

The study of the PCBs distribution in the blood, and the trial of the PCBs analysis based on specific isomers by HRGC/HRMS

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Introduction

When human and animal are exposed to the internal secretion disturbance chemical material, a bad influence on their reproduction and the nerve is concerned. PCBs is suspected endocrine disrupting chemicals. We need to measure the concentration of PCBs in the human blood sample to know accumulation of human exposure. This paper describes the comparison of the PCBs concentration in the whole blood and the plasma, the study of the PCBs distribution in the blood, and the trial of the analysis of PCBs based on UNEP-7, 30 isomers by HRGC/HRMS

Methods and Materials

Extraction method

A serum sample of 5g was transferred to a 50mL centrifuge-tube. 25μL of 50 times diluted ¹³C₁₂-PCBs solution (TK-BPA-LCG, Wellington, US) and 15mL of 1mol/L KOH/EtOH were added to the sample. The sample was mixed, and stood for 18 hours at room temperature, then 10mL of distilled water and 10mL of hexane were added. Sample was shaken for 5 minutes, upper layer was transferred to another tube. Lower layer was extracted by hexane twice again. Combine the upper hexane layers rinse by 10mL of distilled water 3 times, and add anhydrous Na₂SO₄, the hexane solutions were concentrated to about 2mL by a rotary evaporator.

Cleanup and analysis

A florisil column composed of 5g of florisil was conditioned by hexane 100mL. Concentrated solution was applied to the florisil column, and eluted by 50mL of hexane. The fraction was evaporated to approximately 1mL and then transferred to a pointed bottom test tube. The internal standard 50μL of 100 times diluted ¹³C₁₂-PCBs (WP-ISS, Wellington, US), was added. The solvent was removed with a nitrogen gas flow and evaporated to approximately 50μL. 20μL of the sample in 50μL of final solution was injected into the PTV injector to analyse by HRGC-HRMS. The HRGC/HRMS analysis was performed on AutoSpec-Ultima high resolution mass spectrometer, (Micromass, UK) coupled to a Agilent 6890 Plus GC Agilent Technologies inc, USA. Table 1 shows HRGC/HRMS condition. Figure 1 shows the chromatogram of PCBs in blood.

Results and Discussion

Measurement of the PCBs concentration was done on 6 samples to compare the PCB concentration of the whole blood between the plasma. PCBs concentration in the plasma showed higher value than in the whole blood, and the correlation of $R = 0.99$ was recognized (figure2.).The PCBs concentration of the whole blood was converted by the following hematocrit conversion-formula. The PCBs in the plasma, and the distribution of PCBs in the blood were calculated.

<Conversion-formula>

Plasma PCBs conversion value = whole blood PCBs concentration / (1 - hematocrit value)

PCBs concentration in the whole blood converted by the hematocrit value was very close to PCBs concentration of the plasma (figure5.). It was expected that small amount PCBs was contained in the blood cell and many PCBs existed in the plasma.

Muir and Morita et.al focused on PCBs isomers of 7(#28/31, 52, 101/90, 118, 138, 153, 180) and 30 (#8/5, 18, 28, 31, 44, 49, 52, 95/66, 87, 99, 101, 105/132, 110, 118, 128, 146, 149, 151, 153, 138/163, 156, 183, 187, 201/157, 170, 180, 194, 195, 206, 209) in UNEP (the United Nations environment plan). And we measured these specific PCB isomers, and compared them with the measurement value of all isomers. Correlation $R > 0.99$ was recognized on PCBs of the whole blood between the plasma respectively in UNEP-7, 30isomer, and all isomers. UNEP-30isomer was occupying 80% amount of all PCBs isomers in blood, and the utility of the simplified analysis by specific isomer was recognized (figure3. and figure4.).

We obtained positive correlation $R > 0.99$ in the whole blood and the plasma, in PCBs measurement.

The utility of the simplified analysis which used the specific congeners was recognized.

Table 1. The condition of HRGC/HRMS

MS	AutoSpec-Ultima(Micromass)
Ionization mode	EI
Electron Energy	38eV
Trap current	700 μ A
Accel Voltage	8kV
Ion source Temp	280
Interface Temp	300
Resolution	M/ M >10,000 (10% Valley)

GC	Agilent 6890 Plus GC (Agilent Technologies inc)
Column	HT8-PCB(Kanto kagaku,Japan) 60m x 0.25mmI.D.
Injector	PTV(Agilent, Technologies inc, USA)
Injection Volume	5 μ L
Oven Temp	60 (2.5min)-20 /min-180 -2 /min-260 - 5 /min-300 (4min)

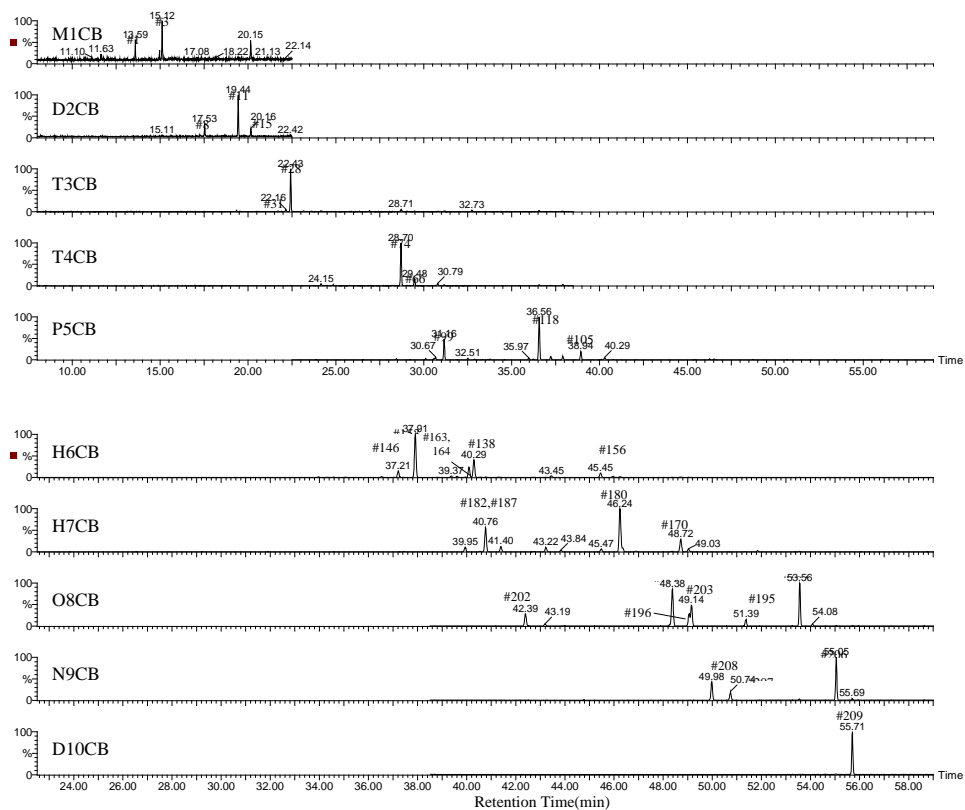


figure1. An example chromatogram of PCBs in blood

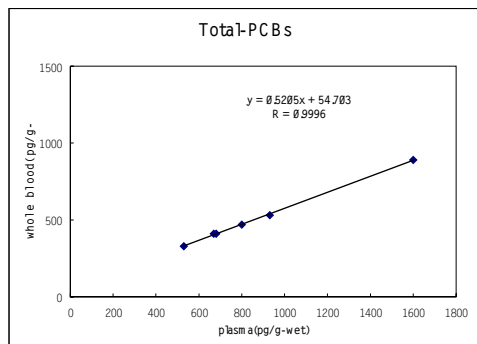


figure2. The correlation figure of the PCBs concentration of the whole blood between the plasma

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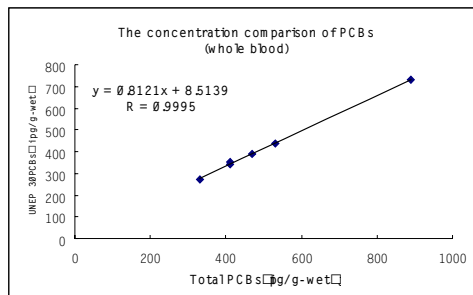
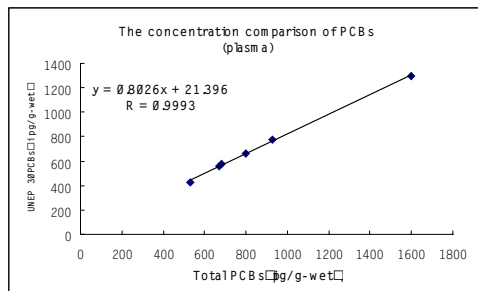


figure3. The comparison of the PCBs concentration by the analysis of all isomers and the analysis of UNEP 30 isomers: Plasma (the left) and whole blood (the right)

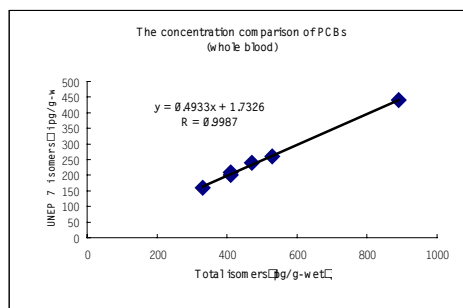
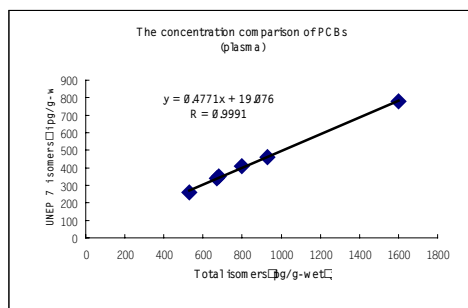


figure4. The comparison of the PCBs concentration by the analysis of all isomers and the analysis of UNEP 7 isomers: Plasma (the left) and whole blood (the right)

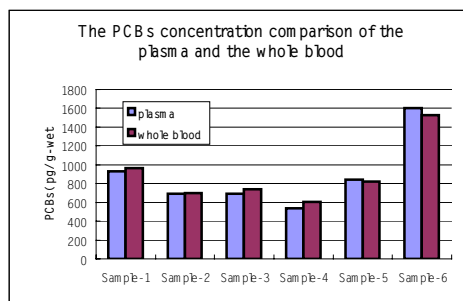
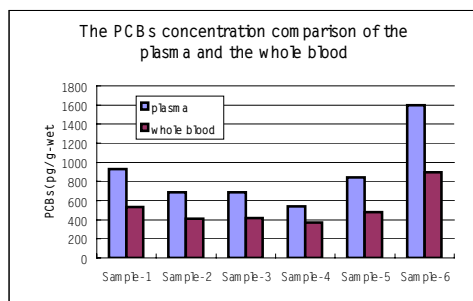


figure5. The comparison of the PCBs concentration between the whole blood and the plasma : before (the left) and the after (the right) of the hematocrit conversion.

References

- 1 Matsumura T, environment chemistry Vol.12, NO.4, p855, 200
- 2 Kadokami K, the 26th meeting on Japanese Environment Chemistry Program and Abstracts, p109, 1998
- 3 D. Muir and M. Morita, Substances and Analytical Techniques, Background Paper for the UNEP POP Workshop, 24-27 March 2003.