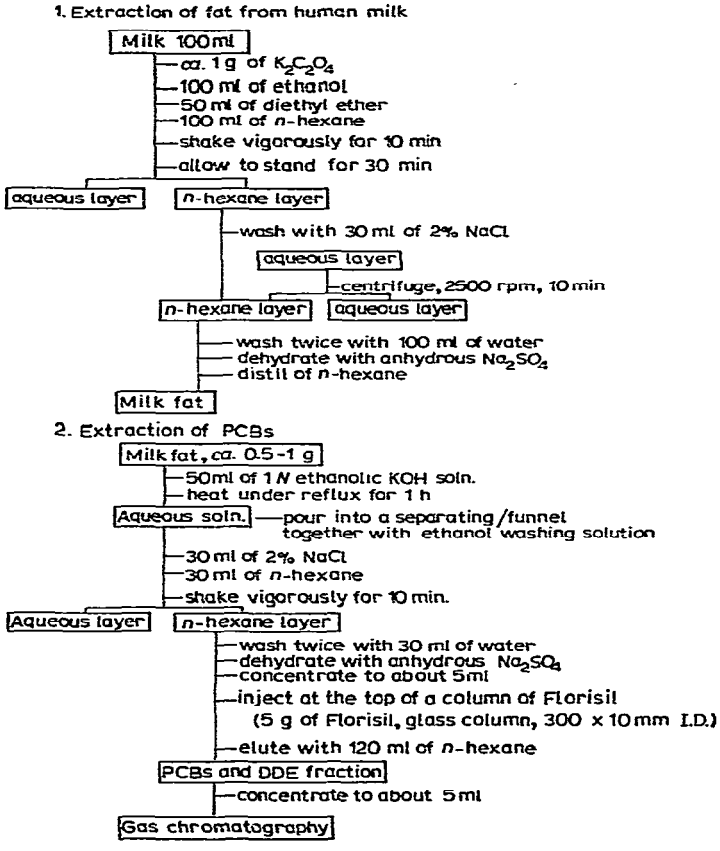

Fig. 1. Analytical method for PCBs.

temperatures were as follows: oven 160°, injector 250°, separator 260°. The carrier gas was helium at a flow-rate of 30 ml/min. Other conditions were: chamber voltage (CV) 70 eV; trap current (TC) 120 μA; accelerator voltage 3.5 kV; *m/e* 256, 258, 290 and 292 (Fig. 3). For GC–MS analysis, 2% Apiezon L was used as the stationary phase. The following temperatures were used: oven 250°, injector 300°, separator 300°. The carrier gas was helium at a flow-rate of 6 ml/min. Other conditions were: CV 20 eV; TC 60 μA; accelerator voltage 3.5 kV; interval 6 sec (Figs. 4 and 5).

Quantification

The quantification of PCBs with Kanechlor 500 is not accurate as the patterns of PCBs extracted from human milk are different from that of Kanechlor 500. Therefore, the 2% OV-1 column analysis proposed by Ugawa *et al.*[16] was used for the calculation of PCB residues.

On the other hand, for the detailed analysis of individual components, the 2% Apiezon L column analysis, proposed by Jensen and Sundström[7] and applied by Nakamura and Kashimoto[17] for the calculation, was used. The system for the numbering of peaks follows these methods.

RESULTS

Fig. 2 shows the GC separation on 2% OV-1 of samples (2) and (3). Peaks 15–23 have already been identified and it was recognized that they did not contain any contaminants that were sensitive to the electron-capture detector. However, peaks 4, 9 and 9′ have not been identified and could lead to serious errors in quantifications.

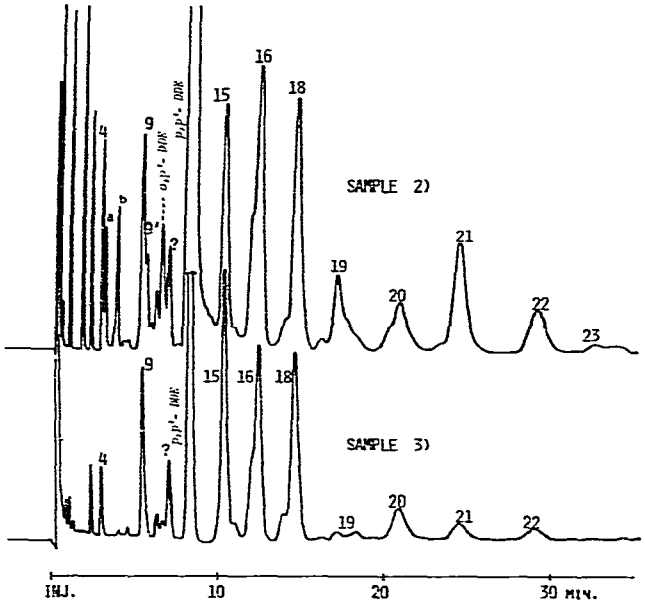

Fig. 2. GC separation on a 2% OV-1 column of PCB residues in samples (2) and (3). Internal standard: (a) 2,3′,4′-tri-CB; (b) 2,4,2′,4′-tetra-CB.

The identification of *o,p′*-DDE was confirmed as follows. After the clean-up of the PCB fraction on a Florisil column, it was heated under reflux for 1 h with chromic anhydride, the *o,p′*-DDE being completely degraded. In this reaction, PCBs were almost stable except for a slight loss of peaks 4, 9 and 9′. The same result was obtained on the hydrolysis of *o,p′*-DDT, which was completely hydrolysed and converted into *o,p′*-DDE.

To ensure the correct identification of peaks 4, 9 and 9′, internal standards [(a) 2,3′,4′-trichlorobiphenyl; (b) 2,4,2′,4′-tetrachlorobiphenyl] were added to the PCB fraction of sample (2). Both the qualitative and the quantitative analysis were carried out by mass fragmentography using 2% OV-1 as the stationary phase.

In a previous study, the retention times of peaks 4 and 9 agreed with those of 2,4,4′-tri- and 2,4,2′,4′-tetra-CB, respectively. Therefore, a mixture of four pure PCBs, the 2,4,4′-, 2,3′,4′-, 2,4,2′,4′- and 2,4,3′,4′-chloro compounds, were used as the standard. Fig. 3 shows the mass fragmentograms of sample (2). The components of peaks a and b in the sample correspond to those in Fig. 2. The peak height ratio of peaks 1, a and b in the sample is similar to that in the standard. From these mass fragmentograms, it became clear that the peak 1 was tri-CB and did not contain

contaminants. Further retention studies were made in order to clarify the structure of peak 1. It was recognized that the retention time of synthesized 2,4,4′-tri-CB agreed with that of peak 1 in the GC separation on 2% OV-1, 2% Apiezon L and OV-101 capillary columns. This was also confirmed by the retention indices reported by Welti and Sissons[18,20], who determined the composition of Aroclor 1254 and the retention indices of its components.

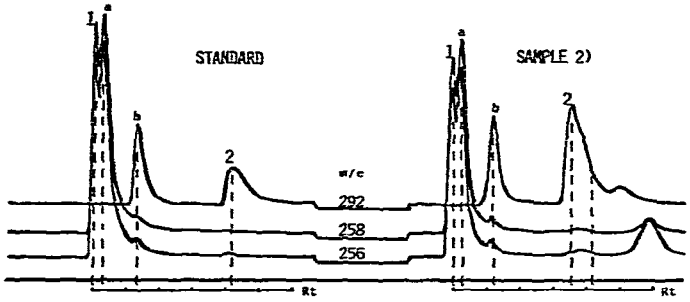

Fig. 3. Mass fragmentograms of PCB residues in sample (2) at *m/e* 256, 258 and 292. Internal standard: (a) 2,3′,4′-tri-CB; (b) 2,4,2′,4′-tetra-CB.

In the case of peak 2, however, a 2- to 3-times higher shoulder peak was detectable at a slightly shorter retention time in the sample. Other mass fragmentograms, obtained at *m/e* 290 and 292, showed the isotope peaks of chlorine. It was therefore recognized that peak 2 and the higher peak were tetra-CBs. The same result was obtained by the GC–MS analysis of sample (1).

From these identifications by mass fragmentography using an OV-1 column, it was confirmed that considerable amounts of tri- and tetra-CBs remained in human milk. Peak 4 in Fig. 2 can be assigned to 2,4,4′-tri-CB and peak 9′ to 2,4,3′,4′-tetra-CB. The latter was also confirmed by capillary column chromatography using OV-101.

Peak 9 was presumed to be 2,4,5,4′-tetra-CB from the retention indices reported by Welti and Sissons[18,20]. For comparison purposes, 2,4,5,4′-tetra-CB was therefore synthesized by the method of Cadogan[22]. The latter reaction also gave 2,4,5,2′- and 2,4,5,3′-tetra-CBs from which the 2,4,5,4′-tetra-CB was separated on a silica gel column. The retention time of the synthetic compound agreed with that of the unknown in human milk on 2% OV-1, Apiezon L and OV-101 capillary columns. In another reaction in which 2,3,5,4′-, 2,3,6,4′- and 2,4,5,4′-tetra-CBs were synthesized, the same result was obtained. The details of the synthesis, purifications, separation and the identification by GLC and UV and NMR spectroscopy will be published separately.

To confirm the results for residues of low chlorinated biphenyls in human milk, especially the identification of the unknown tetra-CB, a 2% Apiezon L column was used for the analysis of sample (3) by GC–MS.

As can be seen in Fig. 4, the peaks on the Apiezon L column that correspond to peaks 4, 9 and 9′ on the OV-1 column are peaks *k* and 9. The application of an Apiezon L column to the detailed analysis of individual components in PCB residues will ensure a more accurate identification of the components that might be responsible for specific biological effects. A commercial PCB mixture, Kanechlor 300, 400, 500 and 600 (1:1:1:1), can be separated into about 70 components on an Apiezon L

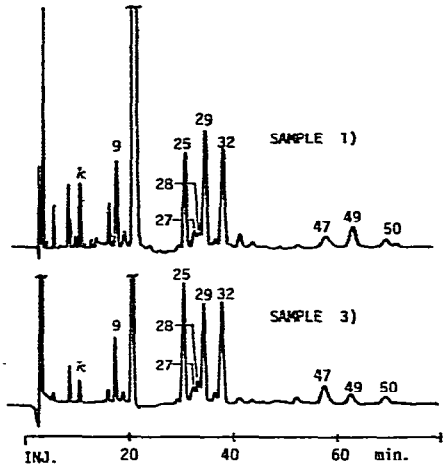

Fig. 4. GC separation on a 2% Apiezon L column of PCB residues in samples (1) and (3).

column[7,17], whereas peaks 9 and 9′ obtained on an OV-1 column become a single peak on an Apiezon L column, so that their identification by GC–MS will be difficult. In this instance, however, sample (3) does not contain peak 9′ shown in Fig. 2. The absence of peak 9′ has also been recognized in the blood of Yusho patients. The details of this and the stability of the components in human tissues will be published separately[19]. Thus, only the component corresponding to peak 9 was identified on an OV-1 column. Fig. 5 shows the mass spectra of peaks *k* and 9. No contaminants were detected.

Fig. 6 shows the isotope peaks of chlorine (*m/e* 256, 258, 290 and 292), as recognized above. In addition, the contents of peaks *k* and 9 determined by GC–MS were almost identical with those determined by GC using an electron-capture detector.

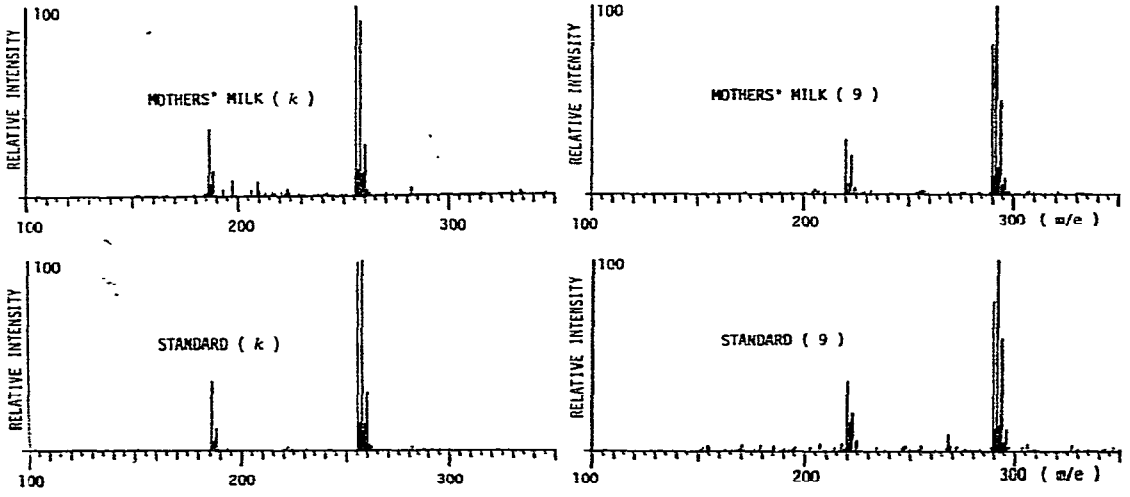

Fig. 5. Mass spectra of peaks *k* and 9 in sample (3) (2% Apiezon L column).

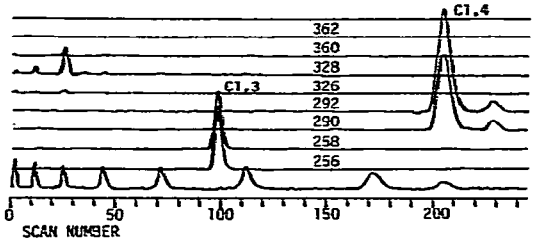

Fig. 6. GC–MS analysis of PCB residues in sample (3) (2% Apiezon L column).

DISCUSSION

Low chlorinated biphenyls have long been considered to be rapidly excreted from the body. In this work, however, it was confirmed that 2,4,4′-tri-CB, 2,4,5,4′-tetra-CB and 2,4,3′,4′-tetra-CB remain in considerable amounts in human milk. The mean content of these components of 40 milks collected from women in Osaka in 1975 was about 30% of the total amount of PCBs calculated from the Apiezon L column analyses reported by Nakamura and Kashimoto[17].

This result might be important in studies of the mechanism of biological degradation[20,21]. According to our recent studies on the individual components in human milk, all of the major residual components have specific structures, with two chlorine atoms *para* to the biphenyl bridge, *i.e.*, the 2,4,4′-, 2,4,5,4′-, 2,4,3′,4′-, 2,4,5,3′,4′-, 2,3,4,3′,4′-, 2,4,5,2′,4′,5′-, 2,3,4,2′,4′,5′-, 2,3,4,5,3′,4′-, 2,3,4,5,2′,4′,5′- and 2,3,4,2′,3′,-4′,5′-compounds. The 2,4,5-substitution pattern in one ring and 4-substitution in the other ring are assumed to be one of the most stable structures in the human body. Further studies should be made in order to confirm the mechanism involved.

ACKNOWLEDGEMENTS

We gratefully acknowledge and thank Messrs. K. Yamase and K. Aoki of Japan Food Research Laboratories, Mrs. S. Kon and Mr. K. Ishidzu of Sakai City Institute of Public Health and Mr. S. Takahashi of Shimadzu Seisakusho Ltd. for their technical assistance with the GC–MS analysis.

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