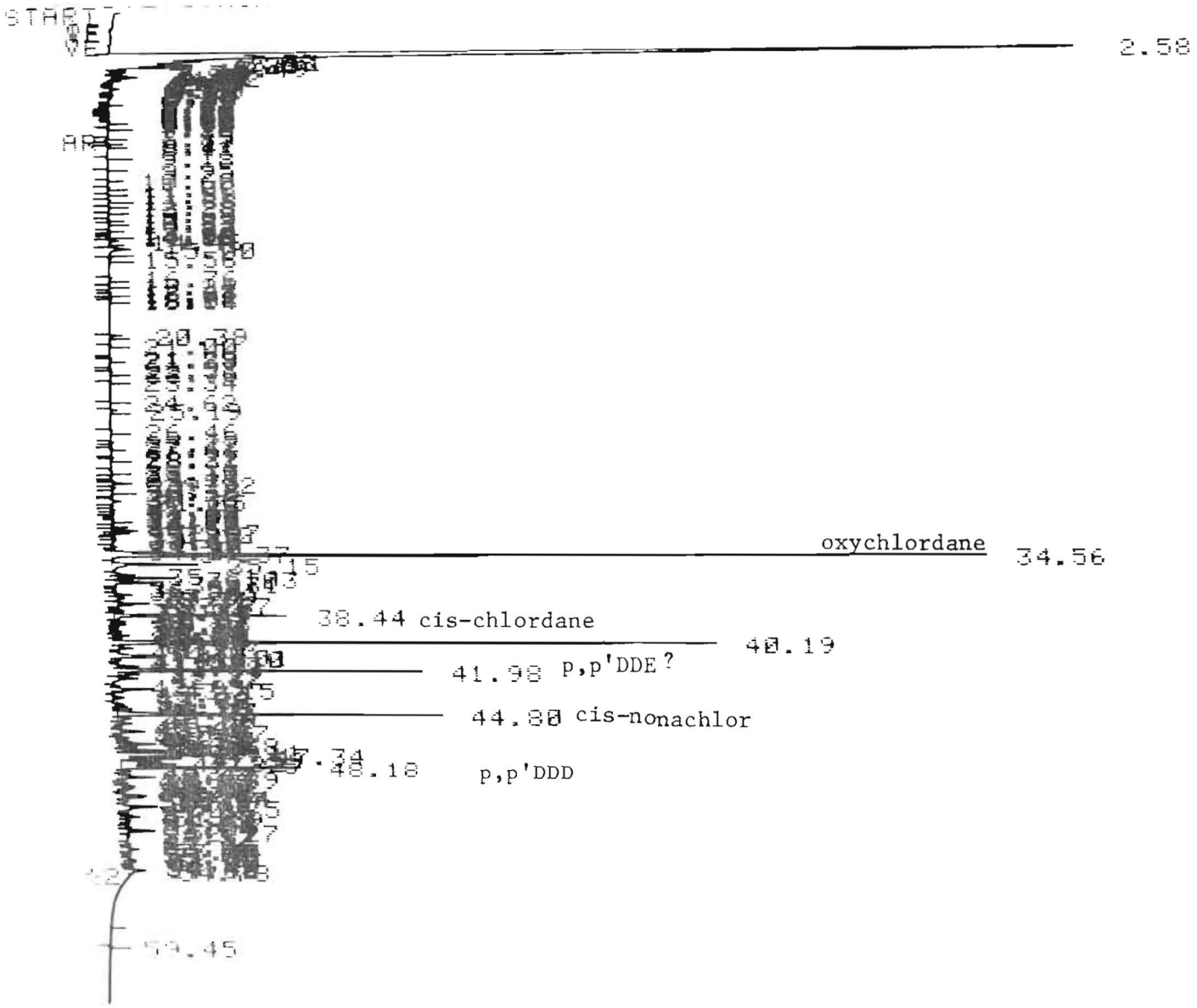

Figure 10

Chromatogram of Third Fraction, Pool No. 1
Att. 2↑10

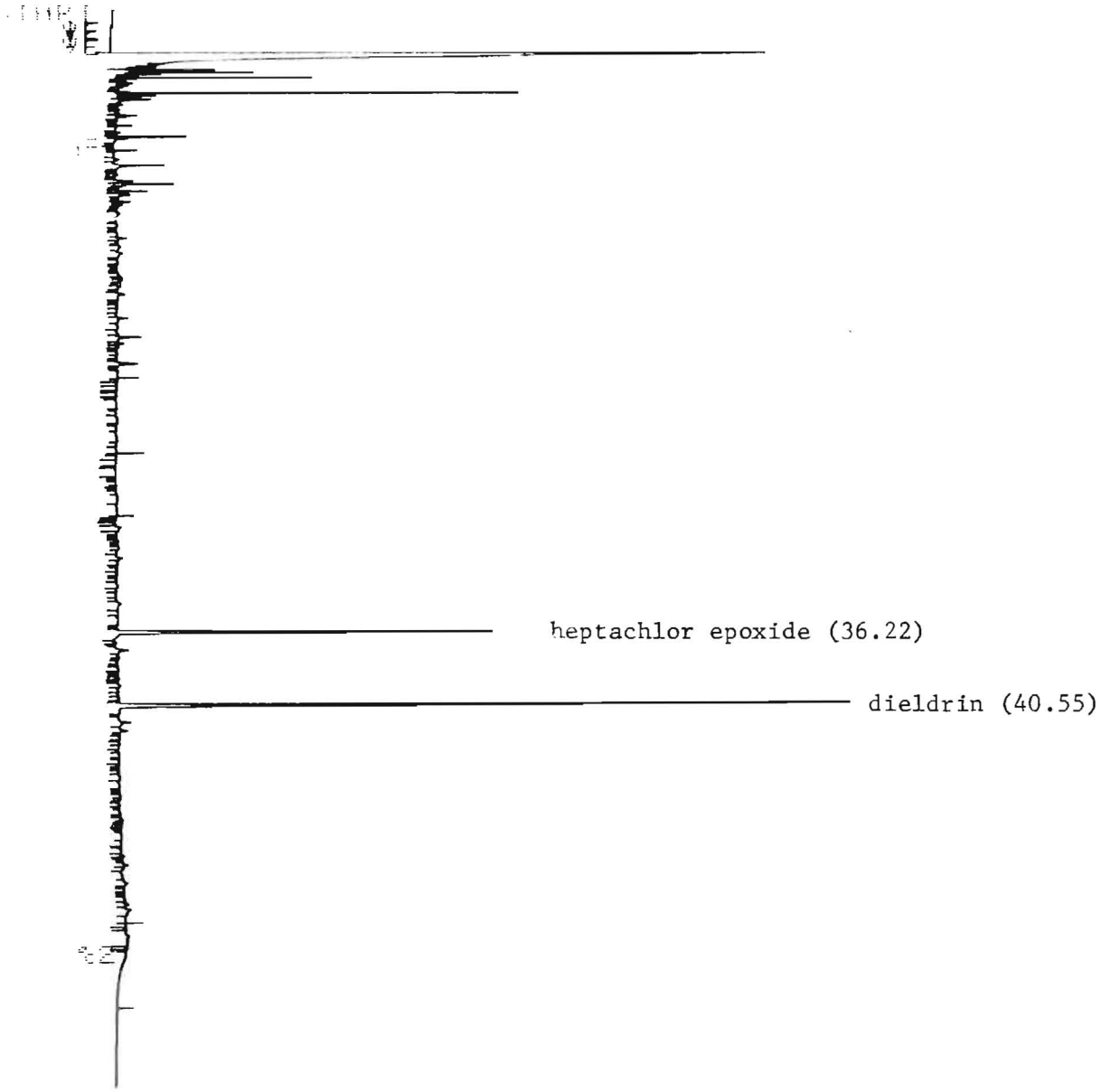

Figure 11

Chromatogram of Standard Mixture of Nineteen Compounds.
Conc. 30 pg/ul Range (p,p'DDE)
Att. 2↑8

RT	ID
13.05	Pentachlorobenzene
20.33	HCB
25.10	∝-BHC
28.30	Heptachlor
29.02	∫-Bhc (Lindane)
29.58	Aldrin
32.70	Octachlorostyrene
34.47	Oxychlordane
36.22	Heptachlor epoxide
37.58	trans-Chlordane and trans-Nonachlor
38.36	cis-Chlordane
40.55	Dieldrin
41.91	p,p'DDE
44.71	cis-Nonachlor
46.10	Photomirex (8-monohydromirex)
48.09	p,p'DDD
48.69	10-monohydromirex
50.12	Mirex