MONSANTO CHEMICAL COMPANY
W. G. KRUMMRICH PLANT LABORATORY

STANDARD ANALYTICAL METHOD

DATE ISSUED	PAGE	NUMBER
12-17-70	1 OF 10	A-1099
WRITTEN BY: HLCampbell	APPROVED BY: HJHorner	
ATTACHMENTS:		

PRODUCT: Polychlorinated Biphenyls (PCB)

TEST: Determination In Water, Sediment and Air Samples

THIS FORM: ☐ SUPERSEDES METHOD _____ ISSUED ON NEW
☐ REVISES INSTRUCTIONS

SCOPE

This method describes the preparation and subsequent gas chromatographic determination of trace amounts of polychlorinated biphenyls in ecological samples.

For convenience it is written in three parts according to sample type.

EQUIPMENT

A. General

a. Gas Chromatograph, Model 5750, with high temperature Ni^{63} electron capture detectors (two) and required accessories. Hewlett Packard Co., Avondale, Penn.

b. Syringe, 10 ul, Cat. No. 701, Hamilton Co., Whittier, Calif.

c. Column (two), 6 ft x 6 mm glass, 3% XE-60 on 80/100 mesh chromsorb-W, HP, AW-DMCS.

Precondition columns at 225°C for at least twenty-four hours with carrier gas flowing. Do not connect column to the detector during conditioning period.

d. Water bath, Thelco Model No. 84, Fisher Scientific Co., St Louis, Mo.

e. Kuderna-Danish Evaporative Concentrators, 500 ml capacity equipped with 3-ball Snyder columns and graduated 5 ml capacity receivers, Cat. No. 6707, Ace Glass Inc., Louisville, Ky.

f. Liquid chromatographic columns, glass, 10 inch x 20 mm (OD) with 5 inch by 50 mm (OD) reservoir at the top and equipped with teflon stopcocks. Prepared by laboratory glassblower.

g. Separatory funnels, 125, 250, 500 and 1000 ml capacities equipped with ground glass stoppers and teflon stopcocks. Fisher Scientific Co.

h. Volumetric flasks, 25, 50, 100 and 250 ml capacities. Fisher Scientific Co.

i. Usual laboratory glassware and equipment.

REAGENTS

A. General

a. Hexane, Pesticide grade, Cat. No. H-300, Fisher Scientific Co.

b. Alcoholic Potassium Hydroxide, 2.5% (W/v).

Dissolve 12.5 grams of reagent grade KOH in 500 ml of Formula 2-B ethanol.

c. Sulfuric Acid-water solution, 9/1 (v/v). Carefully add 270 ml of reagent grade H_2SO_4 acid to 30 ml of distilled water in a 500 ml iced beaker.

d. Sodium Sulfate, reagent grade, Anhydrous granular, Cat. No. S-421, Fisher Scientific Co.

e. Alumina, chromatographic grade, 80/200 mesh de-activated. Fisher Scientific Co. Cat. No. A-540. Heat 190 grams at 400°C for a minimum period of 4 hours.

De-activate by adding 10 grams (5%(w/w)) of distilled water, shake well until lumps can no longer be observed. Let stand for two hours before using.

f. Distilled water, Hexane washed. Extract 500 mls of distilled water with several 50 ml portions of pesticide grade hexane to remove electron capturing impurities.

g. PCB Standard Stock Solutions (Aroclor 1242, 1248, 1254 and 1260). Dissolve a known weight (20 to 25 mgs) of the appropriate Aroclor in 100 ml of Hexane in a volumetric flask. Mix thoroughly and store in a refrigerator.

h. PCB Standard Solutions.

These solutions are prepared as needed. Dilute 0.5 ml of the PCB stock solution to 100 ml with hexane in a volumetric flask. The solutions should contain 1.0 to 1.25 nanograms (10^{-9} gram) per microliter (ul) of the appropriate Aroclor.

SAFETY

Be aware of the toxicity and corrosiveness of the reagents being used. Hexane is a highly flammable solvent. Keep away from heat, sparks and open flame. Perform all solvent evaporations in a well vented hood.

INSTRUMENTATION

Temperatures:

Column oven: ~~250~~ 165°C
Detector: 275°C
Injection Port: 200°C

Flow Rates:

Helium carrier: ~60 ml/min.
Argon-methane: purge~120 ml/min.

Electrometer & Recorder:

Pulse interval: 150
Range: 10
Attenuation: 16
Recorder: 1 mv @0.25 or 0.5 inch/min.

Note: Elution times for highly chlorinated PCB (Aroclors 1254 & 1260) can be decreased by raising column oven temperature~20°C.

The instrumentation described in this method utilizes an electron capture detection system which contains the radioisotope nickel 63. As a consequence its operation is subject to all rules, regulations and orders of the Atomic Energy Commission (AEC). Among the more important of these are the following:

a. Operation of the instrument is restricted to trained personnel under the guidance of the licensee (H. L. Campbell and/or H. J. Horner).

b. Effluent gas must be piped to a fume hood, and leak tests performed by licensee at six month intervals.

c. Repair, modification or removal of detectors is prohibited except by authorized personnel.

Since abuse or failure to comply with all regulations can result in the loss of license; it is recommended that in case of instrument malfunction the licensee be notified and the instrument shut down immediately.

DETERMINATIONS

MONS 074684

Part A - Water Samples

Procedure

1. Transfer the entire aqueous sample or a 250 ml aliquot of very large samples 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 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 layer separation, add the initial hexane extract to the second funnel and transfer the water layer back to the original separatory funnel.

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

6. Filter the hexane extracts through a funnel plugged with glass wool covered with sodium sulfate.

7. Collect the filtrate in a Kunderna-Danish evaporative concentrator, add a 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 (HOOD).

8. After cooling, remove the 5 ml graduated receiver and transfer the concentrate to a previously prepared alumina adsorption column, washing it in with several 5 ml portions of hexane.

9. Carefully add 100 ml of hexane to the column reservoir and collect the total eluent in a Kunderna-Danish concentrator.

   Note: If the sample is known to contain 1 ppm or more of PCB, concentration is not required at this point. Instead collect the eluent in a 250 ml volumetric flask and dilute to volume with additional hexane.

10. If the column eluent has been collected in the evaporative concentrator, reduce solvent volume to less than 5 ml, cool, and dilute to a suitable volume in a volumetric flask with additional hexane.

11. Inject 1 to 10 ul of the hexane solution into the chromatograph operating at the prescribed conditions - see instrumentation.

12. Determine the type of PCB by comparing the sample chromatogram to those of various Aroclor standards.

13. Once the PCB unknown has been identified, inject 1, 2 and 3ul volumes of the appropriate Aroclor standard solution into the the chromatograph.

14. From the sample chromatogram, select a peak free from interference and measure its height in millimeters (mm). Record also the volume injected.

15. Locate the corresponding peak in the standard chromatograms and measure the peak heights at the various volumes injected. Prepare a plot of peak height (in millimeters) vs. concentration (nanograms) from the volumes of standard solution injected.

Note: In all cases, the response of the electron capture detector must be linear for quantitative analysis. With the instrumentation and procedures described here, any response less than 30 percent full scale at an attenuation of 16 x 10 falls within the linear response range at a pulse rate of 150. This corresponds to about 5 nanograms (5×10^{-9} g) of PCB. Samples with greater response must be re-analyzed from a more dilute solution and/or with a smaller injection volume.

CALCULATION

a. Determine the nanograms (ng) of PCB in the unknown by referring peak height to the standard curve. Divide this quantity by the volume injected to obtain nanograms per microliter (ng/ul).

b. To obtain total micrograms (ug) of PCB in the Chromatographic solution:

PCB in ug = nanograms/microliter X volume of sample sol'n(mls)

Note:

1. The concentrations ng/ul and ug/ml are equivalent.
2. Volume of sample solution is obtained from Steps 9 or 10 of the Procedure Section.

c. Amount of PCB in sample:

1. PCB in ppm = $\frac{\text{PCB in ug}}{\text{Sample volume or weight (mls or gr)}}$

2. PCB in ppb = ppm of PCB X 1000

Report results to nearest part per billion (ppb).

Part B - Sediment or Soil Samples

EQUIPMENT

A. General MONS 074686

See Page 1.

B. Specific

a. Pyrex baking dishes, 8 x 12 x 2 inch (2 1/2 quart capacity), Fisher Scientific Co.

b. Mortar and pestle, all glass, 32 oz. capacity, Fisher Scientific Co.

c. Filter funnels, sintered glass, 600 ml capacity 90 mm ID, medium porosity, Fisher Scientific Co.

d. Sieve, U. S. Standard No. 30, Fisher Scientific Co.

e. Shaker, reciprocating, variable speed, Cat. No. 14-260, Fisher Scientific Co.

f. Flasks, flat bottomed, boiling, capacity 125 ml, Cat. No. 6896 (code-04), Ace Glassware Co.

g. Condenser, Liebig, 200 mm length, Cat. No. 5915 (code-12), Ace Glassware Co.

REAGENTS

A. General

See page 2.

B. Specific

a. Acetonitrile, pesticide grade, Cat. No. A-999, Fisher Scientific Co.

SAFETY

Acetonitrile is a toxic and flammable solvent. Avoid inhalation or prolonged contact of the skin. Keep away from heat, sparks and open flame. Handle only in a well vented hood.

PROCEDURE

1. Decant any excess water and transfer the entire soil or sediment sample to a glass baking dish. Air dry at room temperature.

2. When dry, transfer the sample to a mortar and pestle and grind. Sieve the ground material through a No. 30 mesh screen.

3. Weigh 250 g (to the nearest 0.1 g) of the sieved sample into a 16 oz. narrow-neck, screw cap (aluminum foil liner) glass bottle.

4. Moisten the sample with distilled water (ca 10 ml) and add 150 ml of acetonitrile. Cap the bottle tightly, and mechanically shake for at least 1 hour.

5. Quantitatively transfer the acetonitrile extract into a 600 ml sintered glass filter funnel containing a 1/4inch layer of anhydrous sodium sulfate. Collect the filtrate in a 600 ml beaker.

   Note: Vacuum filtration may be necessary, in this case, collect the filtrate in a 500 ml suction flask.

6. After the acetonitrile has completely drained, wash the bottle twice with 50 ml portions of acetonitrile, adding each wash to the funnel after the previous has completely passed through the filter.

7. Quantitatively transfer the extract to a Kunderna-Danish evaporative concentrator, add a boiling chip, put the Snyder column in place and reduce the acetonitrile volume to about 5 ml by heating the apparatus in the water bath (HOOD).

8. After cooling, remove the 5 ml graduated receiver and transfer the concentrate to a 125 ml extraction flask, washing it with several small portions of solvent.

9. Evaporate the extract just to dryness with a gentle stream of dry nitrogen, and add 25 ml of 2.5% alcoholic KOH.

10. Add a boiling chip, attach the flask to a water cooled condenser and allow the solution to reflux for 45 minutes.

11. After cooling, transfer the solution to a 250 ml separatory funnel with the aid of 25 ml of distilled water. RINSE EXTRACTION FLASK W 25ml of HEXANE. ADD HEXANE WASH TO THE SEPARATORY FUNNEL

12. Extract, by shaking vigorously, for at least one minute. Allow the layers to separate and transfer the lower aqueous layer to a second 250 ml separatory funnel.

13. Extract the aqueous layer a second time with a 25 ml portion of fresh hexane. After layer separation, add the initial hexane extract to the second funnel and transfer the aqueous layer back to the original separatory funnel.

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

15. Carefully add 25 ml of the 9:1 sulfuric acid-water solution to the combined hexane in the separatory funnel.

16. Extract by shaking vigorously for at least 1 minute. Allow the layers to separate and discard the lower acid-water layer. Repeat this step until acid layer is colorless!

17. Wash the hexane with 25 ml portion of distilled water and discard the water wash.

18. Filter the hexane through a filter funnel plugged with glass wool covered with sodium sulfate.

19. Collect the filtrate in a Kunderna-Danish evaporative concentrator, add a boiling chip, put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in the water bath.

20. After cooling, remove the 5 ml receiver and transfer the concentrate to a previously prepared alumina adsorption column, washing it in with several 5 ml portions of hexane.

21. Proceed according to Part A, Procedure, beginning with Step No. 9.

Part C - Air Scrubber Samples

Equipment

See Page 1.

REAGENTS

A. General

See Page 2

B. Specific

a. Toluene, pesticide grade, Cat. No. 8092, Mallinckrodt Chemical Works, St. Louis, Mo.

SAFETY

The scrubber solvent toluene is a toxic and flammable liquid. Avoid prolonged inhalation and contact with the skin. Keep away from heat, spark and open flame.

PROCEDURE

The airborne PCB's have been absorbed in toluene by drawing a known quantity of air through gas scrubbers filled with the solvent.

1. Obtain and record the volume of air sampled.

2. Quantitatively filter the scrubber solvent through a sintered glass filter funnel containing a 1/4 inch layer of anhydrous sodium sulfate. Collect the filtrate in a round bottom flask.

3. Wash the scrubber with several fresh volumes of toluene or hexane, adding each wash to the funnel after the previous has passed through the filter.

4. Reduce the scrubbing solvent volume to about 2 ml by heating on a steam bath and blowing with a gentle stream of dry nitrogen.

5. Dilute the concentrate with hexane to a suitable volume in a volumetric flask.

6. Inject 1 to 10 ul of the solution into the chromatograph. If no interferences are observed, proceed according to Part A, Procedure, beginning with Step No. 12.

   Note: If interferences are present proceed with the alumina column clean up procedure beginning with Step No. 8.

CALCULATION

Report the results as micrograms (ug) of PCB per unit volume of air sampled.

DISCUSSION

Almost all samples from an environmental source will require some type of clean-up procedure before analysis by electron capture chromatography. Unless experience has shown otherwise, all hexane solutions of the samples should be taken through the alumina column cleanup prior to injection into the chromatograph. Interferences which remain after alumina column clean up can oftentimes be eliminated by chemical treatment-saponification with 2.5% alcoholic KOH.

Laboratory sources of contamination can be a major problem in trace determinations by electron capture chromatography. 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 procedure to insure against the possibility of PCB contamination.

Column performance is the key to effective gas chromatographic analysis. Since all liquid substrates bleed to one degree or another and columns eventually degrade, continual observation of of the chromatograms for satisfactory oolumn performance is necessary. Signs of degradation are a loss in resolution and the tailing of peaks. When these conditions are observed, a new column should be prepared and put into use.

REFERENCES

a. Organic Research Analytical Method No. 69-13.