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# A TENTATIVE PROCEDURE FOR THE DETERMINATION OF AIRBORNE-POLYCHLORINATED BIPHENYLS.

## SCOPE

This procedure is based on techniques originally developed for the isolation and determination of polychlorinated biphenyls (PCBs) in water, soil/sediment, and biological materials (Analytical Method Nos. 69-13 and 70-1).

Absolute confirmation of PCB structure is not obtained with this method. Where needed, additional structure proof should be obtained using techniques such as mass spectrometry to further identify GC fractions.

## PRINCIPLE

Airborne PCBs are absorbed in toluene by drawing the air through one or more fritted bubblers or impingers in cylinders filled with toluene. After sampling a suitable amount of air the scrubbing solvent is diluted or concentrated and interfering components if present, are removed by chemical treatment and column absorption chromatography. The amount and type of PCB present is determined by electron capture chromatography (EC/GC).

## REAGENTS

| | |
|---|---|
| Hexane | Nanograde, Mallinckrodt Chemical Works Works, Catalog No. 4159. |
| Toluene | Nanograde, Mallinckrodt Chemical Works, Catalog No. 8092. |
| Sodium Sulfate | Anhydrous, granular: AR grade, Mallinckrodt Chemical Works, Catalog No. 8042. Heat at 400° C for one hour prior to use. |
| Alumina Adsorption | (for chromatographic analysis) 80/200 mesh, Fisher Scientific Co., Catalog No. A540. Heat at 400°C for a minimum period of 4 hrs. and deactivate with 5% (w/w) distilled water. |
| | Alumina column preparation: Fill a chromatographic column with hexane up to the point where the reservoir joins the column and push a glass wool plug to the bottom with a glass rod. In a 50 ml beaker measure 35 ml of deactivated alumina (∿30g), and pour this slowly into |

| | |
|---|---|
| | the column. Tap or vibrate the column to settle the alumina and top the alumina with 2-3 cm of anhydrous sodium sulfate. Wash the column with 50-100 ml of hexane prior to the addition of the sample. |
| Distilled Water | Extracted with hexane to remove hexane soluble electron capturing impurities. |
| Sulfuric Acid | Analytical Reagent Grade, SG = 1.84 |
| Potassium Hydroxide | Analytical Reagent Grade |
| Ethanol | Formula 2B |
| 2.5% (w/v) Alcoholic Potassium Hydroxide | Dissolve ~12.5 grams of AR grade KOH in 500 ml of ethanol |
| 9/1 (v/v) Sulfuric Acid - Water | Carefully add 270 ml of AR grade sulfuric acid to 30 ml of distilled water in a 500 ml iced beaker |
| PCB Standards | Aroclor 1221, 1242, 1248, 1254 and 1260 |

APPARATUS

1. Gas Scrubbing Bottled, High form, ground glass joint, Fritted Coarse Discs.

2. Separatory funnels equipped with ground glass stoppers and Teflon stopcocks: 125, 250, 500, 1000 and 2000 ml capacities.

3. Kunderna-Danish Evaporative Concentrators, 500 ml capacity equipped with 3-ball Snyder columns and graduated 5 ml capacity vials: Ace Glassware Company, Catalog No. 6707.

4. Chromatographic columns, glass, 10" x 20 mm (OD) with a 5" x 50 mm (OD) reservoir at the top, equipped with Teflon stopcocks.

5. Flat bottomed boiling flasks, 125 ml capacity: Ace Glassware Company, Catalog No. 6896, Code - 04.

6. Liebig Condenser, 200 mm in length: Ace Glassware Company, Catalog No. 5915, Code - 12.

7. Hot plates, Corning PC-100: Fisher Scientific Company.

8. Water bath, Thelco, Precision Scientific, Model No. 84, Fisher Scientific Company.

9. 10 µl Hamilton Syringes, Catalog No. 701N.

10. Dry Test Meter or Wet Test Gas Meter.

11. Laboratory Vacuum Pump.

12. Rotating vacuum evaporator.

13. Usual laboratory glassware.

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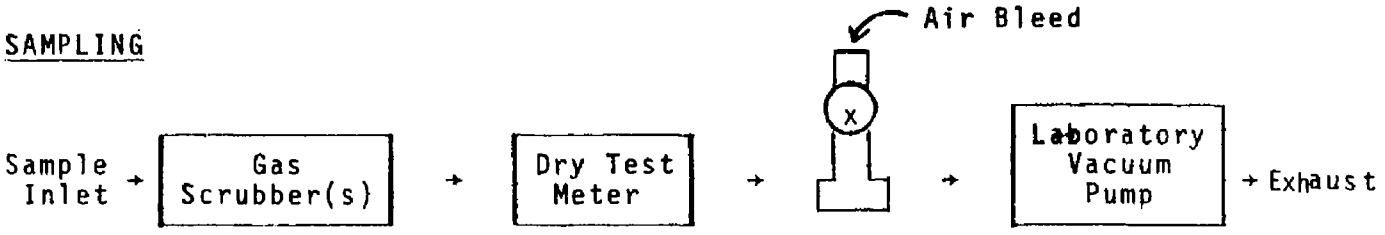
## SAMPLING




SAMPLING TRAIN

The air to be sampled for airborne PCBs is drawn through a gas scrubber(s) and a dry test meter using a laboratory vacuum pump. The sampling flow rate is controlled by bleeding in air via a needle valve located between the pump and the meter. At the end of the sampling period the metered gas volumes are corrected for temperature and pressure to cubic meters at 25° C. and 760 mm. Hg.

It is important to note that neither the capacity nor the efficiency of the gas scrubber(s) for removal of airborne PCBs have been experimentally evaluated. For this reason it is best to minimize the sampling flow rate and maximize the sampling time period to obtain measurable amounts of PCBs. When high flow rates must be employed or a larger capacity may be needed it is recommended that several gas scrubbers be used in tandem.

It is cautioned, that until the efficiency and capacity of the toluene gas scrubber has been experimentally established, this procedure should be only used to measure relative PCB levels sampled under equivalent conditions.

## PROCEDURE

1. After scrubbing the desired amount of air, record the metered volume, pressure, and temperature.

2. Quantitatively transfer the scrubbing solvent to a round bottom flask and reduce the volume to approximately 2 ml by rotary vacuum evaporation.

3. Quantitatively transfer the concentrate to 30 ml beaker with the aid of several small portions of toluene.

4. Inject a fraction of a ul of the concentrate into the gas chromatograph to check for interferences and determine the approximate level of PCBs present. If no interferences are present dilute or concentrate the sample to a known volume, as determined by the electron capture chromatogram, and proceed with the gas chromatographic analysis.



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5. If interferences are present proceed with the chemical treatment and column chromatographic clean up procedures.

6. Transfer the concentrate to a 125 ml extraction flask with the aid of several small portions of solvent.

7. Evaporate the concentrate just to dryness with a gentle stream of dry filtered air and add 25 ml of 2.5% alcoholic potassium hydroxide.

8. Add a boiling chip, put a water condenser in place, and allow the solution to reflux for 45 minutes.

9. After cooling, transfer the solution to a 250 ml separation funnel with the aid of 25 ml of distilled water.

10. Rinse the extraction flask with 25 ml of hexane and add it to the separatory funnel.

11. Stopper the separatory funnel and shake vigorously for at least 1 minute. Allow the layers to separate and transfer the lower aqueous phase to a second separatory funnel.

12. Extract the saponification solution with a second 25 ml portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous alcohol layer to the original separatory funnel.

13. Repeat the extraction with a third 25 ml portion of hexane. Discard the saponification solution and combine the hexane extracts.

14. Carefully add 25 ml of the sulfuric acid solution (9:1 concentrated sulfuric acid/water) to the hexane extracts.

15. Stopper the separatory funnel and shake vigorously for at least one minute. Allow the layers to separate and discard the lower aqueous acid layer. Repeat this step until the acid layer is colorless.

16. Wash the hexane with 25 ml portion of water. Discard the water wash.

17. Filter the hexane extract through a 4" funnel plugged with glass wool which is covered with a layer of sodium sulfate into a Kunderna-Danish evaporative concentrator.

18. Add a small boiling chip, put the Snyder column in place and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90° C. water bath.

19. After cooling, remove the 5 ml graduated tube and transfer the hexane extract to an alumina adsorption column washing it in with several 5 ml portions of hexane.



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20. Carefully add 100 ml of hexane to the column reservoir and collect the total eluent in either a 250 ml volumetric flask or a Kunderna-Danish evaporative concentrator.

21. If the column eluent is collected in a volumetric flask, dilute to volume with hexane and proceed with the gas chromatographic analysis.

22. If the column eluent is collected in a Kunderna-Danish evaporative concentrator, reduce solvent volume, cool, dilute to volume and proceed with gas chromatographic analysis.

Electron Capture Gas Chromatographic Procedure:

Instrument: F&M 402 Biomedial Gas Chromatograph

Detector: High Temperature $Ni^{63}$ Electron Capture Cell
6 mm x 6' Glass Column, 4% XE-60 on 80/100 mesh
Chromosorb W, HP, AW-DMCS

Column Temperature: 160-190° C.

Detector Temperature: 300° C.

Injection Port Temperature: 195-215° C.

Pulse: 150

Flow Rates: Helium Carrier ∿60 ml/min
Argon-Methane Purge ∿120 ml/min

Using EC/GC as the determinative step, inject in duplicate 1-10 µl of each solution into the chromatograph. By comparison with standard solutions injected, in duplicate, under the same operating conditions, determine the amount and type of Aroclor using the individual or total peak height and area methods.

DISCUSSION

Sample Concentration

Concentration of sample extracts is necessary, prior to clean up by chromatographic or chemical means, to reduce sample size and increase sensitivity. The preferred method of concentrating allows minimum loss through volatilization or chemical decomposition and requires a minimum time. The three methods of solvent volume reduction most commonly used are evaporation by exposure to a stream of air, evaporation employing a Kunderna-Danish evaporative concentrator equipped with a Snyder column, and evaporation under reduced pressure. We have used all three techniques and have not encountered any significant losses from volatilization or chemical alternation.

Column Adsorption Chromatography and Chemical Clean Up

Silica gel, Florisil and Alumina deactivated with 0, 1.0, 1.5, 2.0 and



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5% water were investigated as adsorbants for the elimination of interferences. Alumina (5% water) was found to be more effective and reproducible than either silica gel or Florisil. The activity of alumina varies with age and lot, therefore, 5% water was added to the alumina, after heating for a minimum of 4 hours at 400°C, to insure a reproducible activity.

Saponification and subsequent extraction of the sample with sulfuric acid is an effective way to remove a number of chlorinated hydrocarbon interferences as well as other matrix interferences. PCBs are not affected.

Electron Capture Gas Chromatography

Columns

Column performance is the key to effective gas chromatographic analysis and as such the choice of column materials is particularly important. Ideally, the support employed should be inert, mechanically strong, and of high surface area. For these reasons, Chromosorb W, HP, AW-DMCS was used in all of our work.

A variety of polar and non-polar liquid phases were investigated. The following columns were found to provide adequate separation, etc., for use in PCB analysis by electron capture: 4% (w/w) DC-200, SF-96, OV-17, SE-30, SE-54, XE-60, Apiezon L, and 6% QF-1. DC-200 and XE-60 or QF-1 have been found to be the most suitable of these liquid phases.

Another important consideration when working with an extremely sensitive detector and consequently low levels of materials is column conditioning. With polar phases such as XE-60 and QF-1, we have found that operating a new column overnight at a temperature 25-50°C higher than to be used during analysis results in a more stable column. A no-flow conditioning technique is employed to condition non-polar columns. The column is purged with carrier gas, heated for 30 minutes at an elevated temperature without carrier flow and then cooled to room temperature. At the end of this cycle, the carrier flow is resumed and the conditioning is completed as in the case of the polar liquid phase. Two precautions: during conditioning, the column should not be connected to the detector and one should not exceed the maximum safe temperature of the liquid phase.

Since all liquid substrates bleed to one degree or another and columns eventually degrade, we characterize all new columns with two column performance indicators - the number of theoretical plates (N) and a tailing factor (T). p,p'-DDT is employed to check these parameters because it is known to degrade on "poor" columns. In this manner, we can determine if the performance of a new column is satisfactory and when the column performance begins to fall off. We consider a column good if the number of theoretical plates per foot is on the order of 400-500 with tailing factors of 1.0-1.3. Calculation of these parameters is shown in the Appendix. Additionally, there should be no significant

extraneous peaks upon injection of a pure p,p-DDT standard.

Other chromatographic conditions that can be adjusted are column temperature and flow rates. Although resolution of a mixture increases with decreasing temperature, a temperature should be chosen that allows the elution of all components within a convenient time period. The flow rates are optimum for our instrument, column and detector system and, of course, should be adjusted if better results can be achieved.

Two gas chromatographic systems have been used for PCB analysis - F&M Model 402 and 5750. We find that any system of instrument and column suitable for chlorinated pesticides is satisfactory for PCB analysis. The bulk of analyses in our laboratories was carried out using the system outlined. The use of the high temperature $Ni^{63}$ electron capture cell is highly recommended. The ability to operate at higher temperatures prevents maintenance problems due to contamination from high boiling components. Glass columns should also be employed.

Detection and Measurement

Quantitative determinations employing the electron capture detector are non-stoichiometric measurements made by comparing peak heights or areas for known concentrations with those for unknown compositions. Three variations of the peak height or area quantification procedures have been employed.

Case I — EC gas chromatogram of PCB unknown unchanged with respect to standard PCB with no evidence of interferences.

Case II — EC gas chromatogram of PCB unknown altered with respect to standard PCB with no evidence of interferences.

Case III — EC gas chromatogram of PCB unknown unchanged with respect to standard PCB with evidence of interference.

The amount of PCBs in Case I samples were determined by preparing a plot of the major peak height or area vs. concentration. With Case III samples, a peak free from interference was used. When dominant interferences were present, one or more of the chemical clean up procedures was employed.

In all cases, the response of the electron capture detector must be linear for quantitative analysis.

Contamination

In determining PCB's in biological materials by electron capture gas chromatography, laboratory sources of contamination can be a major



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problem. The samples and extracts should never be allowed to come in contact with materials other than glass, Teflon or metal. Laboratory glassware should be thoroughly washed with hot, soapy water, rinsed with distilled water, acetone, and then hexane. All equipment should also be rinsed again with hexane just prior to use and blanks should be frequently carried through all steps of the procedures to insure against the possibility of contamination.

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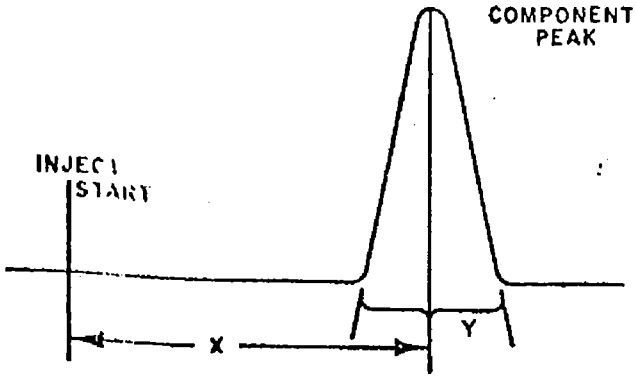
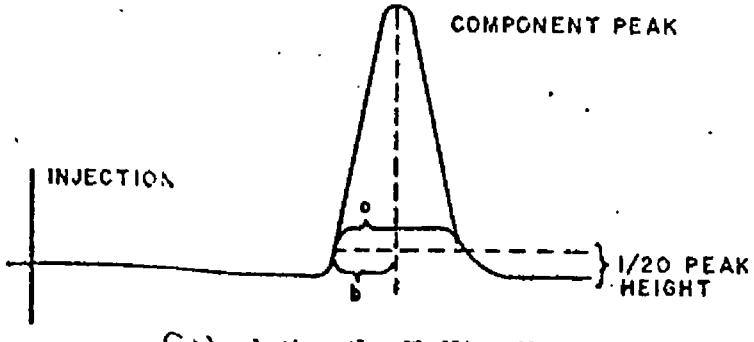
## COLUMN PERFORMANCE INDICATORS




Calculating Column Efficiency

1. Theoretical Plates, N

$$N = 16(x/y)^2$$




Calculating the Tailing Factor

2. Tailing, T

$$T = a/2b$$

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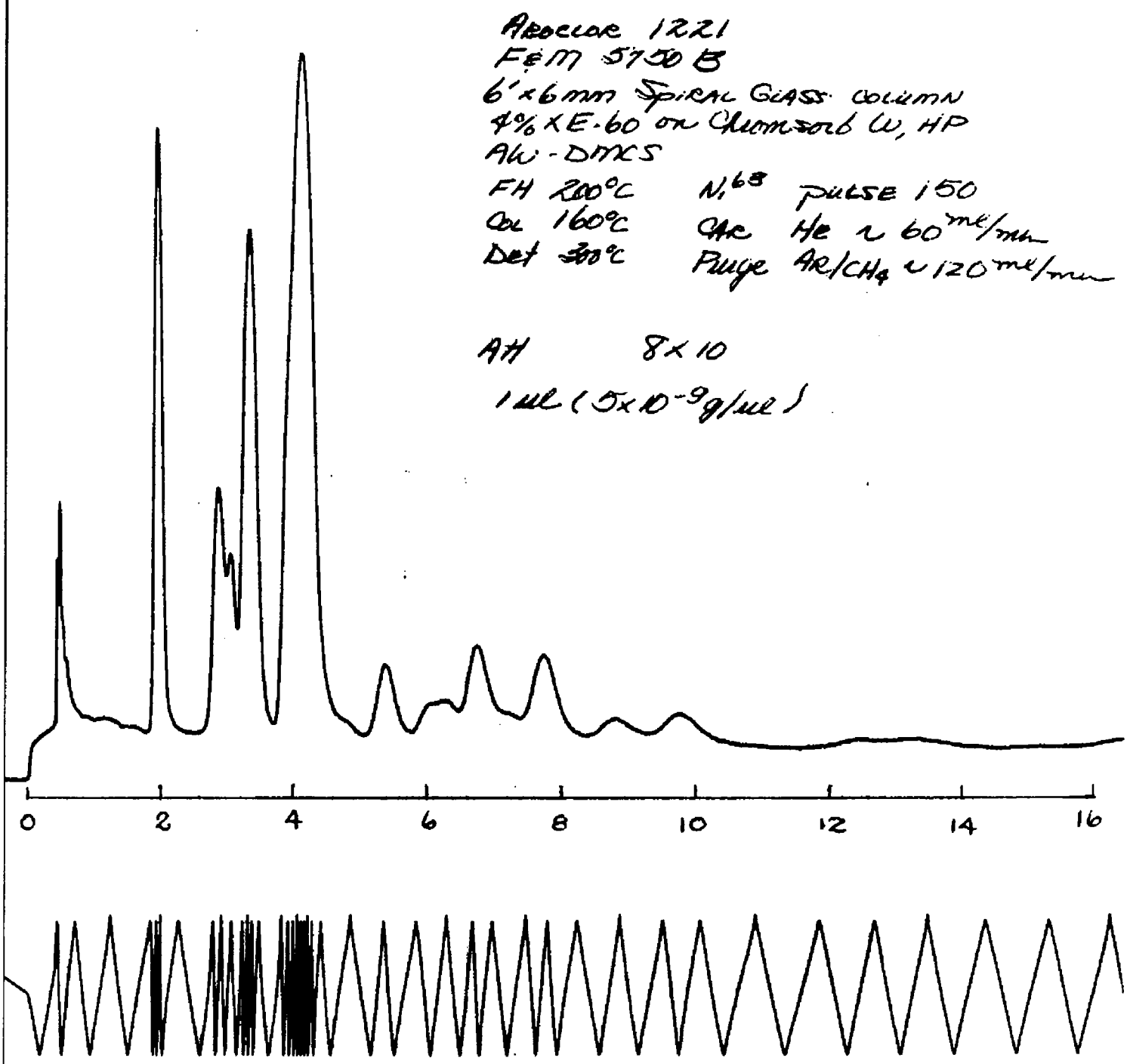
AROCLOR 1221
F&M 5750 B
6'x6mm SPIRAL GLASS COLUMN
4% XE-60 on Chromsorb W, HP
AW-DMCS
FH 200°C
Col 160°C
Det 300°C
Ni63 PULSE 150
CAR He ~ 60 ml/min
Purge AR/CH4 ~ 120 ml/min
AH 8x10
1 ul (5x10-9 g/ul)
0
2
4
6
8
10
12
14
16

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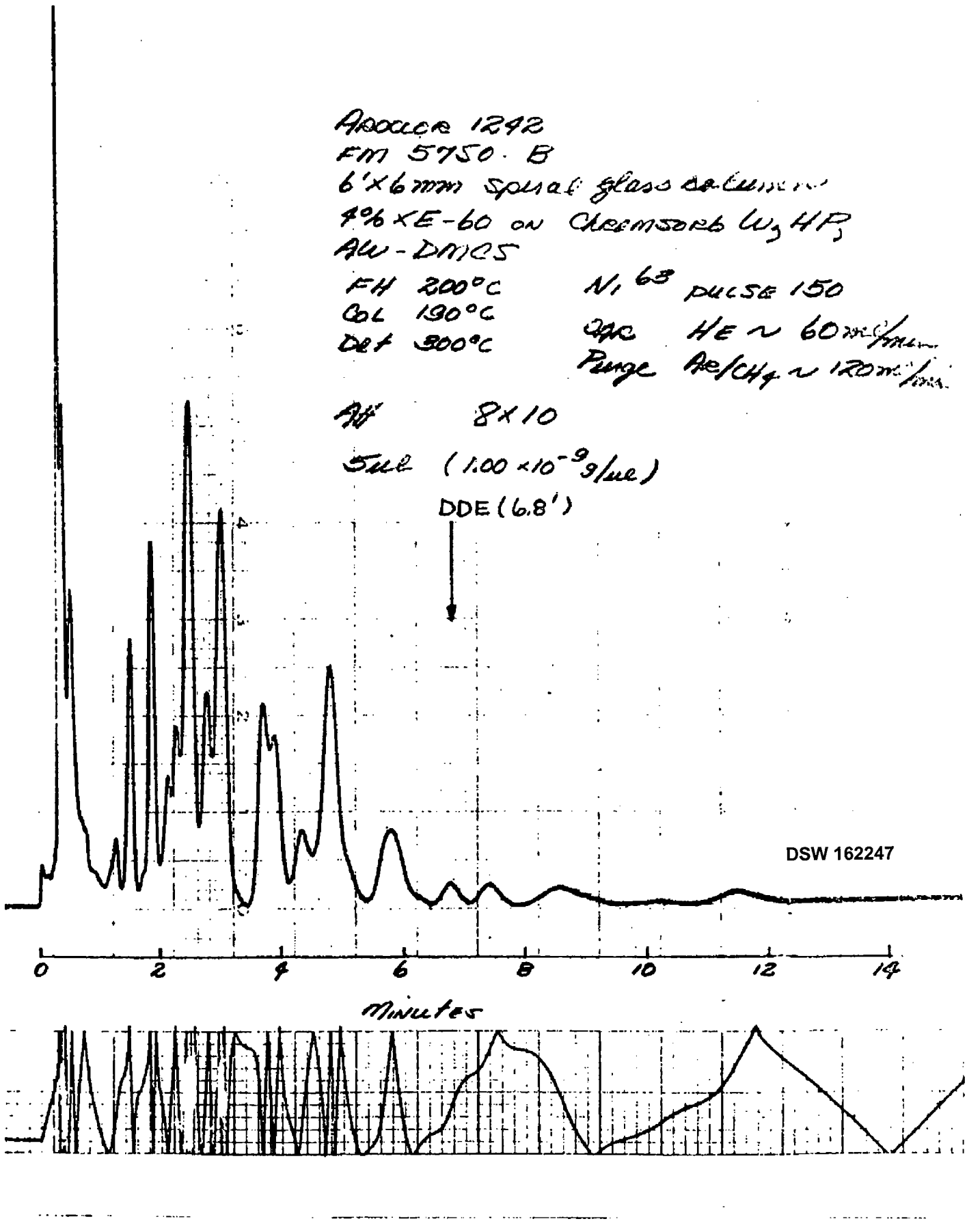
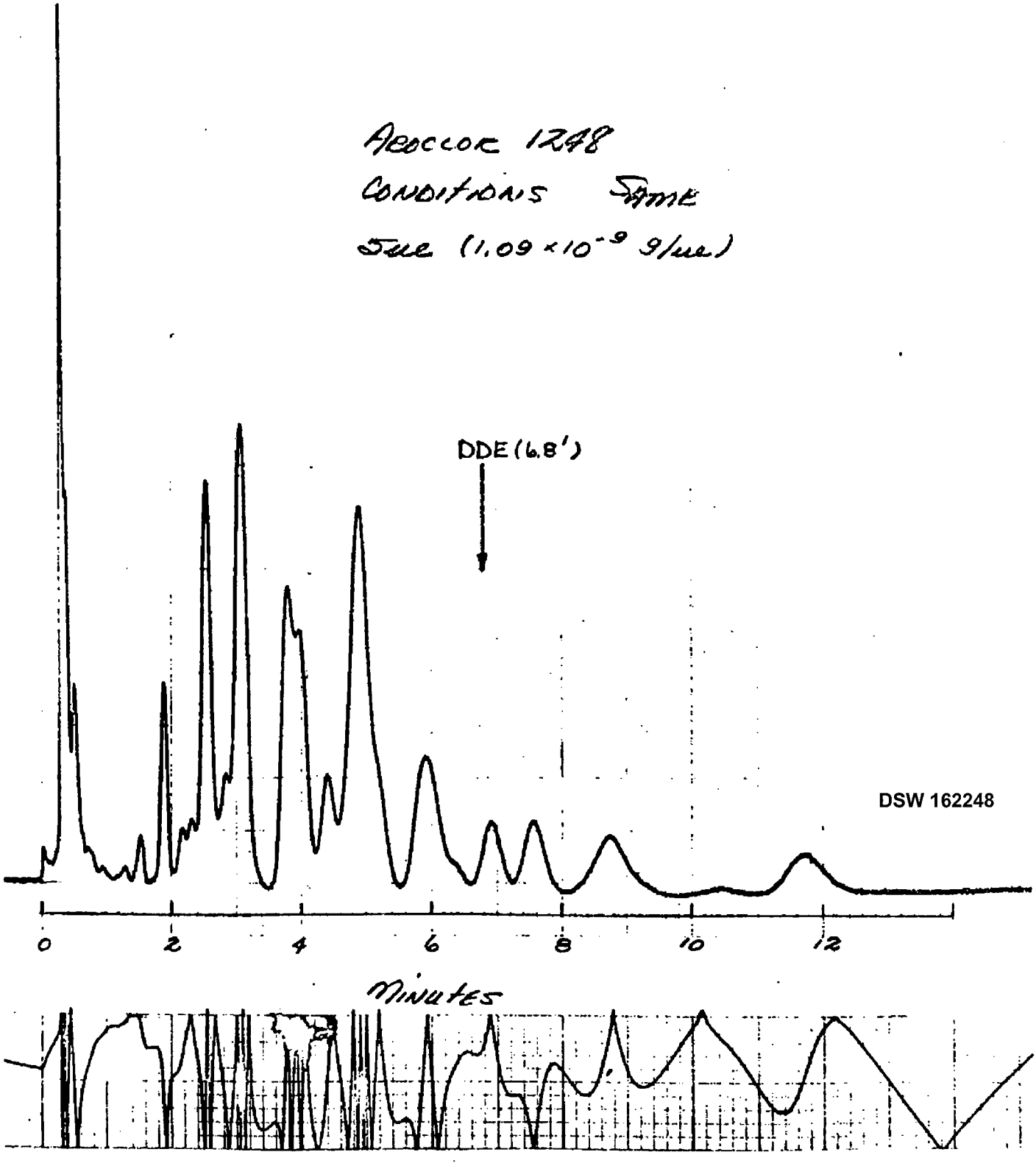
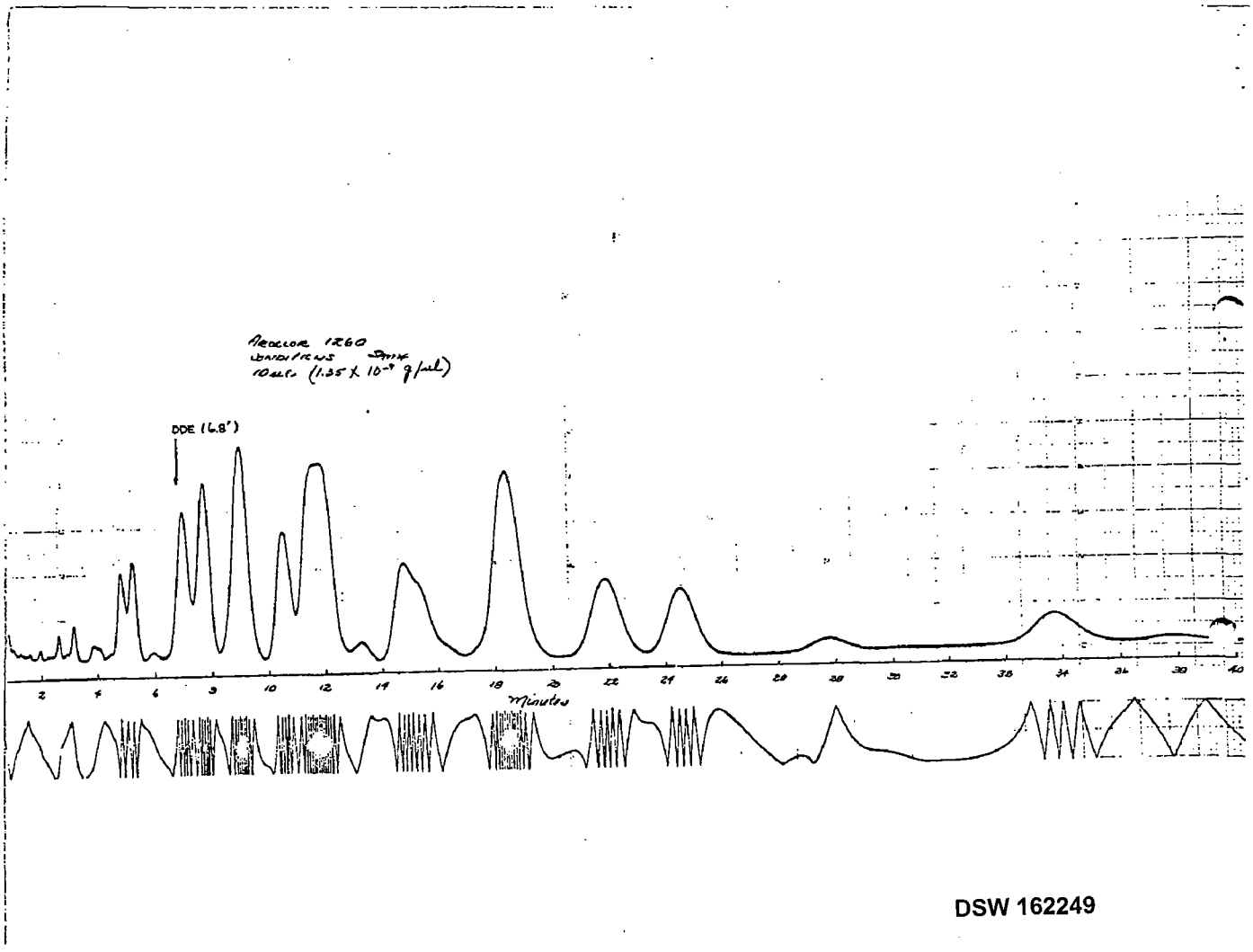

Aroclor 1242
FM 5750 · B
6'×6mm Spiral glass column
4% XE-60 on Chromosorb W, HP
AW-DMCS
FH 200°C
Col 190°C
Det 300°C
Ni 63 pulse 150
Gas He ~ 60 ml/min
Purge Ar/CH4 ~ 120 ml/min
Att 8×10
Full (1.00×10⁻⁹ g/ml)
DDE (6.8')
DSW 162247
0
2
4
6
8
10
12
14
Minutes

Aroclor 1248
Conditions Same
Sue ($1.09 \times 10^{-9}$ g/ml)




Aroclor 1260
Conditions
10 uL (1.35 x 10⁻⁹ g/uL)
DDE (6.8')
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Minutes

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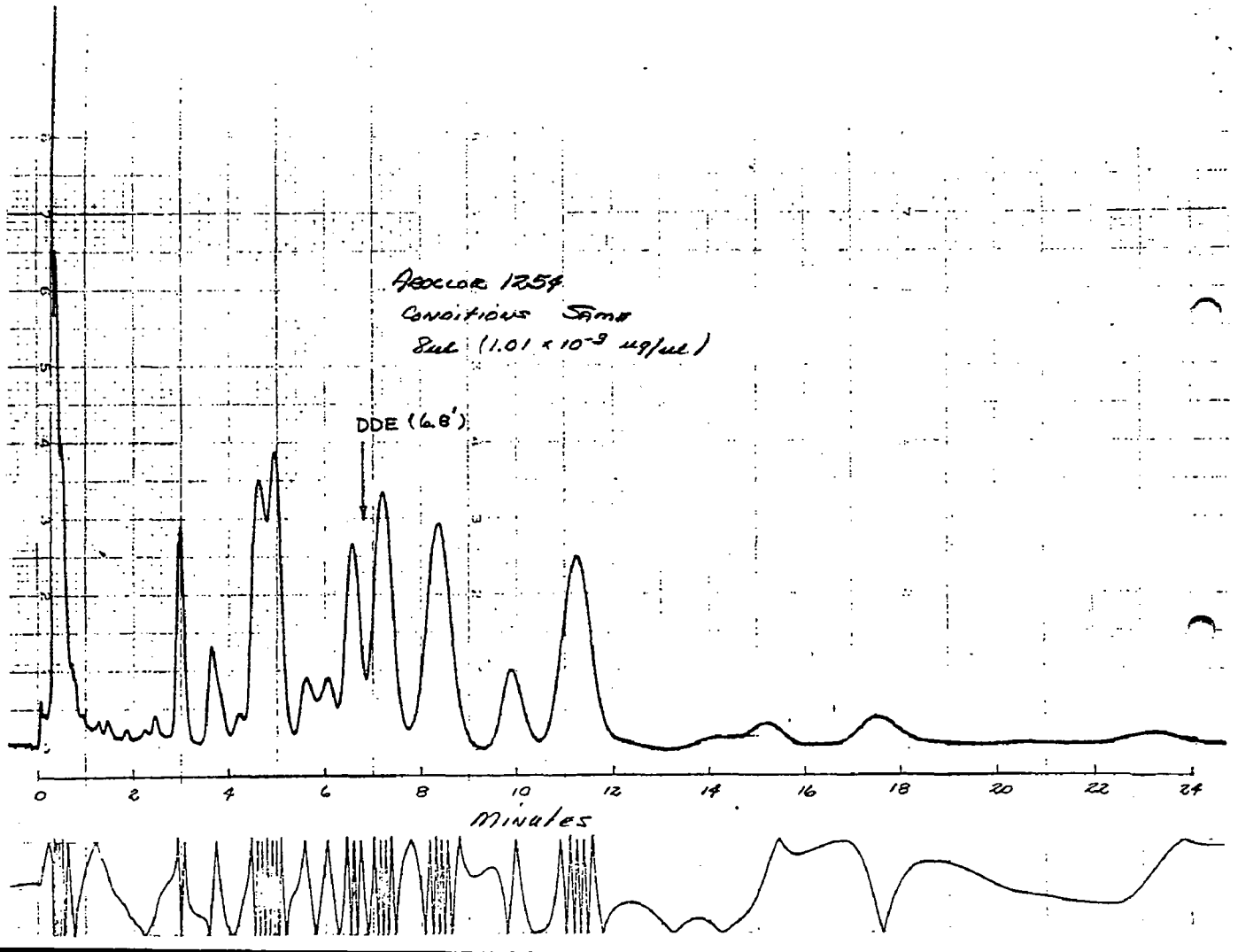
Aroclor 1254
Conditions Same
Std (1.01 x 10⁻³ ug/ul)
DDE (6.8')
0
2
4
6
8
10
12
14
16
18
20
22
24
Minutes

DSW 162250