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TITLE: ANALYSIS OF MOUND SERUM SAMPLES FOR PCBs.

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ABSTRACT: A report on the analysis of PCBs in human serum is presented. Serum samples from 10 donors were analyzed for PCBs. Results are presented in this report.

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ANALYSIS OF MOUND SERUM DONORS FOR PCBs

On 22 June 1982, samples of blood serum were obtained from 10 donors at the Mound facility of Monsanto Research Corporation (MRC-Mound). These samples were then transported to the Dayton Lab (MRC-Dayton) for analysis of serum PCB levels. Full QA/QC was run on this set of samples as discussed in the meeting of 14 April 1982 in St. Louis. This entailed duplicate analyses as well as low and high spikes for each sample. Samples were analyzed according to the attached method (MRC Method #24-13-PREP R0).

The low spike of the serum samples was at 42.4 ppb of PCB distributed among 37 separate chromatographable peaks and ranging from 0.10 ppb to 3.22 ppb with a median spike level per peak of 0.94 ppb. The high spike was at a level five greater than the low spike. The results of the analysis for these ten donors are given in Table 1. The average subtracted blank was 1.0 ppb. The minimum detection limit varied from sample to sample in the range of 1 and 2 ppb.

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TABLE 1. RESULTS OF MOUND SERUM DONOR ANALYSIS FOR PCBs

Donor	Sample (ppb)	Replicate (ppb)	Average (ppb)	Low Spike recovery	High Spike recovery
1	4.1	6.6	5.3	87.8%	66.4%
2	5.8	5.6	5.7	100%	68.6%
3	4.1	1.7	2.9	98.7%	73.3%
4	2.4	2.4	2.4	78.2%	57.8%
5	2.0	2.4	2.2	102%	89.7%
6	3.2	2.8	3.2	91.0%	70.3%
7	1.7	2.2	2.0	75.4%	61.1%
8	4.1	4.0	4.1	87.8%	66.4%
9	6.5	4.7	5.6	111%	71.5%
10	6.7	8.1	7.4	74.1%	65.3%
				Ave = 90.7%	69.1%
				σ = 12.4%	8.6%

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MRC Method #24-13-Prep-RO

Title:	Analysis of Polychlorinated Biphenyls in Blood Serum
Nomenclature:	In this paper, the term "PCB" will be used to refer to any combination of the 209 different chlorinated biphenyls. The term "class" will refer to PCB's having the same degree of chlorination regardless of the positional substitution of the chlorines. The term "isomer" will refer to a PCB having a specific arrangement of the chlorines in the biphenyl.
Application:	This method is applicable to the analysis of part per billion (ppb) levels of PCBs in blood serum based on a ng/mL basis. This method can be applied to the analysis of trichlorobiphenyls through decachlorobiphenyl.
Precision and Accuracy:	The precision and accuracy for this analysis will be derived from the QA/QC data.
Safety:	Persons handling PCBs (and any other chemicals) should familiarize themselves with current safe handling procedures for those chemicals.
Authors:	Roy W. Noble, Cynthia L. Heflin
Approved:	Joseph J. Breen
Date:	21 July 1982

Scope and Limitations of Method:

This method is applicable to the analysis of PCBs in blood serum. The method has been successfully applied in this laboratory under the conditions set forth in this paper. However, this does not imply that this method can be used in another laboratory without validation within that laboratory.

Equipment and Reagents:

1. Silica Gel, Fisher chromatographic grade, 100/200 mesh or equivalent.
2. $AgNO_3$, Eastman - Kodak Co. X491
3. Distilled deionized H_2O, supplied in house.

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4. Na_2SO_4, anhydrous, granular. S/P #BAK - 3891.

5. All solvents are Burdick and Jackson, "Distilled in glass grade".

6. Aroclors 1248, 1254, and 1260 from Monsanto Company, St. Louis MO.

7. PCB isomers from Ultra Scientific Inc., Hope, RI.

8. Centrific Centrifuge- Fisher #04-978-50.

9. Vacutainer Blood Collection Tubes - 10mL, plain, sterile red top, S/P - B-2983-54.

10. 5mL Serological pipette, sterile, disposable, glass S/P - P464-15A

11. 15mL culture tube, - (16 X 125 mm) disposable with teflon-lined screw cap. S/P T-1356-5.

12. Genie Wrist Action Shaker. Vortex Mixer

13. Burrell Wrist Action Shaker.

14. Glass Wool - untreated Supelco #2-0384.

15. 50 mL Centifuge tube - disposable, glass Corning 8060.

16. Water Bath - S/P 6990.

17. Sea Sand - Fisher S-25.

18. Auto Sampler Vials - ~1.8 ml glass with teflon - lined screw caps. Supelco #332-86 and 3-3210.

Sampling:

Human blood samples will be drawn by a certified medical technologist, nurse, or medical doctor. Each sample will be drawn in an individual plain, sterile vacutainer and sent to the Dayton Lab following appropriate shipping and preservation procedures. Upon arrival at the laboratory site, each sample will be centrifuged at 3000 rpm for 10 minutes to separate serum from the red blood cells. A 2mL aliquot is then tranferred by a sterile disposable pipet to a 15mL teflon lined screw capped extraction tube. The extraction tube was previously cleaned with hexane and baked over night at 600°C in a glassware oven. Refrigerate samples at 4°C until proceeding with extractions.

Extraction and Clean-up Procedure:

1. Remove samples in extraction tubes from refrigerator and allow to warm to room temperature. If appropriate spike the sample with a standard solution of PCBs in dimethyl sulfoxide (DMSO).

2. Add 1mL of methanol to the samples to denature serum proteins.

3. Add 5mL of hexane to the extraction tubes and mix on a vortex mixer.

4. Agitate on a waist action shaker at maximum speed for 10 minutes.

5. Separate serum and hexane layers by centrifugation at 3000 rpm for 10 minutes.

6. Pool hexane extracts for each sample in a second 15mL screw cap tube containing 1mL of nonane, and marked at a volume of 1mL.

7. Repeat steps 3 through 6 two times.

8. Concentrate all extracts to 1mL by evaporation under purified nitrogen in a heated sand bath (60°C).

9. Prepare liquid chromatography column by inserting a glass wool plug into a 5mL disposable pipet, then filling the pipet with 2 cm of anhydrous sodium sulfate, 8 cm of 10% silver nitrate deactivated silica gel previously prepared according to the method of Lamparski et al.[1] Top this column with an additional 2 cm of anhydrous sodium sulfate and prerinse the column with 10mL of hexane.

NOTE: In preparation of the $AgNO_3$-silica gel column it is important that the Lamparski procedure be followed exactly to obtain reproducible column packing. For that reason, a brief outline is given below but one should familiarize themselves with the full procedure. Chromatographic grade 100/200 mesh silica gel is rinsed with ~75mL methanol, and then with ~75mL methylene chloride, and baked overnight at 180°C under nitrogen purge. The silica is allowed to cool to ambient temperature under nitrogen purge and a known amount is weighed out. Silver nitrate is then weighed into a separate beaker so that the amount of silver nitrate equals ten percent of the total weight of the silver nitrate plus the silica gel. Similarly distilled deionized water is weighed out so that the weight of the water equals 30% of the total weight of the silica gel plus the water. The water is then added to the silver nitrate and this solution is then slowly added to the silica with stirring to produce a uniformly coated silica gel which is allowed to stand for a minimum of 30 minutes. The silica gel prepared by this method is then baked in an oven under nitrogen

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purge at 60°C for 10 minutes and the temperature is then increased at 0.2°C/minute until reaching a final temperature of 125°C where it is held for a minimum of 15 hours. The 10% $AgNO_3$ - silica gel is stored in an amber glass bottle inside a desicator until use.

10. The sample is then placed on the head of the prerinsed column and eluted with 30mL of hexane which is collected in a 50mL centrifuge tube premarked to a volume of 1mL.

11. The eluant is then concentrated to 1mL as in step 8 and submitted for GC-EC analysis.

12. A known amount of internal standard, octachloronapthalene(OCN), is added to account for differences in final volume and variation in the electron capture response over the course of the analytical runs.

13. The sample is analyzed by capillary column gas chromatography-electron capture detection with splitless injection technique using the following conditions:

gas chromatograph: Varian 3700
column: 30 m wide bore SE-54 (or DB-5) sold by J&W Scientific

injector temp: 280°C
detector temp: 350°C
initial temp: 140°C
program rate: 3°C/min.
final temp: 275°C
linear flow velocity: 20.8 cm/sec of 10% Methane 90% Argon
head pressure: 9 psi

A minimum of three standards at different levels are run for each set of samples. These standards contain the OCN and a mixture of PCBs representing a broad range of PCBs in the chlorine classes analyzed. These standards were previously calibrated by GC-MS and GC-FID against individual isomer standard for each class.

14. Calculations are performed on a peak by peak basis using a least squares regression analysis. Prior to the regression analysis all areas are normalized using the OCN to account for differences in final volumes and electron capture sensitivity changes over the course of the GC runs.

Reference:

1. L. L. Lamparski, T. J. Nestrick and R. H. Stehl, Anal. Chem., 51, 1453 (1979).

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