Bull. Environm. Contam. Toxicol. 21,428-431 (1979)

# A Rapid Method for the Analysis of Polychlorinated Biphenyls in Milk

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There has recently been much concern expressed over the possibility of contamination of human milk by various persistent organochlorine chemicals. One group of compounds which has been prominently mentioned is the polychlorinated biphenyls (PCBs). Analytical methods which have been used [CAHILL et al. (1970), HUCKINS et al. (1974)] have posed problems in both qualitation and quantitation. They have also been lengthy and subject to error because of the many manipulations involved. The method presented here has the advantages of being rapid, sensitive, and requiring a minimum of manipulations while simplifying quantitation.

The method is based on extraction and simultaneous cleanup using a microFlorisil column followed by perchlorination of PCB by antimony pentachloride to yield decachlorobiphenyl. Quantitation is accomplished by comparison of the perchlorinated PCB from the sample with a perchlorinated standard of Aroclor 1254. Results are calculated as Aroclor 1254 equivalent. In cases where the identity of the PCB is known to be other than Aroclor 1254 the appropriate PCB may be substituted.

## MATERIALS AND REAGENTS

1. Microcolumns, Kontes K-420100 size 22
2. Glass stoppered graduated centrifuge tube, Kontes K-410550, 13 mL
3. Glass wool, pre-extracted with hexane
4. Florisil activated at 130°C at least overnight and stored in a 130°C oven
5. $Na_2SO_4$, Anhydrous Granular, pre-extracted with hexane
6. n-hexane, pesticide quality
7. Antimony pentachloride
8. Glass mortars and pestles, 2 ounce size

0007-4861/79/0021-0428 $01.00

9. Sand bath adjustable to 195°C

10. Vortex Mixer

11. HCl solution prepared by mixing 1 volume of concentrated reagent grade HCl with 1 volume of deionized water

## ANALYSIS

The following stepwise procedure is used. With each set of samples run a reagent blank and a standard. For the quantitation standard prepare a solution of Aroclor 1254 in hexane at a concentration of 0.05 µg/mL. Use two mL of this solution and carry it through the procedure in exactly the same manner as the sample. This standard is equivalent to 50 ppb in the sample. The reagent blank is prepared by substituting 2 mL of n-hexane for the sample.

Note: Because of the ubiquity of PCBs and the sensitivity of the procedure, it is sometimes difficult to achieve good reagent blanks. Therefore all glassware must be scrupulously cleaned prior to use including a final thorough rinsing with n-hexane. Antimony pentachloride is a powerful chlorinating reagent and the conditions used in this procedure are forcing. Because of this it is essential that no contact with plastics occurs anywhere in the procedure.

1. Place 10 g of $Na_2SO_4$ in a glass mortar.

2. Add 2 mL of milk to the $Na_2SO_4$ and grind the mixture to a dry, free flowing powder.

3. Fit a loose plug of glass wool into the tip of a micro column and pour 2 g of Florisil into the column. Do not pack the column tightly as this will result in a very slow flow.

4. Add the ground $Na_2SO_4$-milk mixture to the top of the Florisil. Do not prewet the Florisil.

5. Place a graduated centrifuge tube under the column. Add 20 mL of n-hexane to the top of the column and collect the first 12 mL of eluate. A properly packed column will flow at about one drop/sec.

6. Concentrate the eluate to 1 mL using a gentle stream of nitrogen and a 40°C water bath. Inject for qualitation.

7. When qualitation is completed evaporate the samples just to dryness using a gentle nitrogen stream and 40°C water bath.

8. Add 0.05 mL of $SbCl_5$ to the tube, stopper tightly with

a ground glass stopper and place in a 195°C sand bath overnight.

9. After overnight heating remove the tubes and allow them to cool to room temperature.

10. Cautiously remove the stoppers in a hood (there may be some slight pressure in the tube) and add 2 mL of HCl solution, using the acid to rinse the stopper and tube walls. Mix briefly on a vortex mixer.

11. Add 3 mL of n-hexane to the tube and "vortex" for 60 sec. Allow the layers to separate and transfer the n-hexane layer to a graduated centrifuge tube using a disposable pipet.

12. Repeat step 11 two additional times.

13. Concentrate the combined hexane extracts to 1 mL for initial injection for quantitation.

## GAS-LIQUID CHROMATOGRAPHY

Two columns are used in this procedure.

Qualitation is performed on a 91 cm x 6 mm OD glass column packed with 4% SE-30/6% OV-210 on Chromosorb W, H.P., 80/100 mesh. The column is conditioned at 245°C for 72 h with a nitrogen carrier flow of 60 mL/min. At the end of the conditioning period the temperature is lowered to 205°C, the carrier gas flow is adjusted to 85 mL/min and four consecutive injections of 25 µL of Silyl 8 are made at 1/2 h intervals. Allow the Silyl 8 to elute from the column overnight before connecting it to the detector. This column operated at 205°C with a carrier flow of 85 mL/min completely elutes Aroclor 1254 in about 12 min with adequate peak separation for qualitation.

Quantitation is performed on a 91 cm x 6 mm OD glass column packed with 5% OV-210 on Chromosorb W, H.P., 100/120 mesh. The column is conditioned as previously described, then operated at 196°C with a nitrogen carrier gas flow of 85 mL/min. Under these conditions decachlorobiphenyl has a retention time of just over 11 min.

Detection for both qualitation and quantitation is by electron capture.

## DISCUSSION

Tracings of typical chromatograms of milk fortified with Aroclor 1254 at a level of 53 ppb are shown before and after perchlorination in Figure 1.

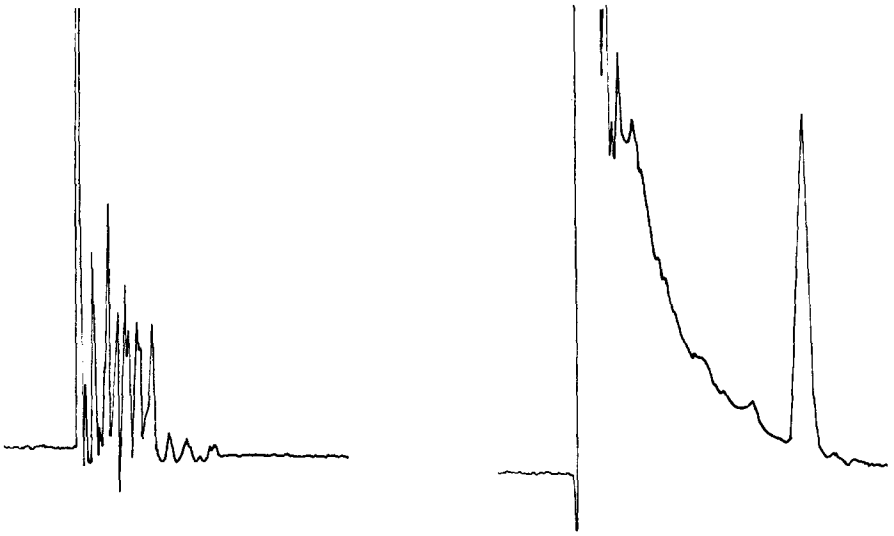

Figure 1

Before Chlorination After Chlorination

The procedure as written yields residue of PCB equivalent to Aroclor 1254 in whole milk. If residues based on lipid content are desired it is necessary to perform lipid determination on a separate subsample.

PCB commonly identified in milk have been Aroclor 1242, 1254 and 1260. The choice of Aroclor 1254 as a quantitation standard was made as a compromise typical of this range of Aroclor.

## ACKNOWLEDGEMENTS

The work was supported by the Epidemiologic Studies Program, Human Effects Monitoring Branch, Technical Services Division, Environmental Protection Agency under contract # 68-01-4303.

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