# GENDER SPECIFIC DYNAMICS OF PCDD/DFs, PCBs, PBDEs AND ORGANOCHLORINES IN BLOOD OF JAPANESE FAMILIES OVER TWO-YEAR STUDY PERIOD

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## Introduction

Contamination and health impacts of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (dioxin-like PCBs), total PCBs and polybrominated diphenyl ethers (PBDEs) have been studied(for example1-3). Since Baughman and Meselson reported the occurrence of PCDDs/DFs in human tissues in the early 1970's, several studies have described the occurrence of these compounds in environmental samples and biological tissues collected from many regions of the world[4].

On the other hand, organochlorine compounds were produced in large quantities in the 1930's and the global production then increased year by year. Because of their toxic biological effects, they were banned or severely restricted during the early 1970's in most developed countries. Japan is one of the developed nations in Far East that used large quantities of these chemicals during 1950's and 1960's. Among the organochlorine pesticides (OCPs) used, DDTs (*o,p'*- and *p,p'*-compounds of DDT, DDE and DDD), HCHs (hexachlorocyclohexane), HCB (hexachlorobenzene) and CHLs (chlordane compounds) are known for their contamination in the food chain, and long-term health effects on wildlife and humans. Their exposure to humans and animals is of serious concern owing to chronic toxic effects. Since these chemicals have persistent properties and long-term effects, regular monitoring of these chemicals is essential for understanding their behavior and to prevent health hazards.

In order to avoid future exposure and to provide healthy environment to humans, biological degradation by efficient dioxin decomposing alternative should be introduced. In rats, dietary fiber and chlorophyll shown to activate fecal excretion of PCDD/DFs probably due to inhibition of their absorption in the digestive tract and consequent decrease in liver. For instance, FBRA the brown rice fermented with *Aspergillus oryzae* and rich dietary fiber seems to a major tool for this particular concern[5].

Based on these observations, we studied the dynamics PCDD/DFs, PCBs, PBDEs and OCPs in male and female of different families as they have slightly different habitat such as FBRA exposed and non FBRA exposed cases. Mainly we conducted dynamics of PCDD/DFs, PCBs, PBDEs and OCPs in exposed individual and non-exposed individual from nine Japanese families over two year period. Based on these findings we estimated varied dynamics of these chemicals by food habit and impact of FBRA. Furthermore, current toxic equivalency (TEQ) also calculated. In addition, comparison was made in male and females of same family inter family and temporal variation also discussed.

## Materials and Methods

*Sample collection*

FBRA has been manufactured for 30-years by Genmaikouso Corporation located at Sapporo, Japan and about lakhs of peoples consuming this staple food. Nine married couple of 37 to 48 year old were voluntarily participated for this study and they were further grouped in to two categories that match age and sex. First group consumed FBRA immediate after their meal for two-year period and second group didn't consumed. Whole blood (approximately 50 g) of each volunteers were collected for 5 times [before start the study (*n*=2), ½ an year after study, 1-year after study (*n*=2), 1 and ½ year after study and 2-year after study (n=2)] using clean micro syringe which is heparinized. The collected blood was transported to the laboratory and stored at –20 °C until chemical analysis.

*Analysis*

After adding 17 species of $^{13}C_{12}$-labeled 2378-PCDD/DFs and 22 species of $^{13}C_{12}$-PCBs including dioxin-like PCBs and to the whole blood samples as internal standards, the blood lipid was extracted according to the method previously reported[6] ($^{13}C_{12}$-PBDEs and $^{13}C_{12}$-DDE were spiked after extraction), determined gravimetrically and subjected to a column chromatographic clean-up procedure[7]. Analysis of PCBs and PBDEs was slightly modified and was reported elsewhere[8-9]. Identification and quantification of 2378-substituted congeners of PCDD/DFs, PCBs, PBDEs and OCPs was performed using Hewlett Packard 6890 Series high-resolution gas chromatography interfaced with a Micromass Autospec - Ultima high-resolution mass spectrometer.

**Results and Discussion**

*PCDD/DFs*

Concentrations of PCDD/DFs were greatly pronounced in between FBRA exposed individuals and non-exposed individuals (results not shown). In general, average PCDDs (470) was predominant accumulants than PCDFs (49) on pg/g fat wt basis (Table 1). Particularly, females from 6 families accumulated greater levels of PCDD/DFs (results not shown). The ratio of PCDDs to PCDFs were varied depending up on the families, time and in between gender. Strikingly, accumulation pattern in both sex of same family had similar accumulation levels. The levels were slightly decreased comparatively to the beginning of the study especially for exposed individuals (Figure 1). OCDD was prevalent congener in all the samples analyzed and which is common for human samples.

*Dioxin-like PCBs*

Concentrations of dioxin-like PCBs were several magnitudes greater than PCDD/DFs (mean 25,000 and median 18,000-pg/g fat weight, Table 1). In addition, with contrast to the PCDD/DFs accumulation pattern, males from 8 among 9 families accumulated greater levels of dioxin-like PCBs when compare to females (results not shown). There is no pronounced decrease of dioxin-like PCBs concentrations in blood of humans until to-date (figure 1). Notably, accumulation pattern is slightly varied in between sex of same family. In general, contribution to the total dioxin-like PCBs by non-, mono- and di-*ortho* PCBs was 0.21, 32, 67 %, respectively.

*Toxic Equivalency (TEQ)*

Fluctuated TEQs were observed in between families, gender and time either for PCDD/DF TEQ as well as dioxin-like PCBs TEQ (Table 1). Maximum TEQ of PCDD/DFs and dioxin-like PCBs was several orders greater than minimum TEQs on pg/g fat wt. basis. However, irrespective to the accumulation pattern, TEQ pattern in either sex was same in particular families. On the whole, 23478-PeCDF, 12378-PeCDD and 123678-HxCDD among PCDD/DFs contributed greater TEQs. While non-*ortho* PCBs IUPAC #126 was prevalent and IUPAC #105 and #118 was prevalent among mono-*ortho* PCBs.

**Table 1.** Average, mean, and range of PCDD/DFs, dioxin-like PCBs, TEQ, total PCBs PBDEs and OCPs concentrations in Japanese human blood for two-year study period.

| | Average | Median | Min. | Max. |
|---|---|---|---|---|
| Total PCDDs | 470 | 390 | 79 | 2,100 |
| Total PCDFs | 49 | 34 | 10 | 250 |
| Dioxin-like PCBs | 25,000 | 18,000 | 4,500 | 100,000 |
| Total TEQ | 29 | 25 | 7 | 110 |
| PCBs | 270,000 | 210,000 | 62,000 | 970,000 |
| Total DDTs | 350,000 | 260,000 | 61,000 | 1,600,000 |
| Total HCHs | 140,000 | 68,500 | 14,000 | 710,000 |
| Hexachlorobenzene | 18,000 | 15,000 | 2,700 | 120,000 |
| Total Chlordane | 43,000 | 34,000 | 7,600 | 150,000 |
| DiBDE(#15) | 770 | 100 | 53 | 20,000 |
| TriBDE(#28) | 410 | 125 | 65 | 8,600 |
| TeBDE(#47) | 830 | 1,200 | 100 | 14,000 |
| PeBDE(#99) | 210 | 670 | 130 | 63,000 |
| PeBDE(#100) | 260 | 140 | 57 | 1,200 |
| HxBDE(#153) | 670 | 510 | 370 | 2,100 |
| HpBDE(#183) | 160 | 140 | 56 | 520 |
| Total PBDEs | 3,550 | 1,902 | 951 | 37,250 |

Concentrations express on pg/g fat weight basis

*PBDEs*

There was no notable difference of concentrations in between exposed and non-exposed peoples (data not shown). Considerably, males had comparatively greater concentrations than females. Mean and ranges of total PBDEs were in males and females were 1900-4400 and 1400 to 2100 pg/g fat wt., respectively. TeBDE #47 was highly accumulated congener followed by HxBDE (#153), HpBDE (#183), PeBDE (#99), PeBDE (#100), TrBDE (#28), DiBDE (#15), HxBDE (#154) and TeBDE (#49) (Table 1). This is the first report of mono-through hepta-BDEs in human blood. We also found higher deca-BDEs concentration in all the samples. However, blank samples also contained considerable levels of this congener so we neglected to include their value in this abstract. Thus, we establish the quantification method for deca-BDEs and further studies is on the way. Greater correlations have been noticed for total PCBs and TEQ of dioxin-like PCBs (Figure not shown). Further, slightly good correlation has been observed for TEQs of dioxin-like PCBs and PCDD/DFs. However, there was not apparent correlation in between total TEQ Vs TeBDE, TEQ Vs PeBDE, TEQ Vs HxBDE and PCDD/DF TEQ Vs PCDD/DF concentrations. The exposure routes of PBDEs probably suggestive from working conditions of males and females.

*Total PCBs*

Males from 8 families accumulated greater levels of total PCBs when compare to females. The concentrations were ranged from 62,000 to 970,000 pg/g fat wt, basis (Table 1). There is no pronounced decrease of PCBs concentrations in blood of humans until to-date. It is noteworthy only this study conducted total PCBs of DiCBs to DecaCBs for the first time. Notably, accumulation pattern is slightly varied in between sex of same family. These trends probably reflect the intake and excretion condition between males and females and in between family may be different.

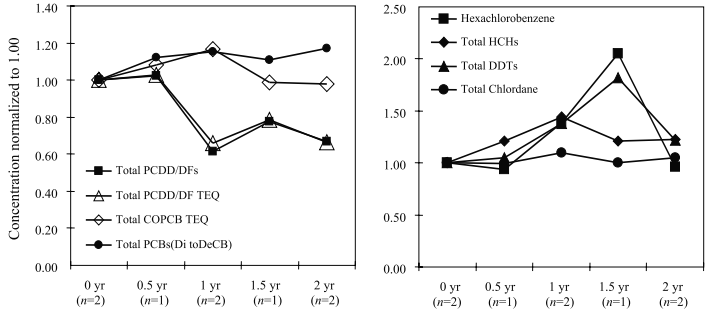


**Figure 1.** Normalized concentrations of PCDD/DFs, dioxin-like PCBs, TEQ and OCPs in Japanese human blood for 2-year study period.

*OCPs*

Among the organochlorine pesticides analyzed, DDTs and its metabolites recorded greater concentrations 350,000 (ranges: 61,000 to 1,600, 000) on pg/g fat wt (Table 1). HCHs recorded second highest concentrations of 140, 000 (ranges: 14,000 to 710, 000) followed by CHLs 43,000 (ranges: 7,600 to 150,000) and HCB 18, 000 (ranges: 2,700 to 120, 000) pg/g fat wt. basis (Table 1). Uniformly either gender of same family had similar OCPs exposures. This trend was different from PCBs accumulation in which males had greater accumulations. Furthermore, PCDD/DFs showed similar results of PCBs pattern in our another study (Figure 1). DDTs levels were doubled in 1.5-year samples and levels were decreased same as 0.5 year samples (Figure 1). Similarly HCHs and HCB were increased until 1-year period and decreased (Figure 1). Only CHLs levels didn't changed during entire period of study (Figure 1). There was not much pronounced decrease in exposed individuals and non-exposed individuals (Figure 1).

Similar accumulation pattern of OCPs in same family possibly suggests the similar food intake by husband and wife. Slightly varied results of PCBs accumulation in between sex is reflective of reduced burden during childbirth in females. Correlation results demonstrated less significant correlations in between TCDD-like TEQ Vs HCB, TCDD-like TEQ Vs HCHs, TCDD-like TEQ Vs DDTs, TCDD-like TEQ Vs CHLs, DDTs Vs total PCBs, DDTs Vs CHLs and coplanar PCB-TEQ Vs total PCBs. These observations indicated that TEQ mainly contributed by PCDD/DFs and dioxin-like PCBs rather than any of analyzed organic pesticides. The correlation discussion in thus study was applicable for both exposed and non-exposed peoples.

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