ENANTIOSELECTIVE ANALYSIS OF PCB CONGENERS IN BREAST MILK

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Introduction

In a previous study on the secular changes of the concentration of PCB congeners in human breast milk, authors showed that the declining rate of high chlorinated PCB congeners is slower than that of low chlorinated congeners[1,2]. The composition of detected PCB congeners in breast milk indicated that not only the number of chlorine atoms but its substitutional position affected the rate of decrease[3]. Enantiomer is also a class of structural isomers, which consists of (+) and (-) (figure 1). Among PCBs, 19 congeners which are chlorine-substituted in 3- or 4- ortho position are known to have enantiomers[4]. In general, physical properties in chiral molecules (enantiomers) such as density, melting point, boiling point, refractive index, thermal conductivity are identical. However physiological behaviors of enantiomers are quite different *in vivo*, since functional biomolecules like metabolic enzymes distinguish their three dimensional structures precisely. Whereby, chiral separation is an important issue of pharmaceuticals and agricultural chemicals. It is also considered that enantiometric POPs would show similar characteristics and it closely relates to their physiological effect such as stablity in body and the strength of toxicity. For the precise estimation the risk to human health caused by POPs in breast milk and food, it is necessary to analyze enantiomers separately and to caluculate enantiomer fraction value (EF value) (figure 2). In this study, we analyzed enantiometric PCB #183 (2,2',3,4,4',5',6-hepta CB) in human breast milk and total diet study (TDS) fish samples and confirmed the time trends of the EF value, then, we evaluated the relationship among food intakes and human metabolism of chiral PCBs

Materials and methods

Breast milk specimens were collected from women living in Osaka Prefecture, Japan. To minimize maternal factors, milk specimens were obtained from the women who were primiparous and whose age ranged from 25 to 29 years old. The milk specimens were collected between 1973 and 2008 and kept in frozen storage at -20 ºC until use. Equivalent amounts of milk fat from 30 to 40 milk specimens were mixed and named for each year's representative sample. Samples were prepared in accordance with the "Dioxins measurement manual of breast milk sample" which was used in "Research on the establishment of biological exposure monitoring methods and ensuring reliability in a biological sample inspection and food chemicals such as dioxins." The human breast milk samples were obtained with the donor's informed consent via the Health Prevention Section of the Osaka Prefectural Health Division.

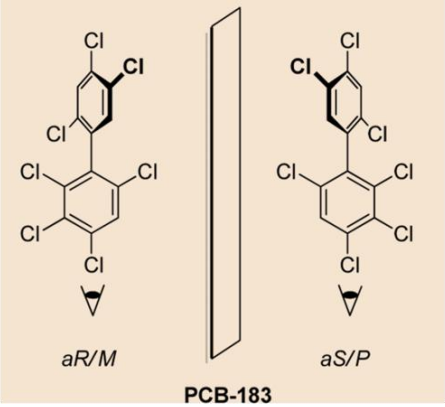


Figure 1. Absolute configuration of atropisometric PCB 183

Table 1. Analytical conditions of HRGC/HRMS for analysis of chiral PCBs

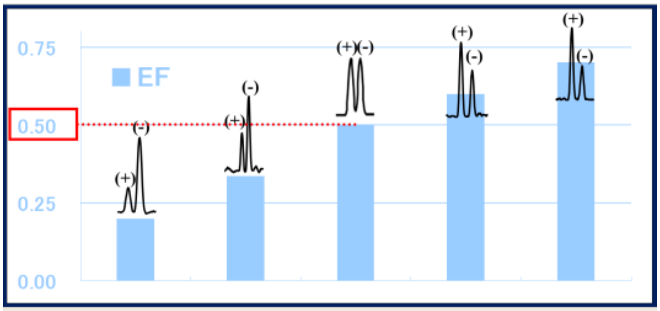
GC	MS
Device : HP-6890N(HP) Column : BGB-172*(BGB Analytik) 30m, 0.25mm i.d. film thickness 0.25μm Carrier Gas : He 1.0 mL/min	Device : JMS-800D (JEOL) Ionization : EI Ion source temperature : 260℃ Ionization volt : 38eV Accel volt : 10kV Resolution : >10,000 Selected fragment (m/z) : [M]+, [M+2]+ or [M+2]+, [M+4]+
Temperature Program : 120℃ (2min) → 2℃/min →250℃ (3min) Injector temperature : 230℃ Injection Volume : 2μL	

＊BGB-172 (20% tert-butyldimethylsilyl-b-cyclodextrin dissolved in 15% diphenyl-polysiloxane and 85% dimethylpolysiloxane)

TDS samples were prepared as described below; a variety of fish samples were purchased in supermarket in Osaka. Fish are weighed as national nutrition survey in Japan and they were cooked as usual recipe. Then, they were combined and were homogenized by food processor. Samples were kept at -20 ºC until use. An aliquot of fish mixture was decomposed by potassium hydroxide overnight; then, PCBs were extracted by *n*-hexane and evaporated to dryness. Additional purification procedure was conducted by activated florisil column.
PCB congeners were analyzed using high resolution gas chromatography/ high resolution mass spectrometry (HRGC/HRMS) connected to a capillary column using selected ion monitoring (SIM) method. A BGB-172 column was used for the chiral analysis. Further detail of analytical method was shown in table 1.

Results and discussion

The concentrations of POPs in breast milk were found to decrease annually, but the decreasing rate was differed greatly from the types of POPs. The average concentrations of β-HCH, ΣDDTs and ΣPCBs were decreased to about 1/70, 1/15 and 1/8 respectively, compared to their highest concentration levels in early 1970's[5]. Even if the amounts of continuous dietary intakes of each POPs are taken into consideration, PCBs is considered to be the most stable compounds among these POPs (figure 3). Among 154 congeners from HpCB to TeCB, 48 types of congeners were still detected from a sample in 2000, although 51 types of congeners were detected from the sample in 1973 (figure 4). Determined enantiomers in breast milk were #135, #149 (HxCB) and #171, #183 (HpCB). PCB #183 was highest concentration in these congeners. However, these congeners were 2% or less of the total PCBs. The present analysis conditions, we could completely separate PCB #183 enantiomers, while the separation of others was inadequate (figure 5).



$$EF = \frac{E(+)}{E(+) + E(-)}$$

E(+): amount of (+)enantiomer
E(-) : amount of (-)enantiomer

Figure 2. Enantiomer Fraction (EF)

It is known that seafood is the largest source of PCBs' exposure. PCBs' intake from seafood has

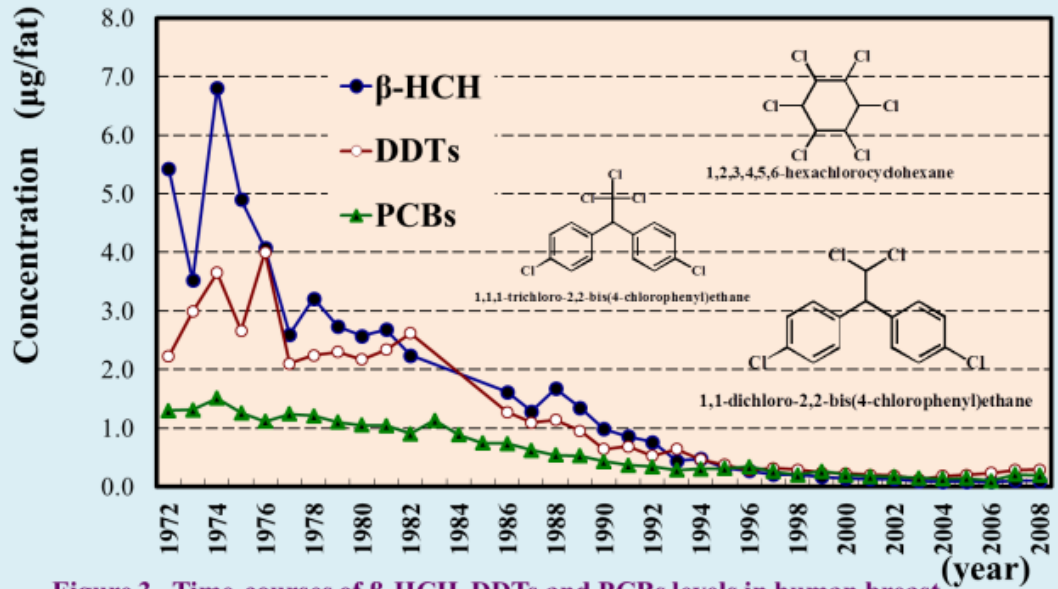
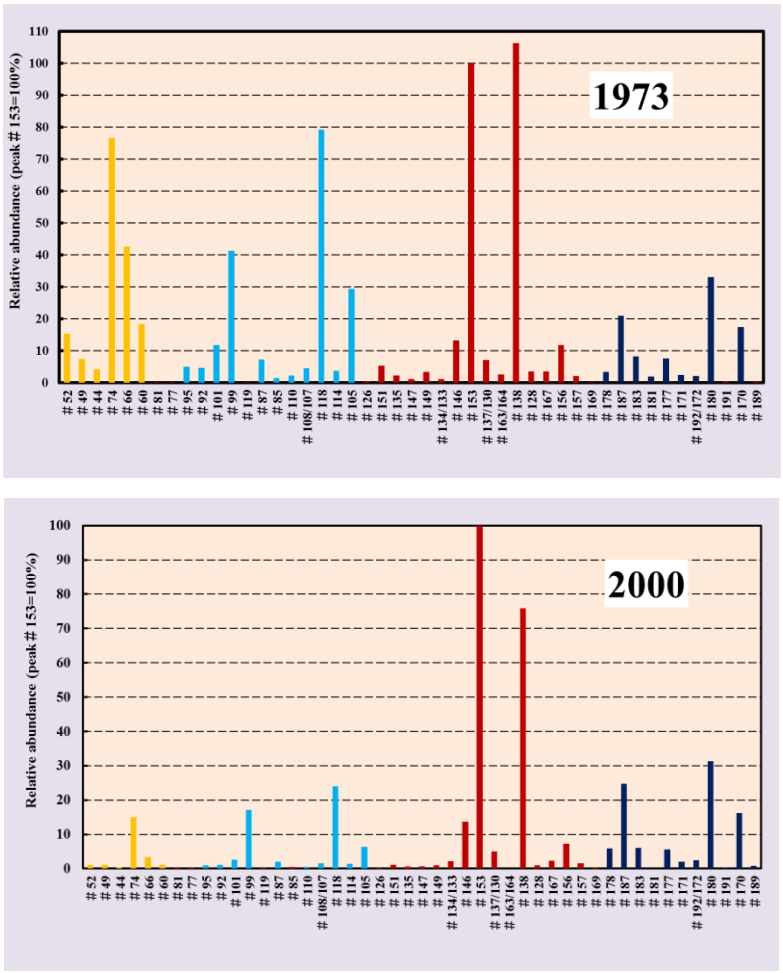


Figure 3. Time-courses of β-HCH, DDTs and PCBs levels in human breast milk from 1972 to 2008 fat basis

been gradually decreased from the first half of the 1990s (figure 6), but surprisingly, EF value sustained close to racemate (EF = 0.5) (figure 7). On the other hand, EF values of #183 in breast milk were elevated year by year (figure 8), despite decreasing its concentration over the time (figure 9). This fact indicated that (-)-PCB-183 is metabolized easily than (+)-PCB-183, therefore, the toxicity of (+)-PCB-183 should be the issue.

In 1974, the Ministry of International Trade and Industry and Ministry of Health and Welfare of Japan designated PCBs as a specified chemical substance. Osaka Prefectural Government researched the "The Project for Breast Feeding Promotion" from 1972 to 2008 when environmental contaminations by POPs have become a serious problem in Japan. In this project, annual monitoring of POPs in human breast milk was performed. Almost thirty years have passed since their use was prohibited, PCBs are still persistent. The source of organochlorine compounds exposed to human body is mostly from food. The contamination of fish and shellfish has been a serious problem for the Japanese, who consume a large amount of seafood in their diet. In our next steps, we will consider the separation of another enantiometric PCBs, # 135, # 149 and # 171, and the difference of physiological effect among #183 enantiomers.



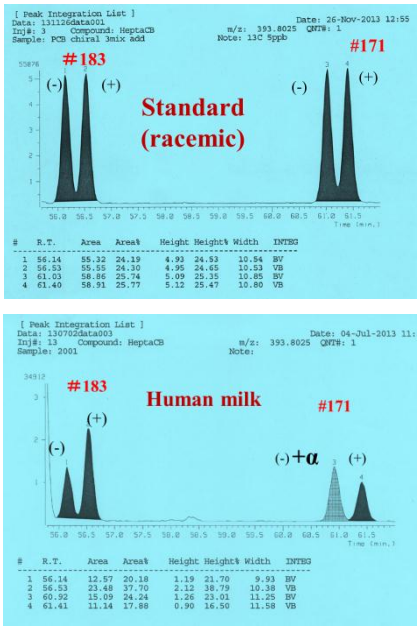


Figure 5. HRGC/HRMS chromatogram for enantioselective analysis in human breast milk

Figure 4. PCB congener composition in human breast milk

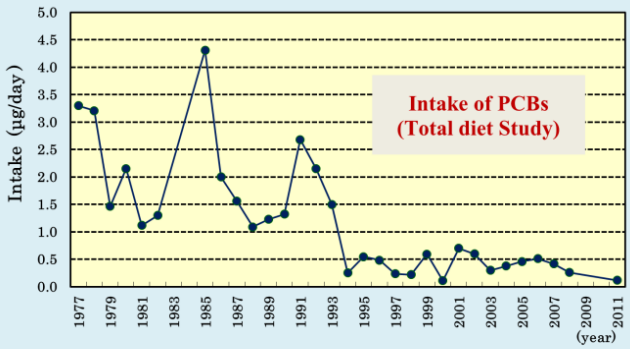


Figure 6. Time-course of the daily intake of PCBs from 1977 to 2011

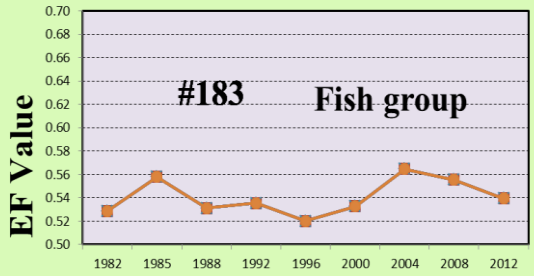


Figure 7. Trends of PCB#183 EF in fish group of total diet study samples

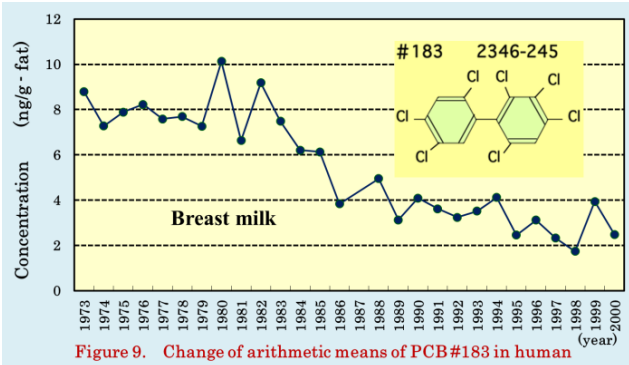


Figure 9. Change of arithmetic means of PCB#183 in human breast milk fat basis,

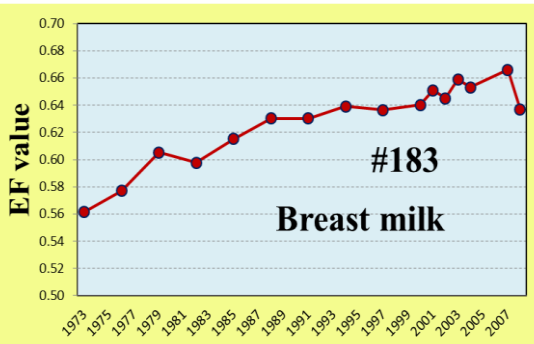


Figure 8. Trends of PCB#183 EF in breast milk

Acknowledgements

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