ANALYSIS OF ENDOCRINE DISRUPTORS IN MOTHER'S MILK AND SERUM OF MOTHER'S BODY / CORD BLOOD USING AN ON-LINE GPC-GC/MS SYSTEM

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Introduction

In recent years, much attention has been paid to endocrine disruptors such as PCB and organochlorine pesticides and their adverse effect on human health. The endocrine disruptors accumulate in the body to a high concentration, causing continuous exposure. The exposure of a fetus or an infant to such chemical substances is a matter of serious concern. In doing toxicological evaluation, it is essential to determine the level of exposure of a fetus or an infant to those substances that come via mother's blood or milk. It is also important to conduct analyses at lowest possible concentrations.

Meanwhile, in most cases, endocrine disruptors have been analyzed individually, requiring a lot of time, organic solvents and complicated procedures. This means that it is difficult to effectively handle many items of endocrine disruptors. Furthermore, an analysis of phthalic esters involves contamination of reagents and other forms of contamination occurring in the course of analysis, making it difficult to obtain accurate data.

We have therefore developed a system whereby we can analyze various types of endocrine disruptors simultaneously, quickly and automatically. Using this system, we have analyzed and compared PCB and organochlorine pesticide levels in serum of mother's body, serum of cord blood and mother's milk, belonging to the same person. As a result, we have clarified the movements of endocrine disruptors from mother's womb to fetus. In particular, we separated 209 different types of PCB isomers and determined their respective concentrations, thereby examining the relationship between their structures and internal movements.

Materials and Methods

Samples and reagents: We analyzed 30 samples, comprising 10 each of serum of mother's body, serum of cord blood, and mother's milk, taken respectively from 10 mothers in 1999 at Tokai University's Medical Department. After obtaining those mothers' consent, the samples were put to tests. As for reagent, we used solvents for analyzing PCB and pesticide residue.

Equipment: We introduced an on-line GPC-GC/MS analytical system as shown in Figure 1. Specifically, the system comprises a gel permeation chromatography (GPC) (Figure 1-A); LC-GC interface (Figure 1-B); and GC/MS with a programmed temperature vaporizer (PTV) (Figure 1-C). Conditions for respective valve units, GPC and GC columns are given in Figure 1.

Each sample (20 μ l) extracted from mother's milk, serum of mother's body and serum of cord

blood is purified in the GPC column (removal of fat, pigment, etc.), stored in the sample loop (200 μ l) and then injected into PTV-GC/MS. In other words, purification, concentration and measurement are conducted automatically in this on-line system. The system is also advantageous in that it prevents contamination at the time of sample preparation (extraction and concentration). Thus, contamination from outside can be effectively prevented in monitoring surveys where many samples are analyzed. The system is therefore especially useful for analysis of minor components.

Calibration curves and detection limits of GC/MS

For PCB, we prepared calibration curves for respective isomers. We used ^{13}C isotopes of 3,3',4,4'-tetrachlorobiphenyl, 3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4',5,5'-hexachlorobiphenyl, HCB and p,p'-DDE as internal standards. The determination was based on relative calibration curves pertaining to approximate internal standards.

All PCB isomers (209 types in total) were separated with capillary columns for determination. The sum of the concentrations of respective PCB isomers was regarded as the total PCB concentration.

Detection limits of this system were as follows:

PCB (tetra-penta-hexa): 5pg (EI),PCB (hepta-octa): 1 pg (NCI),PCB (nona-deca): 0.2 pg (NCI),Hexachlorobenzene: 2 pg (NCI),HCH, heptachlor, dieldrin, and DDT:20 pg (EI)

Preparation of samples: Serum of cord blood (8 ml), serum of mother's body (4 ml), and mother's milk (2 ml) were taken. After extraction of fat according to the Patterson method[1], fat content was measured using the gravimetric method. All amounts of fat were dissolved in 50-100 μ l of GPC mobile phase (acetone:cyclohexane=3:7). Then 20 μ l of the substance was injected into GPC-GC/MS.

Average amounts of fat extracted by this method were 0.197% for serum of cord blood, 0.895% for serum of mother's body, and 3.04% for mother's milk.

Results and Discussions

We examined GPC conditions that would enable us to remove fat and pigment and, at the same time, recover PCB and organochlorine pesticides effectively. As a result, we decided to take a fraction (200 μ l) 3.2-5.2 minutes after injection.

Figure 2 shows average concentrations in fat of PCB and organochlorine pesticides contained in mother's milk, serum of mother's body, and serum of cord blood.

Measurements of PCB concentrations were as follows:

In mother's milk: 95 ng/g on average, 42 ng/g minimum, and 192 ng/g maximum.

In serum of mother's body: 99 ng/g on average, 34 ng/g minimum, and 222 ng/g maximum.

In serum of cord blood: 61 ng/g on average, 15 ng/g minimum, and 126 ng/g maximum.

As for organochlorine pesticides, high concentrations of DDE and β-HCH were detected.

We compared PCB/organochlorine pesticide concentrations in the identical person's milk, body serum and cord blood serum. We found that concentrations in fat were similar for mother's milk and serum of mother's body. However, we found that concentrations in serum of cord blood were somewhat lower than in the above two categories.

On the whole basis, PCB/organochlorine pesticide concentrations in serum of cord blood were approximately one-fourth the concentrations in serum of mother's body. This is because fat content of serum of cord blood is about one-fourth the fat content of serum of mother's body.

Among various PCB isomers, high concentrations of hexa- and hepta-PCBs were found in mother's milk, serum of mother's body and serum of cord blood. There was no significant

concentration difference between serum of mother's body and serum of cord blood in relation to the number of chlorine atoms contained in respective PCB isomers. However, concentrations of #118 (penta chloro), #168 (hexa chloro), #153 (hexa chloro) and #170 (hepta chloro) in serum of cord blood were significantly lower than in serum of mother's body. The confidence coefficient was 95% or more.

Table 1 shows evaluation results of infants' exposure to PCB and organochlorine pesticides via mother's milk. As is clear from the table, daily intake of those substances was between 7.7% and 33.7% of ADI.

Table 1 Daily Intake of Organochlorine Compounds by Infants via Mother's Milk

Compounds	ADI	Concentrationl	Daily intak	Rate(%)
	μ g/kg/day	μ g/g fat	μ g/kg/day	Daily intake/ADI
PCB	5	0.095	0.428	8.6
DDT	20	0.344	1.55	7.7
HCH	8	0.316	1.422	17.8
Chlordane	0.5	0.037	0.167	33.3
HCE	0.1	0.003	0.014	13.5
HCB		0.014		
	pg-TEQ/kg/day			
PCDD/PCDF	4	14.6	65.7	1643
Co-PCB(*non-ortho*)	4	3.2	14.4	360
Co-PCB *(mono-ortho*)	4	3.0	13.5	338
PCDD/PCDF/Co-PCB	4	20.8	93.6	2340

Conclusion

Owing to the development of the on-line GPC-GC/MS system, we are now able to automatically analyze PCB and organochlorine pesticides contained in a small amount of serum.

Concentrations of PCB and organochlorine pesticides in serum of cord blood on the whole basis were approximately one-fourth the concentrations in serum of mother's body.

Exposure of a fetus to PCB and organochlorine pesticides can now be estimated from the flow rate of cord blood.

Exposure of an infant to organochlorine pesticides via mother's milk was between 33% and 8% of respective ADIs.

Acknowledgements

This work was supported in part by the Health Sciences Research Grants from the Ministry of Health and Welfare of Japan.

References

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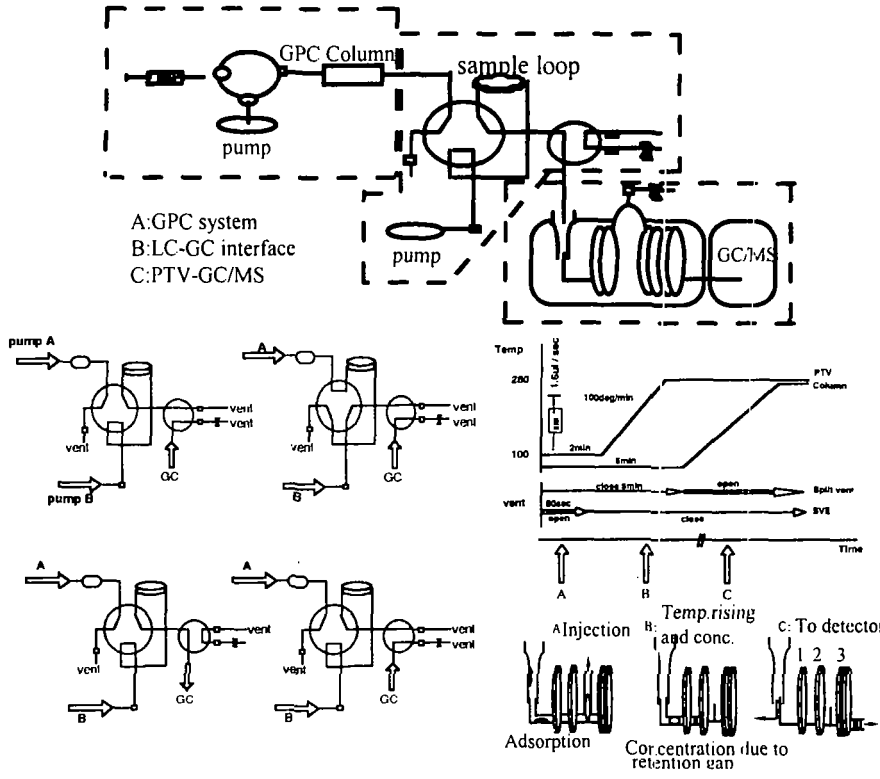

Figure 1 On-Line GPC-GC/MS Equipment

1:J&W deactiveted silica tubing 2m x 0.53mm i.d. 2:J&W DB-5 0.5m x 0.25mm i.d. x 0.25 μ m
3:J&W DB-5 60m x 0.25mm i.d. x 0.1 μ m
GPC column: CLNpak EV-200AC 150mm x 2mm i.d.(Showa Denko,Tokyo, Japan)
GPC mobile phase (acetone:cyclohexane=3:7), flow rate: 0.1ml/min

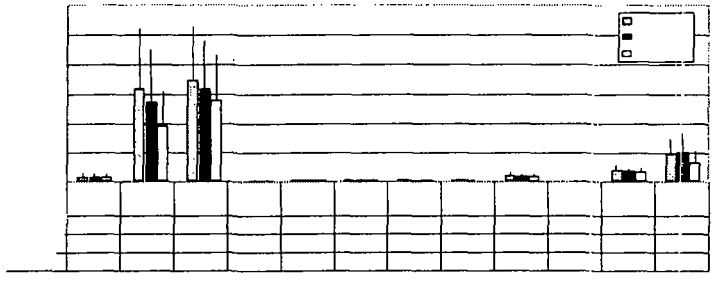

Figure 2 Pesticide/PCB Concentrations