JPM

Designation: D 3304 – 74

AMERICAN SOCIETY FOR TESTING AND MATERIALS
1916 Race St., Philadelphia, Pa., 19103

Standard Method for ANALYSIS OF ENVIRONMENTAL MATERIALS POLYCHLORINATED BIPHENYLS[1]

This Standard is issued under the fixed designation D 3304; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval.

1. Scope

1.1 This method covers the determination of the amount and type of polychlorinated biphenyls (PCB's) in environmental materials, specifically air, water, soils, and sediments. Absolute confirmation of PCB structures is not obtained with this method. Structure proof can be obtained using additional techniques such as mass spectrometry.

2. Applicable Documents

2.1 *ASTM Standards:*

D 510 Sampling Industrial Water[2]

D 1587 Thin-Walled Tube Sampling of Soils[3]

D 1605 Recommended Practices for Sampling Atmospheres for Analysis of Gases and Vapors[2]

D 2928 Sampling Stacks for Particulate Matter[2]

3. Significance

3.1 Since it is possible for minute quantities of PCB's to find their way into the environment, it is desirable to have a method to identify and measure the quantity of such trace amounts.

4. Sampling

4.1 *Water Sampling*—Environmental sampling of natural bodies of water and effluents are covered in many methods under the jurisdiction of Committee D-19 on water. Method D 510 is recommended with the following stipulations:

4.1.1 Under 26.1 a final rinse with Nanograde[4] hexane should be added.

4.1.2 The sample collected should be at least 250 ml.

4.2 *Sediment and Soil Sampling*—A sediment or soil sample of at least 1000 g should be taken according to Method D 1587 or any other acceptable procedure, taking due precautions to minimize contamination.

4.3 *Air Sampling:*

4.3.1 Environmental sampling of ambient air or source sampling is under the jurisdiction of ASTM Committee D-22 on Methods of Sampling and Analysis of Atmospheres. There are various methods of sampling from stacks or ambient air available. See Method D 1605 or Method D 2928, or both.

4.3.2 For the measurements of airborne PCB's the air sample is drawn through one or more fritted bubbler absorbers filled with nanograde toluene. The PCB's are absorbed in the toluene.

5. Principle

5.1 The PCB's present in environmental samples are isolated from the bulk of the sample matrix by solvent extraction. Interfering matrix components and endogenous residues are then removed from the sample

[1] This method is under the jurisdiction of ASTM Committee D-27 on Electrical Insulating Liquids and Gases.
Current edition approved Feb. 27, 1974. Published April 1974.

[2] *1973 Annual Book of ASTM Standards*, Part 23.

[3] *1974 Annual Book of ASTM Standards*, Part 19.

[4] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, D. C. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Reagent Chemicals and Standards," by Joseph Rosin, D. Van Nostrand Co., Inc., New York, N. Y., and the "United States Pharmacopeia."

extracts by a suitable combination of clean-up procedures and the amount and type of PCB's present determined by electron capture gas chromatography (EC/GC).

6. Apparatus

6.1 *Separatory Funnels*, equipped with ground-glass stoppers and TFE-fluorocarbon stopcocks: 125, 250, 500, 1000, and 2000-ml capacities.

6.2 *Kunderna-Danish Evaporative Concentrators*, 500-ml capacity equipped with 3-ball Snyder columns and graduated 5-ml capacity vials.

6.3 *Chromatographic Columns*, glass, 10 in. (254 mm) long by 20 mm in outside diameter with a reservoir 5 in. (127 mm) long by 50-mm in outside diameter at the top, equipped with TFE-fluorocarbon stopcocks.

6.4 *Buchner Filter Funnels*, borosilicate glass, fritted, 90 mm in inside diameter, 600-ml capacity, medium porosity.

6.5 *Boiling Flasks*, flat-bottomed, 125-ml capacity.

6.6 *Liebig Condensers*, 200 mm in length.

6.7 *Hot Plates*.

6.8 *Water Bath*.

6.9 *Reciprocating Variable-Speed Shaker*.

6.10 *Syringes*, 10-μl.

6.11 *Mortar and Pestle*, all glass, 32-oz (or 1-litre).

6.12 *Baking Dishes*, borosilicate, 2½-qt (2.4-litre), 8 by 12 by 2-in. (203 by 305 by 50-mm).

6.13 *Sieve*—U.S. Standard sieve No. 30 (600-μm).

6.14 *Gas Scrubbing Bottles*, high-form, ground-glass joint, fritted-coarse disks, 250-ml capacity.

6.15 *Dry Test Meter*, 0.1 ft[3] per revolution.

6.16 *Laboratory Vacuum Pump*.

6.17 *Rotary Vacuum Evaporator*.

6.18 For the apparatus used in the Electron Capture Gas Chromatographic Procedure, see Section 10.

7. Reagents

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[4] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *Acetonitrile*, Nanograde.[5]

NOTE —Nanograde designates impurities of no more than 10 parts per trillion.

7.3 *Alcoholic Potassium Hydroxide Solution (2.5 % weight per volume)*—Dissolve 12.5 g of potassium hydroxide in 500 ml of ethanol.

7.4 *Alumina* (for chromatographic adsorption), 80/200 mesh. Heat at 400°C for a minimum period of 4 h and deactivate with 5 % (w/w) distilled water.

7.4.1 *Alumina Column Preparation*—Fill a chromatographic column (6.3) 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 (30 g), and pour this slowly into the column. Tap or vibrate the column to settle the alumina and top the alumina with 2 to 3 cm of anhydrous sodium sulfate. Wash the column with 50 to 100 ml of hexane prior to the addition of the sample.

7.5 *Distilled Water*, extracted with hexane to remove hexane-soluble electron-capturing impurities.

7.6 *Ethanol*—Formula 2B.

7.7 *Hexane*, Nanograde.[5]

7.8 *PCB Standards*, production grade. PCB Standards Aroclor 1221,[6] Aroclor 1242, and Aroclor 1254.

7.9 *Potassium Hydroxide* (KOH).

7.10 *Sodium Sulfate*, anydrous, granular. Heat the sodium sulfate (Na_2SO_4) at 400°C for 1 h prior to use.

7.11 *Sulfuric Acid (sp gr 1.84)*—Concentrated sulfuric acid (H_2SO_4).

7.12 *Toluene*, Nanograde.[5]

8. Sample Preparation and Extraction

8.1 *Water:*

8.1.1 Where possible, the entire water

[5] Registered trademark of Mallinckrodt Chemical Works.

[6] Registered trademark of Monsanto Co.

sample is extracted with hexane. With larger samples, where this is not physically possible, the containers are simply agitated and a 250-ml portion used for analysis.

8.1.2 *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.

8.1.3 Rinse the sample container and graduate cylinder with two 50-ml portions of hexane, adding each to the separatory funnel. If the entire sample is not consumed, rinse only the graduate cylinder.

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

8.1.5 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.

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

8.1.7 Filter the combined extracts through a 4-in. (100-mm) funnel plugged with glass wool that 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 an 80 to 90°C water bath. CAUTION—*Solvent vapors must be vented into a hood.* Remove the evaporative concentrator from the water bath and after cooling to room temperature adjust the volume to 5.0 ml.

8.1.8 Inject a fraction of a microlitre of the concentrate into the gas chromatograph to check for interferences and determine the approximate level of PCB's 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.

8.1.9 If interferences are present, proceed with the chemical treatment and column chromatographic cleanup procedures.

8.2 *Sediment and Soil:*

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

8.2.2 Weigh 250 g (to the nearest 0.01 g) into a 16-oz (or 500-ml) narrow-neck screw-cap glass bottle (with an aluminum foil liner).

8.2.3 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 h.

8.2.4 Quantitatively transfer the acetonitrile extract into a sintered-glass filter funnel containing a ¼-in. (6.4-mm) layer of anhydrous Na_2SO_4. Collect the filtrate in a 600-ml beaker (vacuum filtration may be necessary).

8.2.5 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.

8.2.6 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 to 90°C water bath. CAUTION—*Solvent vapors must be vented into a hood.*

8.2.7 Carefully evaporate the acetonitrile extracts to dryness with the aid of a gentle stream of dry nitrogen. Redissolve the residue in maximum of 5 ml of hexane.

8.2.8 Inject a fraction of a microlitre of the concentrate into the gas chromatograph to check for interferences and determine the approximate level of PCB's 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.

8.2.9 If interferences are present, proceed with the chemical treatment and column chromatographic cleanup procedures.

8.3 *Airborne PCB's:*

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

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

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

8.3.4 Inject a fraction of a microlitre of the concentrate into the gas chromatograph to check for interferences and determine the approximate level of PCB's 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.

8.3.5 If interferences are present, proceed with the chemical treatment and column chromatographic cleanup procedures.

9. Supporting Procedures—Sample Clean Up

9.1 *Saponification with Alcoholic Potassium Hydroxide and Extraction with Sulfuric Acid:*

9.1.1 Quantitatively transfer the concentrated extracts to a 125-ml extraction flask with the aid of several small portions of solvent.

9.1.2 Evaporate the extract just to dryness with a gentle steam of dry filtered air (5A molecular sieve of equivalent to remove oil, water, and particles above 12 μm) and add 25 ml of 2.5% alcoholic KOH.

9.1.3 Add a boiling chip, put a water condenser in place, and allow the solution to reflux on a hot plate for 45 min.

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

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

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

9.1.7 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.

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

9.1.9 *Carefully* add 25 ml of concentrated H_2SO_4, in small portions, to the hexane extracts.

9.1.10 Stopper the separatory funnel and shake vigorously for at least 1 min, venting the funnel as necessary. Allow the layers to separate and discard the lower aqueous-acid layer. Repeat this until the acid layer is colorless.

9.1.11 Wash the hexane with a 25-ml portion of water. Discard the water wash.

9.1.12 Filter the hexane extract through a 4-in. (100-mm) funnel plugged with glass wool that is covered with a layer of anhydrous Na_2SO_4. 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 an 80 to 90°C water bath. CAUTION—*Solvent vapors must be vented into a hood.* Remove the evaporative concentrator from the water bath and after cooling to room temperature adjust the volume to 5.0 ml.

9.2 *Liquid-Solid Chromatographic Clean-Up With Alumina:*

9.2.1 After preparation of the alumina column, as described in Section 7, quantitatively transfer the hexane extracts (5 ml or less) on to the column with the aid of several 5-ml portions of hexane (lowering the solvent level to the adsorbant level after each addition of solvent. Do not allow the top of the column to become dry).

9.2.2 Carefully add 125 ml of hexane to the column reservoir and collect the total eluent in a Kunderna-Danish evaporative concentrator.

9.2.3 Add a small boiling chip to the Kunderna-Danish evaporative concentrator;

put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in an 80 to 90°C water bath. CAUTION—*Solvent vapors must be vented into a hood.* Remove the evaporative concentrator from the water bath and after cooling to room temperature adjust the volume to 5.0 ml.

10. Electron Capture Gas Chromatographic Procedure

10.1 *Instruments*—A gas chromatograph equipped with isothermal oven temperature control.

10.2 *Detector*—High-temperature ^{63}Ni Electron Capture Cell

10.3 *Column*—A 6-mm by 6-ft (1.8-m) glass column, 4 % XE-60 on 80/100 mesh Chromosorb W, high-performance.

10.4 *Column Temperature*—The column temperature shall be as follows for the various Aroclors: Aroclor 1221, 170°C; Aroclor 1242, 190°C; Aroclor 1254, 205°C.

10.5 *Detector Temperature*—The detector temperature shall be 250 to 300°C.

10.6 *Injection Port Temperature*—The injection port temperature shall be 220°C.

10.7 *Pulse*—The pulse rate shall be 50 μs.

10.8 *Flow Rates*—The flow rates shall be as follows:

10.8.1 *Helium Carrier*—60 ml/min.

10.8.2 *90% Argon-10% Methane Purge*—20 ml/min.

10.9 Using EC/GC as the determinative step, inject in duplicate 2 to 8 μl of each sample solution into the chromatograph. By comparison with standard solutions injected, under the same operating conditions, determine the amount and type of Aroclor using the total area method.

11. Quantitative Determination

11.1 Quantitative determinations employing the electron capture detector are non-stoichiometric measurements made by comparing the total areas for known concentrations with those for unknown concentrations. Four variations of the area quantification procedure have been employed, as follows:

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

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

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

11.1.4 *Case IV*—EC gas chromatogram of PCB unknown altered with respect to standard PCB with evidence of interference.

11.2 Determine the PCB level in Case I and II samples by comparison of the total area of the peaks in the unknown with a calibration curve prepared by plotting the total area of the peaks in the electron capture chromatogram of the corresponding standard versus the number of nanograms of the standard injected. Initially subject Case III and IV samples to the chemical cleanup procedure and follow by chromatography on alumina. If the interferences are removed by this treatment, calculate the PCB levels for Cases III and IV in the same manner as Cases I and II. If dominant interference(s) is (are) still present, estimate the maximum PCB level in accordance with Cases I and II after correcting the total area for that of the interfering peak(s). In all cases, the response of the electron capture must be linear for quantitative analysis.

12. Calculations

12.1 Assuming that the calibration curve is prepared by plotting the electron capture response (total peak area) versus nanograms of the PCB standard injected, calculate the PCB level present in the air, water, or sediment-soil sample as follows:

12.1.1 *Water and Sediment-Soil Samples:*

$$C = [(A/B)(D \times 10^6\ \mu l)/\text{ml}\ (10^{-6}\ \text{mg/ng})]/E$$

12.1.2 *Air Samples:*

$$F = \frac{[(A/B)(D \times 10^6\ \mu l)/\text{ml}\ (10^{-6}\ \text{mg/ng})]}{G\,(28.32\ \text{litre/ft}^3)(\text{m}^3/10^3\ \text{litre})\,(P_s - P_v/P_s)(T_s/273 + T_s)}$$

where:

A = PCB in unknown from calibration curve, ng,

B = final concentrate injected into the instrument, μl,

C = concentration of PCB's in water, and sediment-soil sample, mg/kg (or ppm),

D = volume of final concentrate, ml,
E = sample weight, kg,
F = concentration of PCB's in air sample, mg/m³, at one atmosphere and 25°C,
G = air sampled from meter, ft³,
P_s = standard pressure, 760 mm Hg,
P_1 = differential pressure of gas stream, mm Hg,
T_s = standard temperature, 298 K, and
T_1 = temperature of sampled gas, °C.

12.2 The corrections for the pressure drop through the scrubbers and for the gas temperature are necessary to get an accurate measurement of the gas volume sampled.

13. Precision and Accuracy

13.1 In order to establish the precision and accuracy of the method used for estimating PCB content in the environment, a round-robin testing experiment was set up. Samples were prepared by spiking water with PCB's at the level of about 500 ppb. Individual preparations included blanks (no PCB), Aroclor 1242 only, Aroclor 1254 only, Aroclor 1260 only, all 50-50 two-component mixtures, and a ⅓, ⅓, ⅓ preparation containing all three Aroclors. Six samples of each mixture were prepared, and tested by two analytical laboratories. Each result, expressed in ppb of the individual components was converted to percent of the total PCB added. Analysis of variance was run on all the data taken together and on each laboratory independently. Two obviously defective samples were deleted from the data.

13.2 The precision and accuracy of this method on standard air and soil-sediment samples have not been established.

13.3 *Conclusions.*

13.3.1 The 95% confidence limits for individual results were as follows:

Either laboratory	87.3 ± 53.2%
Laboratory A	81.3 ± 46.4%
Laboratory B	93.7 ± 19.2%

13.3.2 The repeatability for the individual laboratories, based on repeat tests of samples having identical prepared compositions is as follows:

Laboratory A	±42.0%
Laboratory B	±11.6%

13.3.3 In general, Laboratory B comes closer to properly identifying the individual Aroclors with one exception, Aroclor 1254 found in a mixture of Aroclor 1242 and Aroclor 1260, as follows:

Mixture	Present	Laboratory A	Laboratory B
1	1242	1242, (1254)	1242
2	1242, 1254	1242, 1254, (1260)	1242, 1254
3	1242, 1254, 1260	1242, ( ), 1260	1242, 1254, 1260
4	1254	1254, (1260)	1254, (1260)
5	1242, 1260	1242, 1260	1242, (1254), 1260
6	1260	(1248), 1260	1260
7	1254, 1260	(1248), 1254 1260	1254, 1260

Numbers in parentheses indicate misidentifications.

14. Contamination

14.1 In determining PCB's 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, TFE-fluorocarbon, or metal. Laboratory glassware and sampling gear 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 procedure to insure against the possibility of contamination.

APPENDIX

ELECTRON CAPTURE CHROMATOGRAMS OF COMMERICAL PCB MIXTURES

AROCLOR 1221

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 170°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min.
Purge Gas - 10% CH4/Argon 120 ml/min
Pulse Interval - 50 us
Amt. Injected - 5 ul
Std. Conc. - 1.48 X 10^{-6} g/ml
Range - 10
Attn. - 4

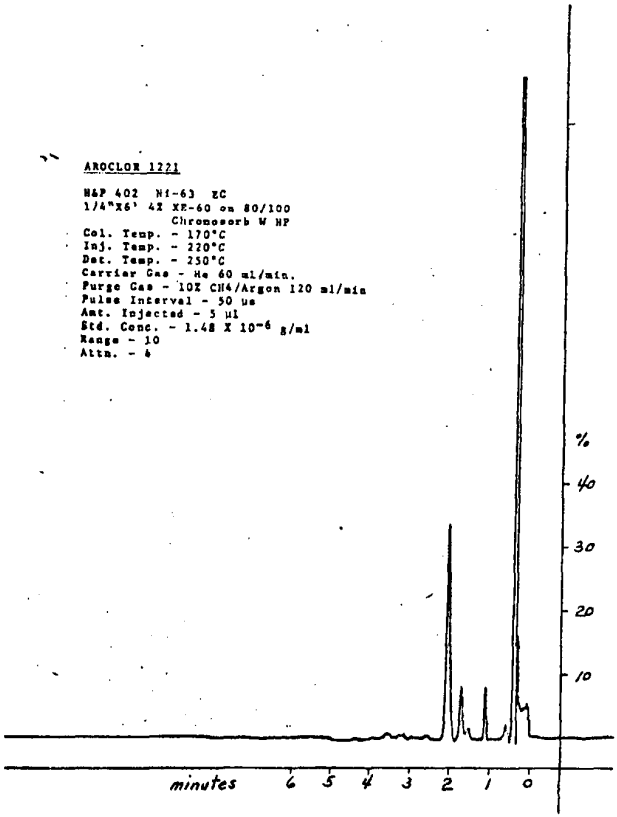

FIG. A1 Aroclor 1221 Electron Capture Chromatogram.

AROCLOR 1242

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 190°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min.
Purge Gas - 10% CH4/Argon 120 ml/min
Pulse Interval - 50 μs
Amt. Injected - 5 μl
Std. Conc. - 1.03 X 10^{-6} g/ml
Range - 10
Attn. - 8

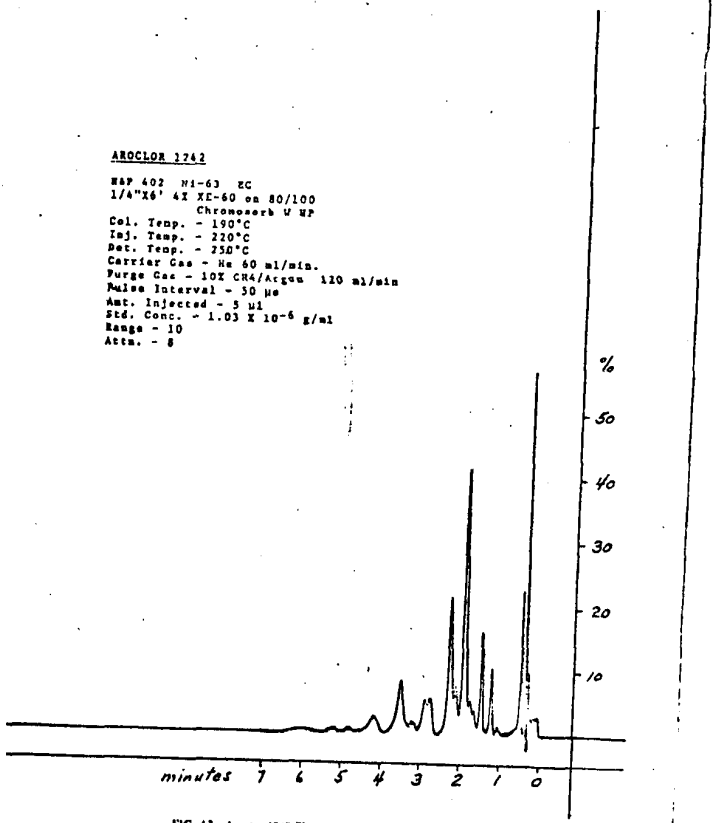

FIG. A2 Aroclor 1242 Electron Capture Chromatogram.

AROCLOR 1254

W&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 205°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min
Purge Gas - 10% CH4/Argon 120 ml/min
Pulse Interval - 50 μs
Amt. Injected - 5 μl
Std. Conc. - 1.02 X 10^{-6} g/ml
Range - 10
Attn. - 8

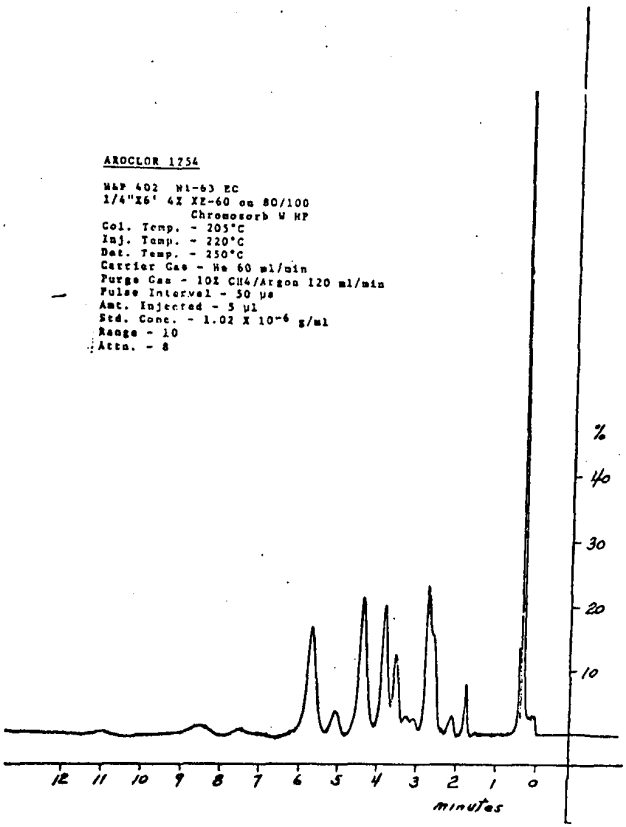

FIG. A3 Aroclor 1254 Electron Capture Chromatogram.