

ANALYSIS OF BIOLOGICAL MATERIALS FOR
POLYCHLORINATED BIPHENYLS

SCOPE

This methodology was developed for the determination of the amount and type of polychlorinated biphenyls (PCB) in biological materials. 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 PCBs present in the biological materials are extracted into hexane and the bulk of the fat removed by partitioning with N,N-dimethyl formamide. Interfering components are then removed from the extracts by chemical treatment and column 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.
N,N-Dimethyl Formamide	Certified, Fisher Scientific Company, Catalog No. D-119. (Saturated in a separatory funnel with hexane.)
Ethyl Ether	Anhydrous, AR grade, Mallinckrodt Chemical Works, Catalog No. 0848.
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 Company, Catalog No. A540. Heat at 400°C for a minimum period of 4 hours 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

PLAINTIFF'S
EXHIBIT

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10. Standard VirTis Macro Flasks, 250 ml capacity, Catalog No. 16-094.
11. 10 μ l Hamilton Syringes, Catalog No. 701N.
12. Grinding Mills, Catalog No. 8-450, and Grinding Unit, Catalog No. 8-455, Fisher Scientific Company.
13. Usual laboratory glassware.

PROCEDURES

Sample Preparation

Whole animal and individual tissue (muscle, liver, heart, lungs, spleen, etc.) samples:

All individual and composite samples weighing more than 50 grams should be weighed and ground by passing through a meat grinder. The samples are then re-ground, with mixing between grindings, until the tissue appears homogenous. A portion of the ground tissue (25-35 grams) is transferred to a tared VirTis flask for analysis.

Samples weighing less than 50 grams are cut into small chunks (1-2 cm³) and the entire sample is transferred to a tared VirTis flask for analysis.

1. Add ~100g of anhydrous sodium sulfate and 100 ml of hexane to the flask. Blend the mixture for 2-3 minutes or until the sample is well homogenized.
2. Decant the hexane extracts into a 350 ml sintered glass filter funnel collecting the filtrate in a 600 ml beaker. A bell jar and glass plate assembly large enough to fit over a 600 ml beaker is employed to allow vacuum filtration.
3. Re-extract the homogenate with two more 100 ml portions of hexane decanting each in turn into the filter funnel. (If the tissue homogenate cakes on the sides of the flask at any time, the sides should be scraped to insure that adequate exposure to the hexane is obtained.)
4. After the final extraction, clean the homogenizer shaft and blades by washing with hexane. Transfer the homogenate and washings from the flask into the filter funnel with the aid of small portions of hexane and press the homogenate with a spatula to remove as much liquid as possible. Complete the filtration by washing the funnel and cake with several portions of hexane.
5. Place the combined hexane extracts on a steam bath and completely evaporate the solvent with the aid of a gentle stream of filtered air. After cooling to room temperature, re-weigh the beaker.

6. Evaporate the solvent, allow the beaker to cool, and re-weigh.
7. Prepare a hexane solution containing a maximum of 3.5 grams of fat per 25 ml and use 25 ml of this solution for the DMF/Hexane partition.

Eggs:

1. Crack fresh eggs and transfer each to an appropriate sized, tared beaker and re-weigh the beaker. Composite egg samples are prepared for analysis by homogenizing the individual eggs and a sample of the homogenate (25-50g), equivalent to an individual egg, is used.
2. Add anhydrous sodium sulfate (3X), re-weigh the beaker, and carefully grind with a heavy, flat end glass rod until a homogenous granular mass is obtained.
3. Weigh ~150-175 grams of the granular mixture into a VirTis flask, add 100 ml of hexane, and blend for 2-3 minutes.
4. Decant the hexane extracts into a 350 ml sintered glass filtering funnel, collecting the filtrate in a 600 ml beaker.
5. Re-extract the homogenate with two more 100 ml portions of hexane decanting each in turn into the filter funnel. (If the tissue homogenate cakes on the sides of the flask at any time, the sides should be scraped to insure that adequate exposure to the hexane is obtained.)
6. After the final extraction, clean the homogenizer shaft and blades by washing with hexane. Transfer the homogenate and washings from the flask into the filter funnel with the aid of small portions of hexane, and press the homogenate with a spatula to remove as much liquid as possible. Complete the filtration by washing the funnel and cake with several portions of hexane.
7. Place the combined hexane extracts on a steam bath and completely evaporate the solvent with the aid of a gentle stream of filtered air. After cooling to room temperature, re-weigh the beaker.
8. Prepare a hexane solution containing a maximum of 1.5 grams of residue per 25 ml and use 25 ml of this solution for the DMF/Hexane partition.

Sample Clean Up

DMF/Hexane Partition, Chemical Treatment, and Column Chromatography:

1. Place an appropriately sized aliquot of the hexane extracts, not exceeding 25 ml, in a 500 ml separatory funnel. If the aliquot taken is some volume less than 25 ml, add enough additional hexane to yield a final volume of 25 ml.

15. 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.
16. Repeat the extraction with a third 25 ml portion of hexane. Discard the saponification solution and combine the hexane extracts.
17. Carefully add 25 ml of the sulfuric acid solution (9:1 concentrated sulfuric acid/water) to the hexane extracts.
18. 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.
19. Wash the hexane with 25 ml portion of water. Discard the water wash.
20. 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.
21. 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.
22. 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.
23. 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.
24. If the column eluent is collected in a volumetric flask, dilute to volume with hexane and proceed with the gas chromatographic analysis.
25. 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 Ni⁶³ Electron Capture Cell
6mm 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

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

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 8×10 fell into the linear response range at a pulse rate of 150. This corresponds to approximately 5×10^{-9} g of Aroclor 1242.

Contamination

In determining PCB's in biological materials 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.

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