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ANALYSIS OF WATER AND SEDIMENT FOR POLYCHLORINATED BIPHENYLS

SCOPE

This methodology was developed for the determination of the amount and type of polychlorinated biphenyls (PCB) in water and sediment samples. Absolute confirmation of PCB structures is not obtained with this method. Structure proof can be obtained using additional techniques such as mass spectrometry to further identify the GC fractions.

PRINCIPLE

The PCB(s) in water and sediment samples are extracted into an organic solvent. Interfering components are then removed from the extracts by chemical treatment and column adsorption chromatography. The amount and type of PCB present is determined by electron capture gas chromatography (EC/GC).

REAGENTS

Hexane	Nanograde, Mallinckrodt Chemical Works, Catalog No. 4159.
Acetonitrile	Nanograde, Mallinckrodt Chemical Works, Catalog No. 2442.
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 1242, 1248, 1254 and 1260

APPARATUS

1. Separatory funnels equipped with ground glass stoppers and Teflon stopcocks: 125, 250, 500, 1000 and 2000 ml capacities.
2. 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.
3. Chromatographic columns, glass, 10" x 20 mm (OD) with a 5" x 50 mm (OD) reservoir at the top, equipped with Teflon stopcocks.
4. Sintered glass filter funnels, 600 ml capacity, 90M.
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. Reciprocating variable speed shaker, Eberbach Corporation, Fisher Scientific Company.
10. 10 µl Hamilton Syringes, Catalog No. 701N.
11. 32 oz. all glass mortars and pestles.
12. 8 x 12 x 2" (2-1/2 qt.) Pyrex baking dishes.
13. U.S. Standard Sieve, No. 30, Fisher Scientific Company.
14. Usual laboratory glassware.

SAMPLING

It is to be assumed that a rather wide variety of sampling techniques were employed in collecting the samples submitted for analysis. In general the procedures used were probably selected for ease of adaption to the local situation. For this reason water and sediment samples were usually treated as follows:

Water

Where possible the entire water sample, including the container in which it was collected, was extracted with hexane. With larger samples, where this was not physically possible, the containers were simply agitated and a 250 ml portion used for analysis.

Sediment

Any excess water was decanted and the entire sediment transferred to a glass baking dish to air dry at room temperature. The dried material was transferred from the dish into a mortar and pestle and ground. The ground sediment was sieved, remixed, and a 250g portion taken for analysis.

PROCEDURES

Extraction of Water Samples

1. Extraction of water samples - after agitating, transfer the entire aqueous sample or a 250 ml aliquot into a graduated glass cylinder. Record the volume of the sample and quantitatively transfer it to a separatory funnel with distilled water.

2. Rinse the graduated cylinder with two 50 ml portions of hexane and add each to the separatory funnel.

3. 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.

4. Extract the water sample a second time with a 50 ml portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous layer to the original separatory funnel.

5. Repeat the extraction with a third 50 ml portion of hexane. Discard the aqueous layer and combine the hexane extracts.

6. Filter the combined extracts through a 4" funnel plugged with glass wool which is covered with sodium sulfate. Collect the filtrate in a Kunderna-Danish evaporative concentrator, 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. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

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

8. 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.

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

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

Extraction of Sediment and Soil Samples

1. Decant off any excess water and transfer the entire sediment sample to a glass baking dish. Air dry at ambient temperature (heat should not be applied).

2. When dry, transfer the soil/sediment to a mortar and pestle and grind. Sieve the ground material through a No. 30 mesh sieve and weigh 250g (to the nearest 0.01g) into a 16 oz. narrow neck screw cap (aluminum foil liner) glass bottle.

3. Moisten the soil with water (~10 ml) and add 150 ml of acetonitrile. Cap the bottle tightly, and mechanically shake for a minimum period of 1 hour.

4. Quantitatively transfer the acetonitrile extract into a 600 ml sintered glass filter funnel containing a 1/4" layer of anhydrous sodium sulfate. Collect the filtrate in a 600 ml beaker (vacuum filtration may be necessary).

5. After the acetonitrile has completely drained into the beaker, wash the bottle twice with 50 ml portions of acetonitrile, adding each wash to the funnel after the previous has completely percolated through the sediment.

6. Quantitatively transfer the extract to a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the solvent volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED INTO A HOOD.)

7. After cooling remove the 5 ml graduated tube and transfer the concentrate of extracts to a 125 ml extraction flask with the aid of several small portions of solvent.

8. Evaporate the extract just to dryness with a gentle stream of dry filtered nitrogen and add 25 ml of 2.5% alcoholic potassium hydroxide.

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

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

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

12. 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.

13. 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.

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

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

16. 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.

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

18. 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.

19. 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.

20. 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.

21. 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.

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

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

Column Temperature: 160°C
Detector Temperature: 300°C
Injection Port Temperature: 195°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 method.

The electron capture detector should also be used to guide the isolation procedures. Water and sediment extracts can be checked for the presence of PCBs and/or interferences by injecting µl portions of the extracts at various points in the extraction and concentration schemes. In this manner, it can be determined if the sample needs to be concentrated or diluted and if the clean up procedures should be employed.

DISCUSSION

Extraction

The extraction of PCB's from water employing hexane as the extractant was found to be quantitative and to be sufficiently simple and rapid for use as a routine procedure.

The evaluation of this method was based on spiking water samples with standard acetone solutions of PCB's. The spiking method consisted of adding the PCB's in acetone (25-50 µl) to 500 ml of tap water in a 32 oz. narrow neck screw cap jar. After thoroughly mixing, duplicate 225-250 ml aliquots were taken and subjected to the proposed sample preparation and work up as outlined. The results were quantified by preparing a calibration curve using standard hexane solutions of the PCB's used to spike the water samples. The major isomer peak height was used to construct the calibration plot.

The average recovery and deviation achieved substantiated the applicability of the method for the quantitative recovery and analysis of PCB's from water at the ppb-ppm level.

No PCB recovery experiments from spiked sediment and soil samples have been performed. Instead, several of the residual solids representative of some of the types of sediment or soil analyzed were re-extracted with hexane/acetone (40/60) in a soxhlet extractor to test for the efficiency of the acetonitrile extraction step. The hexane, after isolation by dilution with distilled water was then carried through the purification steps. Recoveries by soxhlet extraction have indicated that the acetonitrile extraction of PCB's was essentially quantitative in the cases checked.

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. However, the Kunderna-Danish evaporative concentrator and the stream of air were employed because of the ease of use.

Column Adsorption Chromatography and Chemical Clean Up

Silica gel, Florisil and Alumina deactivated with 0, 1.0, 1.5, 2.0 and 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. PCB's 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 temperatures given are optimum for 42% chlorinated biphenyl, temperatures are increased when specifically analyzing for the higher chlorinated biphenyls, i.e., 54%, 60%, etc. 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. Except for sharp peaks, peak area measurements are usually more reproducible than peak height measurements but are extremely time consuming unless a recording integrator is employed. However, peak height measurements are as accurate as disc integration of triangulation and if the peak shape represents a gaussian curve, the height may be considered independent of the base. Consequently, peak height measurements were generally used. Three variations of the peak height quantification procedure were 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 PCB's in Case I samples were determined by preparing a plot of the major peak height vs. concentration; for Case II, a plot of the total sum of all major peaks 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. With our instrument any response less than 50 at an attenuation of 8 x 10 fell into the linear response range at a pulse rate of 150. This corresponds to approximately 5 x 10^{-9}g of Aroclor 1242.

Contamination:

In determining PCB's in water, soil and sediment by electron capture gas chromatography, laboratory sources of contamination can be a major 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.

db

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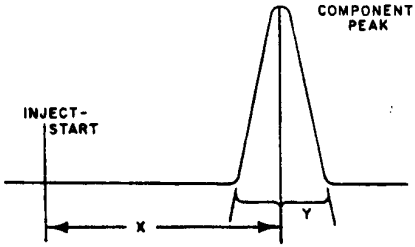
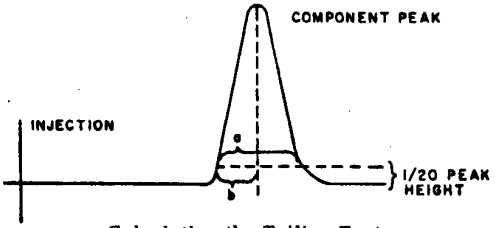
COLUMN PERFORMANCE INDICATORS



Calculating Column Efficiency

1. Theoretical Plates, N

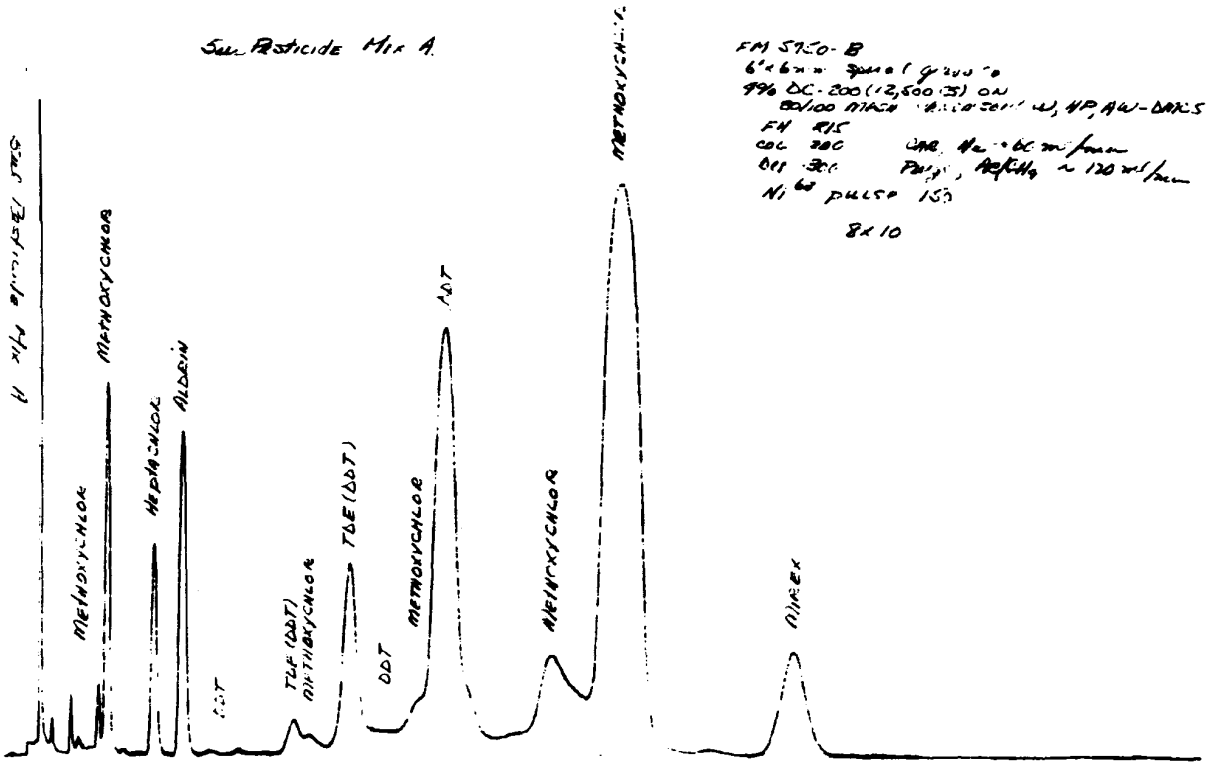
$$N = 16(x/y)^2$$



Calculating the Tailing Factor

2. Tailing, T

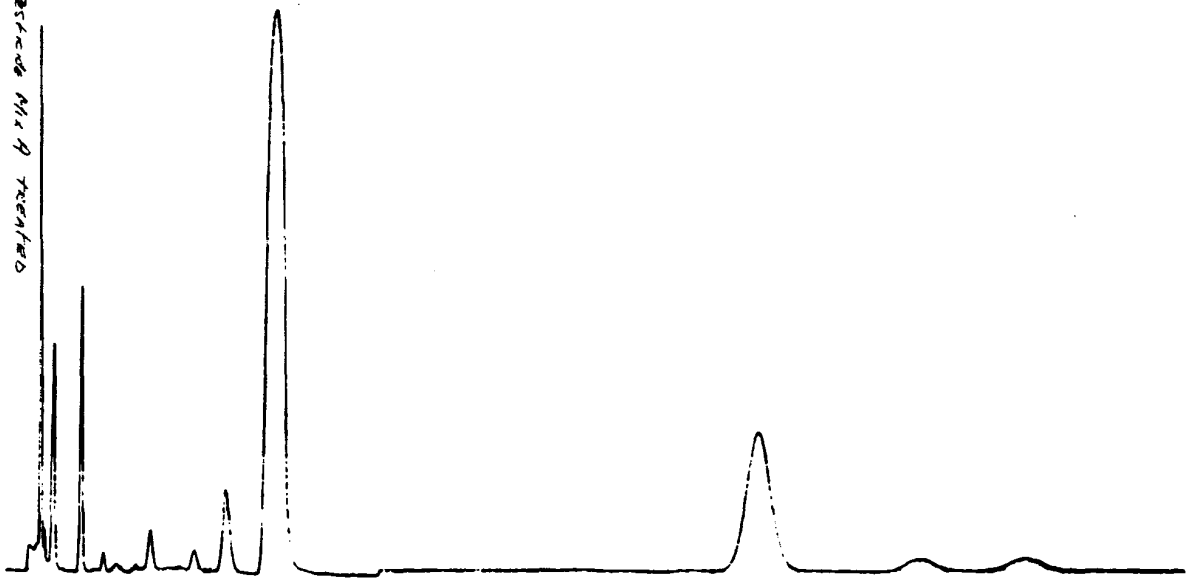
$$T = a/2b$$


Sun Pesticide Mix A
FM 5750-B
6'x6mm Spiral Glass
4% DC-200 (12,500 CS) ON
80/100 MESH
FH R15
COL 200
DET 300
Ni 63 pulse 150
8x10
Sun Pesticide Mix A
METHOXYCHLOR
METHOXYCHLOR
HEPTACHLOR
ALDRIN
DDT
TDE (DDT)
METHOXYCHLOR
TDE (DDT)
DDT
METHOXYCHLOR
DDT
METHOXYCHLOR
METHOXYCHLOR
MIREX

MONS 075525

Std Pesticide Mix 4 Treated

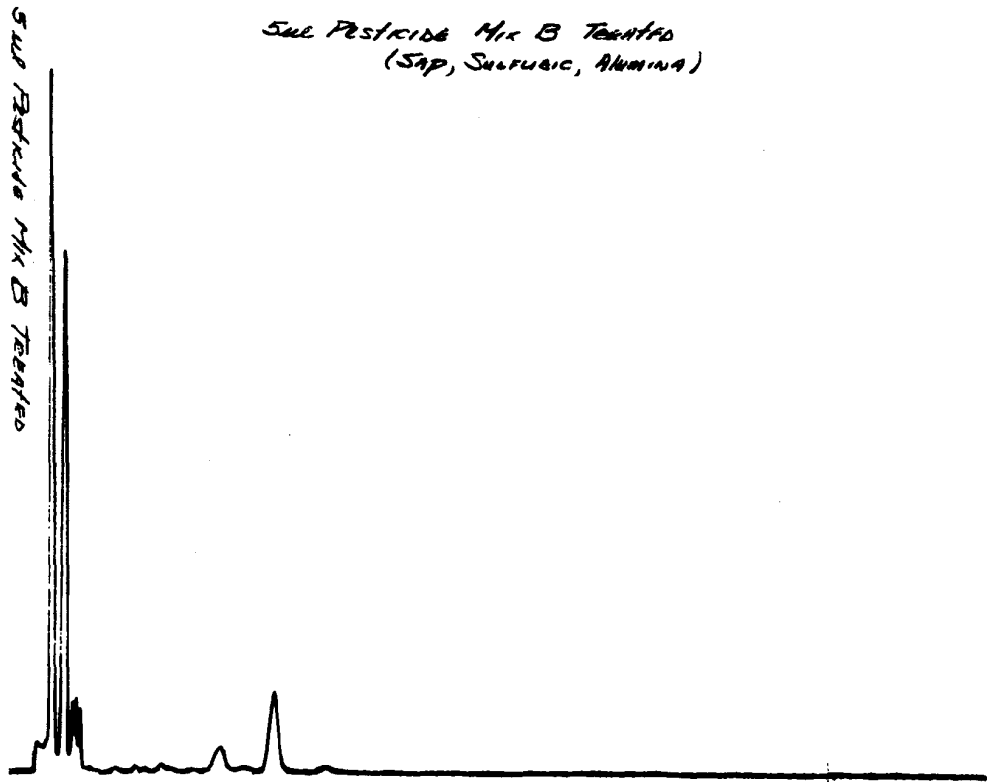
Std. Pesticide Mix 4
Treated (Sat., Sulfuric., Alumina)



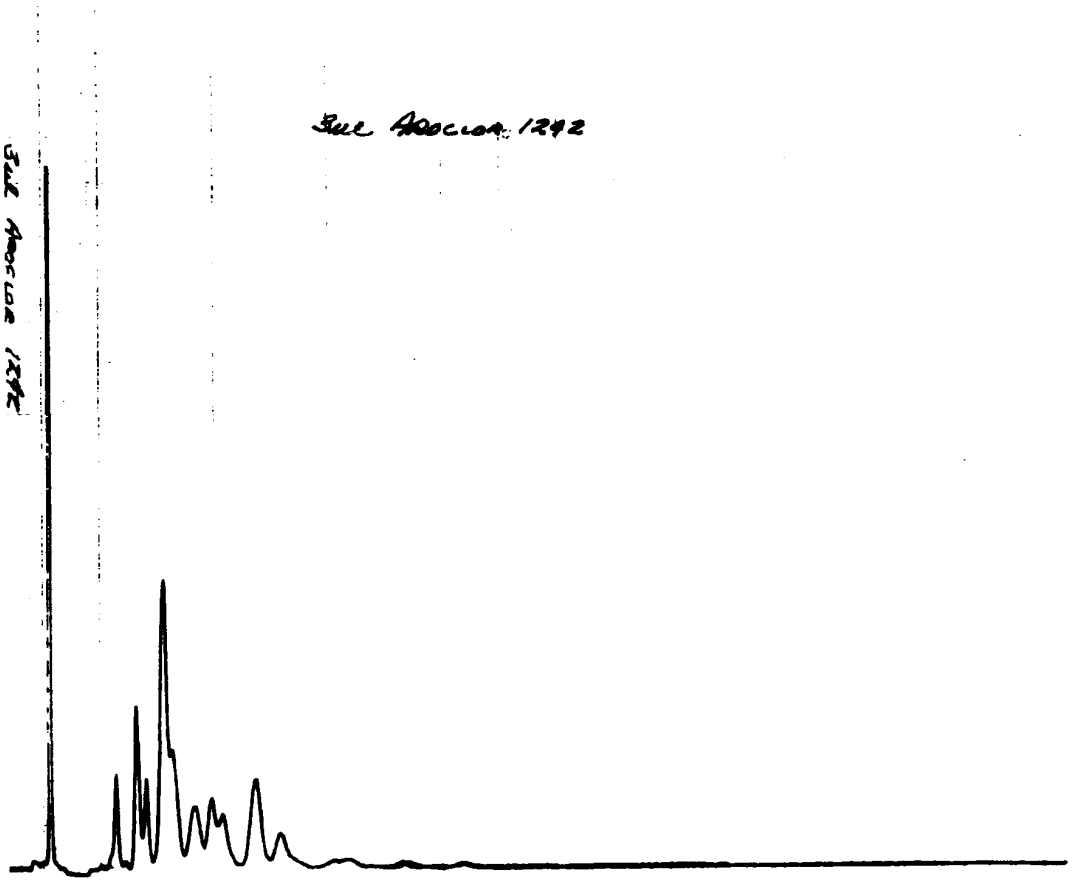
MONS 075526

Std. Pesticide Mix B
Std. Pesticide Mix B
Aldrin
BHC
BHC
Endosulfan
Dieldrin
Aldrin
Endosulfan
Dieldrin

MONS 075527


5ul Pesticide Mix B Treated
5ul Pesticide Mix B Treated
(Sap, Sulfuric, Alumina)

MONS 075528



Sul Aroclor 1242

MCNS 075529

Std Aroclor 1254

Std Aroclor 1254

MONS 075530

3rd Section 1260

MCNS 075531



MONS 075532

10ul Chlordane Treated

MONS 075533

MONS 075534

MCNS 075535

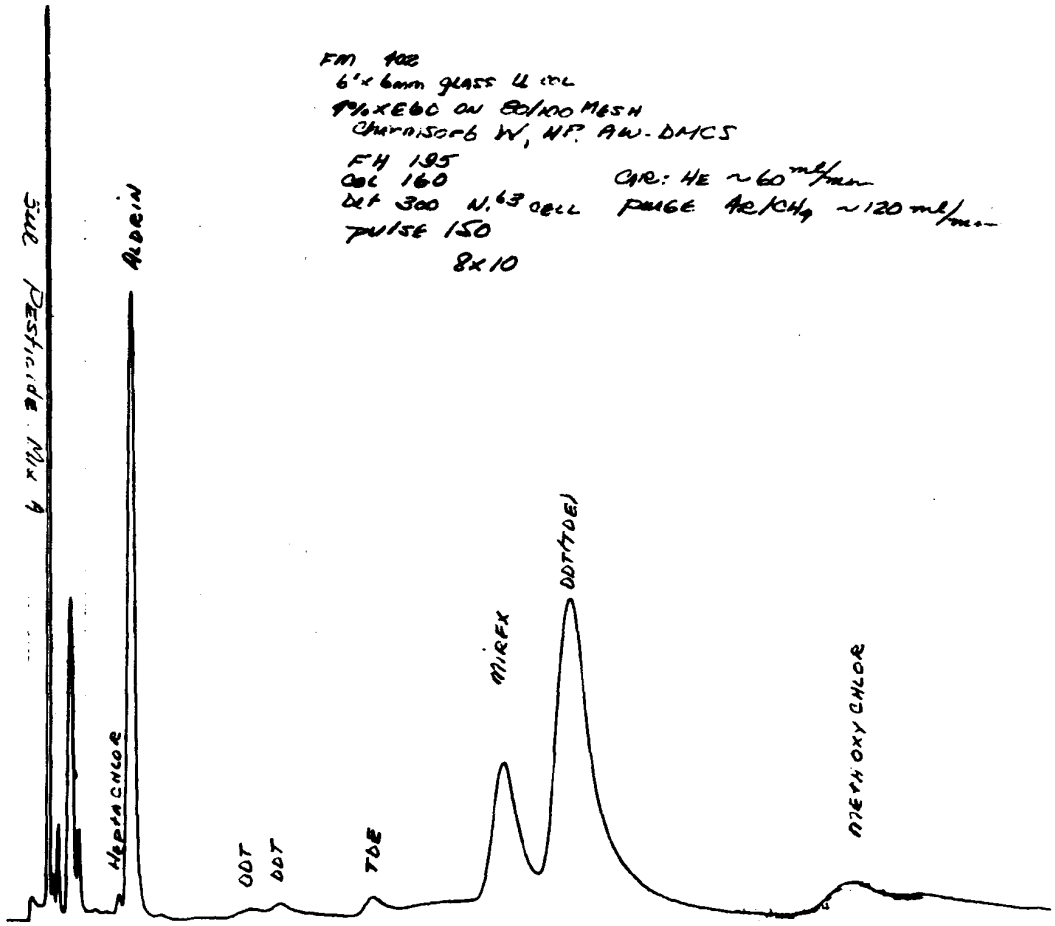
MONS 075536

Sue Shadbane Tested

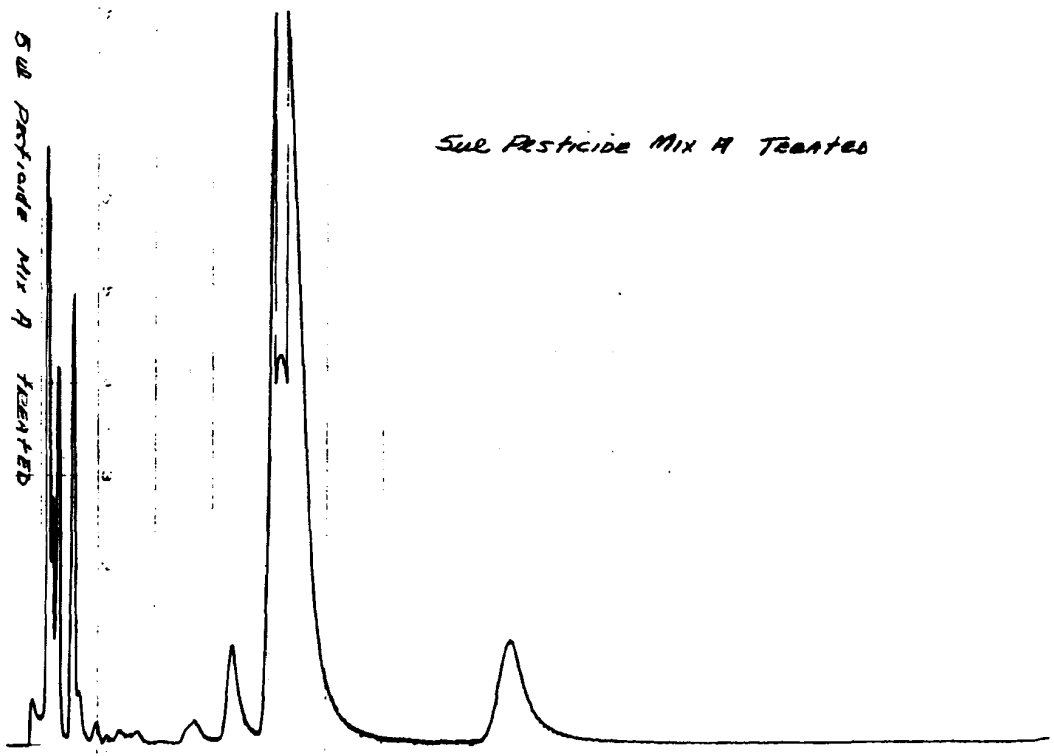
Sue Shadbane Tested

MONS 075537

MDNS 075538


FM 402
6' x 6mm glass U col
4% XE60 on 80/100 MESH
Chromosorb W, HP AW-DMCS
FH 195
Col 160
Det 300 N.63 cell
Pulse 150
8x10
CAR: He ~60 ml/min
PURGE Ar/CH4 ~120 ml/min
3ml Pesticide Mix 4
ALDRIN
HEPTACHLOR
ODT
DDT
TDE
MIREX
DDT(TOE)
METHOXYCHLOR

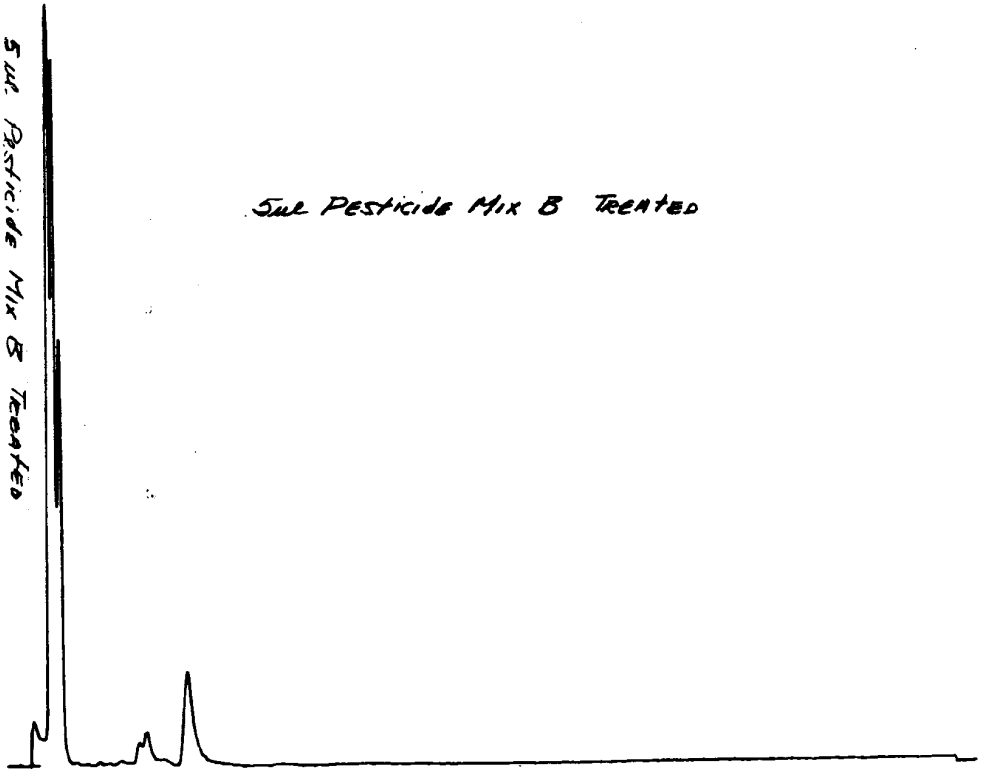
MCNS 075539



MONS 075540

MONS 07554

Std Pesticide Mix B
Perthane
BHC
BHC
Endosulfan (BHC)
BHC
Perthane
Dieldrin
Perthane
Endrin
Endosulfan



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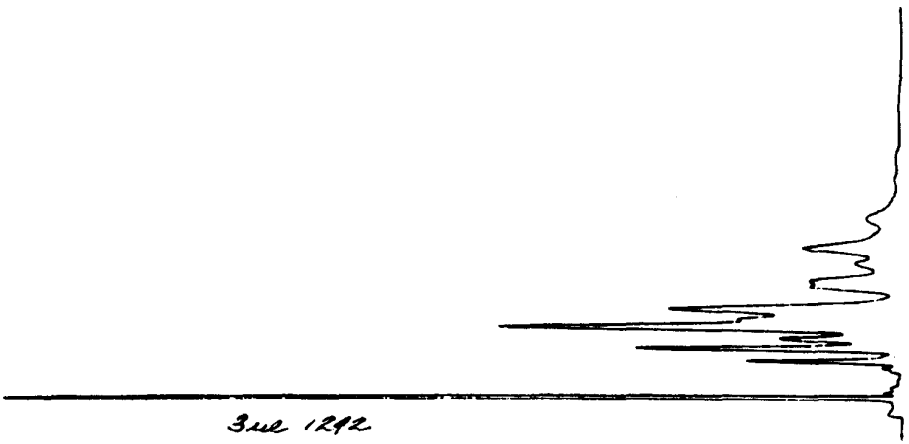
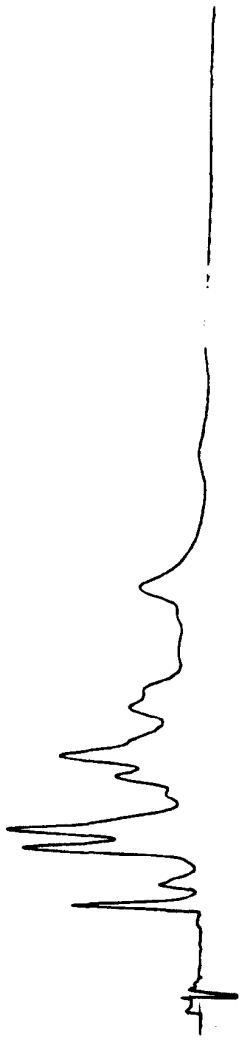


3ue 1292

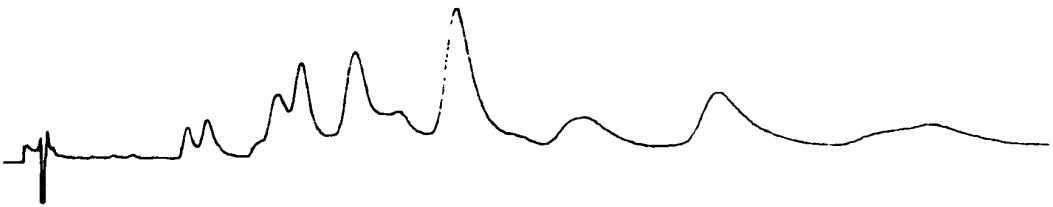


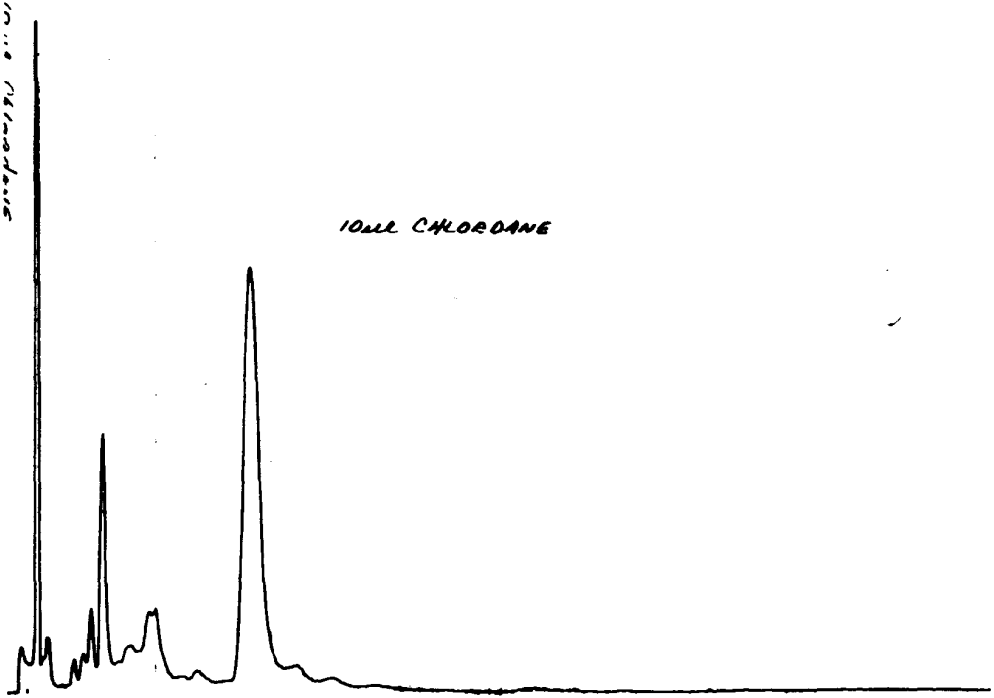
3ml Acetone 1251

MONS 075544

See Aroclor 1260

MONS 075545





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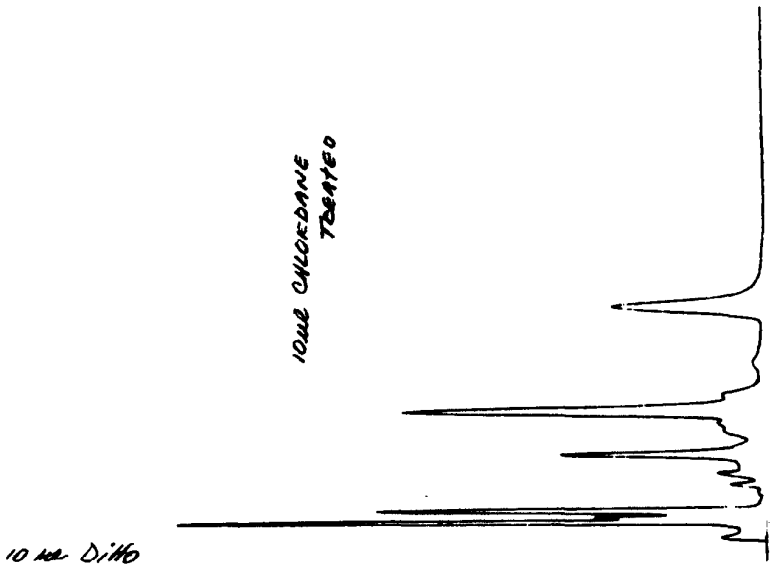

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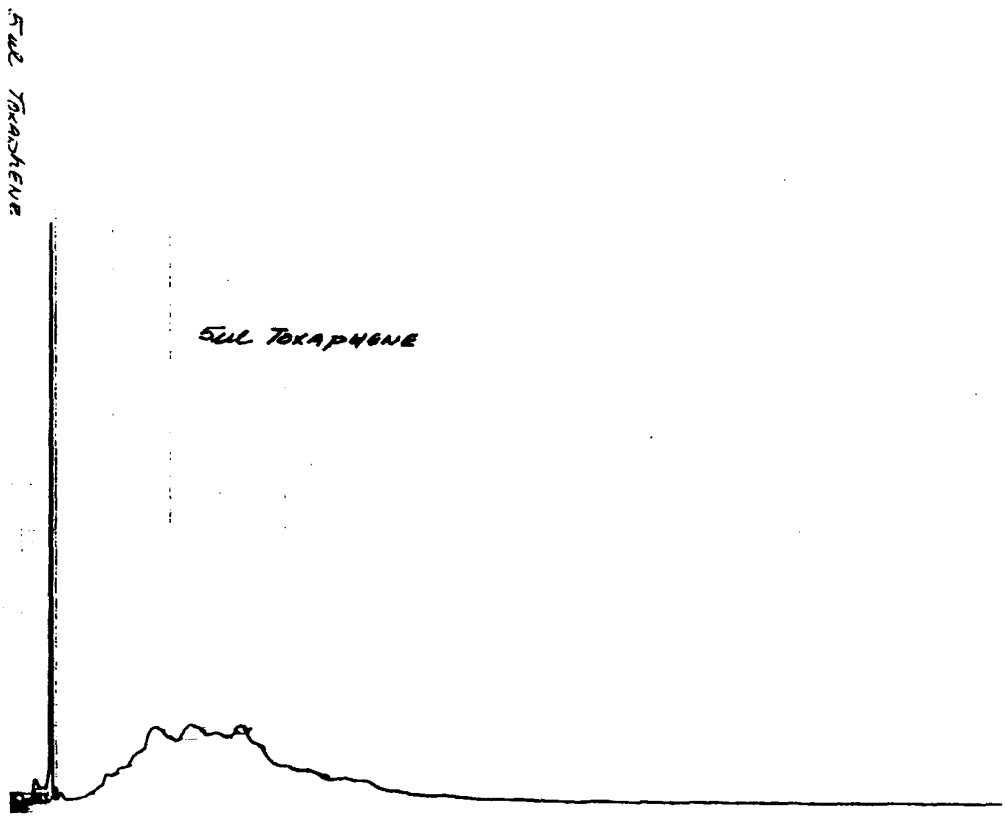


MONS 075548

MONS 025545

Full Strobane

Full Strobane

MONS 075550

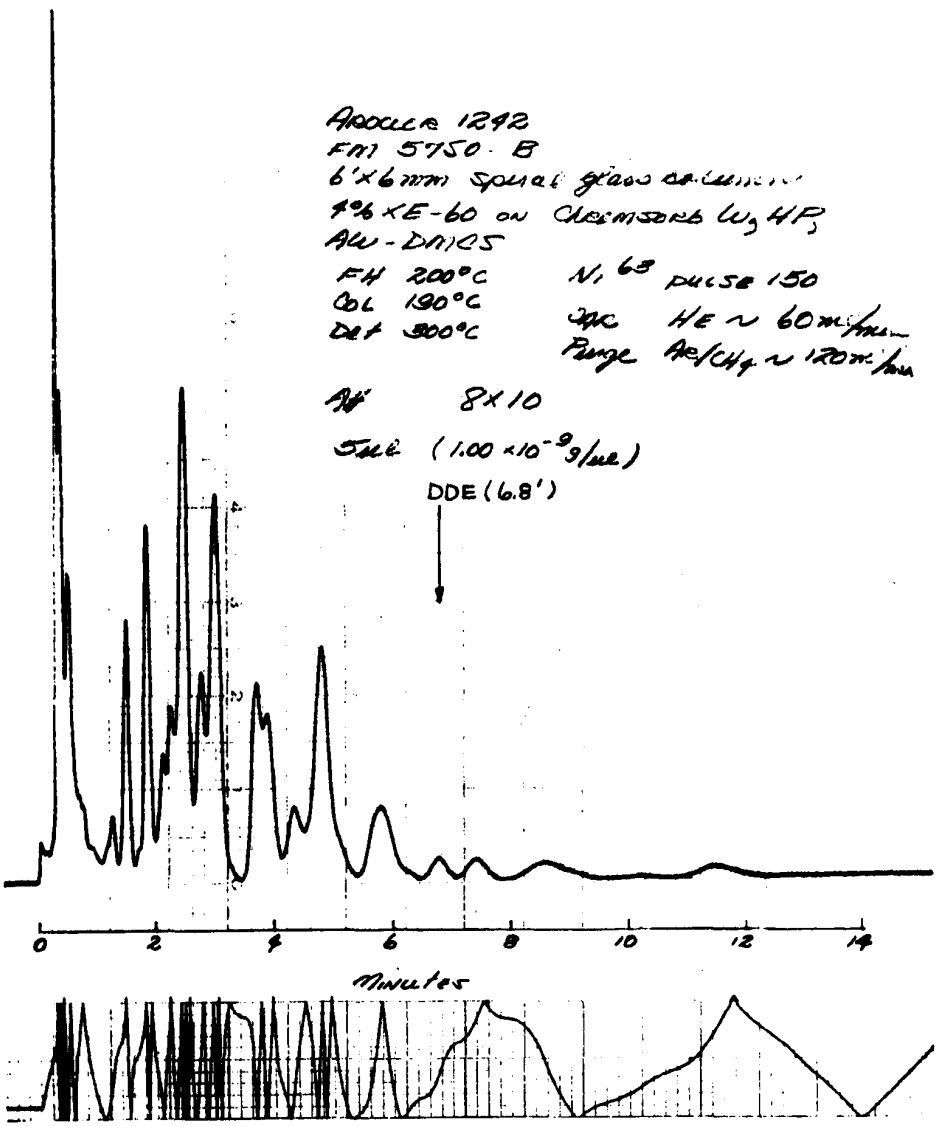
Sue Steadman TREATED

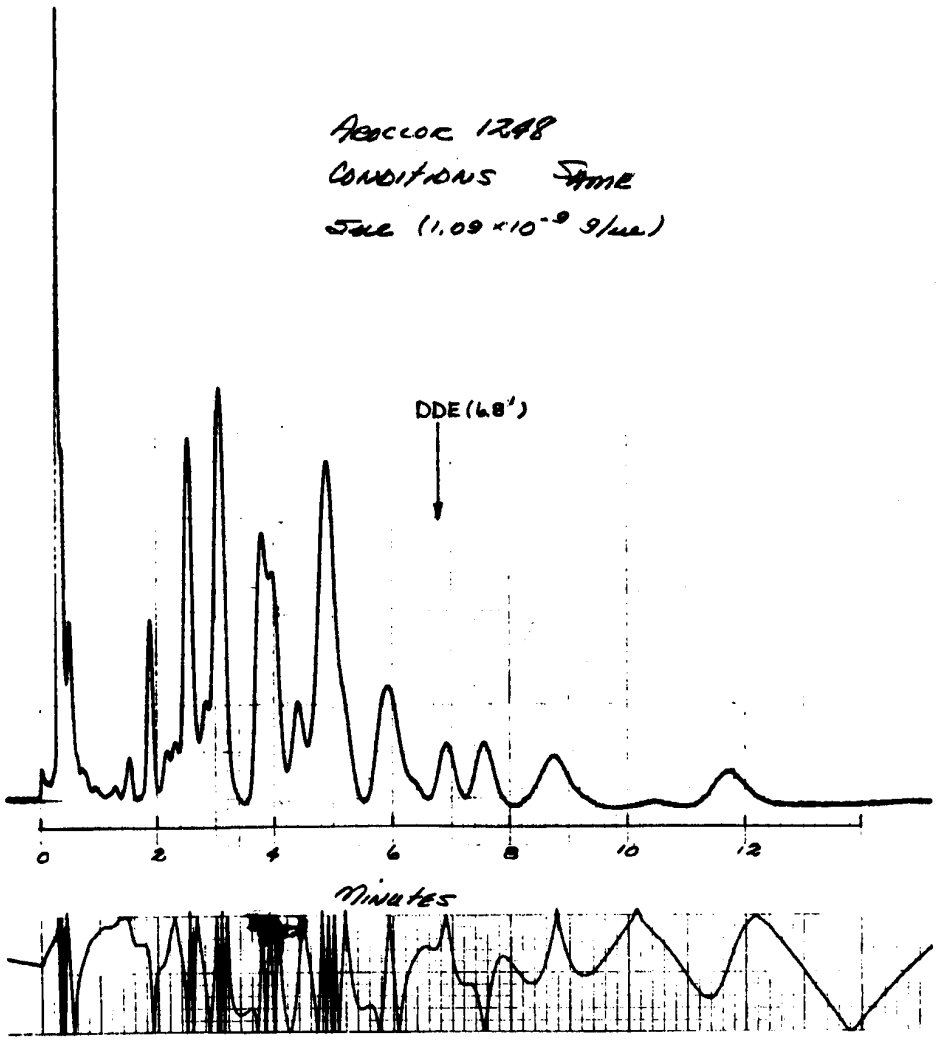
MCNS 025551

10uL Blank

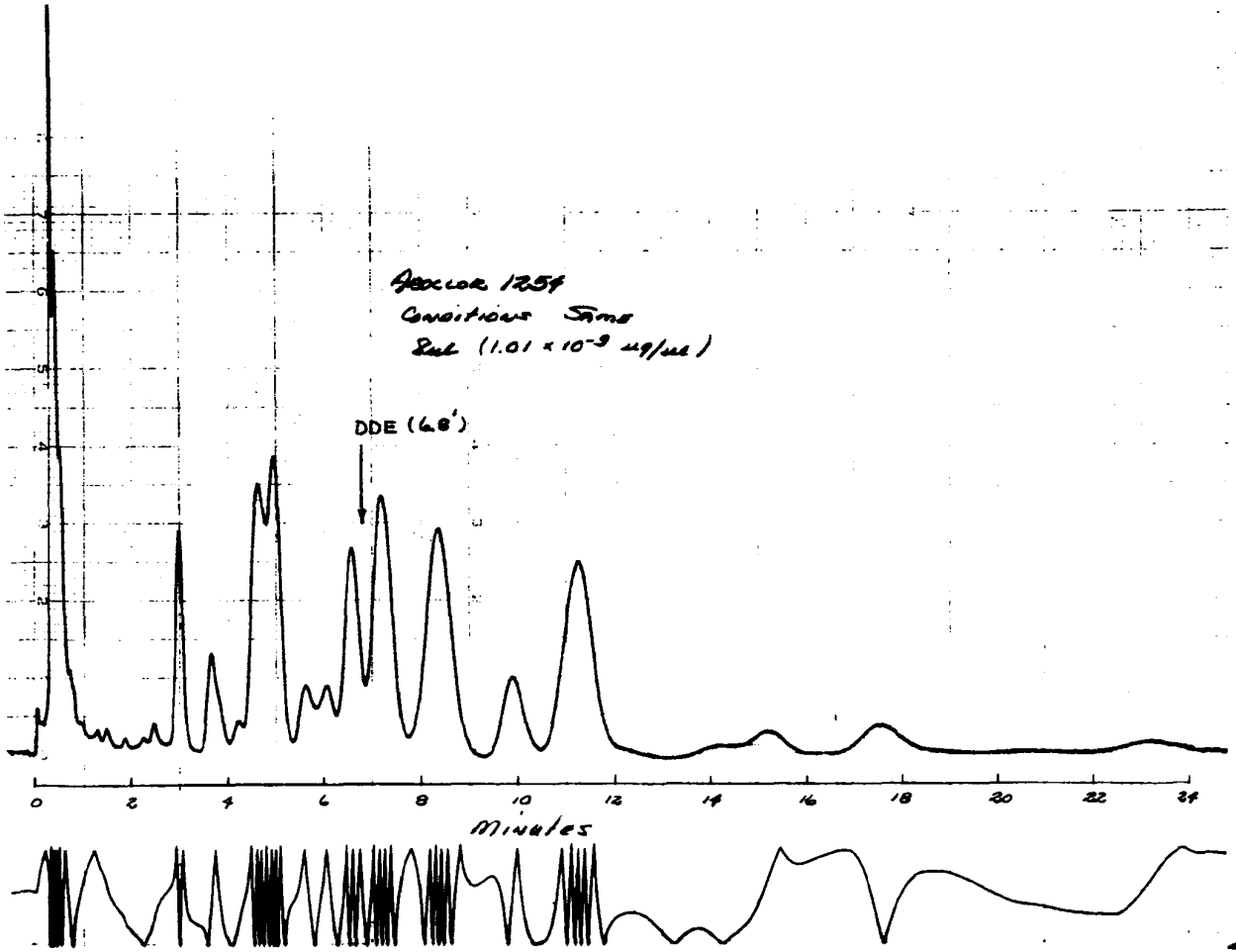
10uL Blank

MDNS 075552

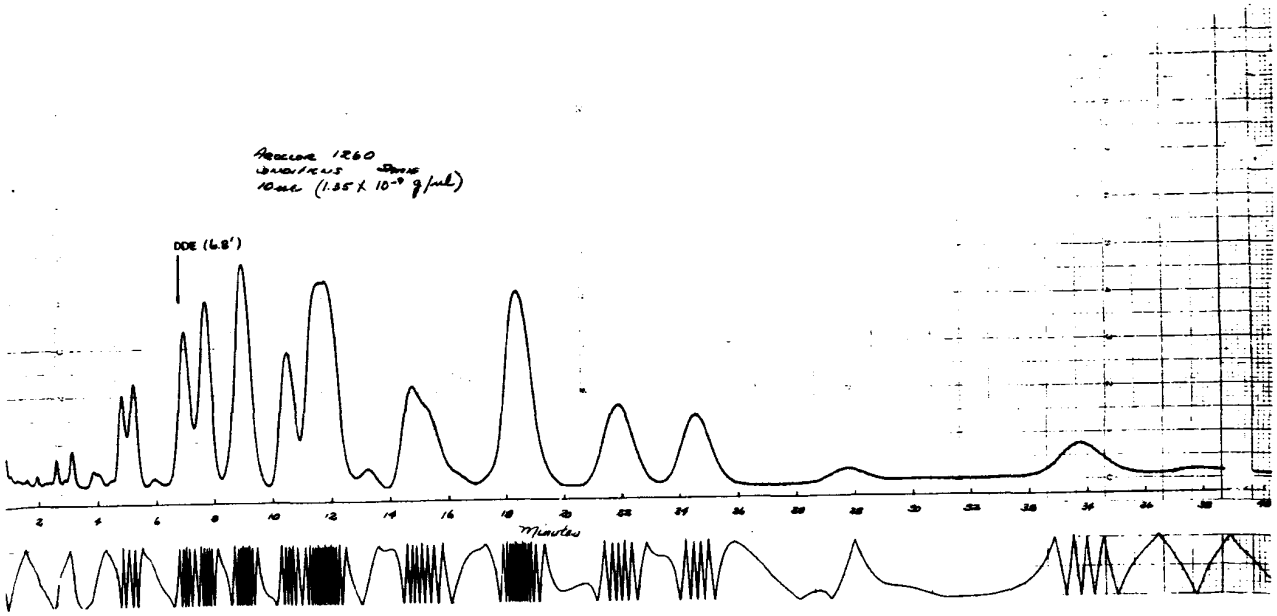

Aroclor 1242
FM 5750 B
6'x6mm Pyrex glass column
4% XE-60 on Chromosorb W, HP
AW-DMCS
FH 200°C
Col 190°C
Det 300°C
Ni 63 pulse 150
Gas He ~ 60 ml/min
Purge Ar/CH4 ~ 120 ml/min
Att 8x10
Std (1.00 x 10⁻⁹ g/ml)
DDE (6.8')
0
2
4
6
8
10
12
14
Minutes


Aroclor 1248
Conditions Same
5 ul (1.09 × 10⁻⁹ g/ul)
DDE (6.8')
0
2
4
6
8
10
12
Minutes

MONS 075554


Aroclor 1254
Conditions Same
Run (1.01 x 10⁻⁹ ug/ml)
DDE (6.8')
0
2
4
6
8
10
12
14
16
18
20
22
24
Minutes

MCNS 075555



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