

## CONCENTRATION AND DISTRIBUTION OF DIOXINS AND RELATED COMPOUNDS IN VARIOUS HUMAN ORGANS

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### Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and non-*ortho* coplanar polychlorinated biphenyls (Non-Co-PCBs) and mono-*ortho* coplanar polychlorinated biphenyls (Mono-Co-PCBs) accumulate in the human body due to their highly lipophilic properties. In recent years, there has been some concern about the potential health effects of dioxins and related chemicals for the general population of humans.

Although there exists an enormous amount of data on this subject, most of it is from breast milk and blood, due to ease of collection; information concerning concentrations and distribution in various human organs hardly exists. Therefore, new data concerning various human tissues is required to evaluate the pathophysiological significance of dioxins and related compounds in humans.

The aim of this study was to investigate the concentration levels and distribution of dioxins and related compounds in various human organ tissues.

We previously reported on the concentration levels in the human liver and adipose tissues from 28 donors<sup>1</sup>.

In this paper, we determined the concentrations of dioxin-like isomers in 8 organs, including blood, lungs, liver, bile, spleen, pancreas, kidney and mesentery fat from 20 donors.

### Materials and Methods

The human organ samples were collected from twenty patients who had died at School of Medicine, Keio University in 1999-2001. The families of the donors

gave their informed consent. The collected organs were blood, lungs, liver, bile, spleen, pancreas, kidney and mesentery fat. The samples were stored at -20 degrees for later analysis. Donor ages ranged from 27 to 90 years; the average age was 65 years.

Each sample was extracted by an accelerated solvent extractor (ASE-200, Dionex, Sunnyvale, CA), weighed to 5 g accuracy, and mixed with 4 g Isolute (International Sorbent Technology Ltd., Hengoed, Mid Glamorgan, UK). After the mixed sample was loaded into the extraction cell,  $^{13}\text{C}$ -labeled-PCDDs,  $^{13}\text{C}$ -labeled-PCDFs, and  $^{13}\text{C}$ -labeled-PCBs, as internal standards, were added. Acetone and n-hexane (1:4, v/v) were used as extraction solvents. The lipid obtained was dissolved in n-hexane and treated with concentrated sulfuric acid. The separated hexane layer was applied to a silver nitrate/silica gel column (0.5 g) and eluted with 15 ml of n-hexane. The eluted solution was loaded into an active carbon column (0.5 g) after being evaporated to 1 ml and separated into two fractions. The first fraction, containing Mono-Co-PCBs, was eluted with 10 ml of 10% (v/v) dichloromethane /n-hexane. PCDDs, PCDFs, and Non -Co-PCBs were eluted with 25 ml of toluene as the second fraction. The method employed here requires only a reduced amount of blood collected from patients compared with the conventional method<sup>2,3</sup>. The column packing (silver nitrate silica gel, active carbon column, and anhydrous sodium sulfate) used in this experiment was washed in order to reduce blank materials by ASE-200 under the same conditions as the lipid extraction with n-hexane or toluene. Concentrations of the PCDDs, PCDFs, Non-Co-PCBs and Mono-Co-PCBs were measured using HRGC/HRMS (Autospec Ultima E, MicroMass Ltd., Manchester, UK) equipped with an SCLV (SGE International, Victoria, Australia) injection system. The column used for solvent-cut was a BPX-5 fused silica pre-capillary column (0.25 mm i.d.×6 m, 0.25  $\mu\text{m}$  film thickness), and for the analytical column was a BPX-5 fused silica capillary column (0.15 mm i.d.×30 m, 0.15  $\mu\text{m}$  film thickness), (SGE International, Victoria, Australia), respectively.

## Results and Discussions

**Concentration of dioxins in various organs:** Table 1 shows the mean concentrations of PCDDs, PCDFs, Non-Co-PCBs and Mono-Co- PCBs in eight organs. The mean TEQ concentrations in blood, lung, liver, bile, spleen, pancreas, kidney and mesentery fat were 119, 178, 228, 50, 113, 163, 138 and 139 pg-TEQ/g lipid, respectively. Parallel levels were seen in the blood, spleen, kidney and mesentery fat; in the lungs and pancreas, levels were somewhat higher. Among the organ tissues sampled, the highest concentration was observed in the liver and the lowest

in the bile. Mean total-TEQ concentration of the liver was about 4.5 times higher than that of bile.

The prevalent congener among PCDDs was OCDD, ranging from 923 to 6837 pg/g lipid; among PCDFs, it was 2,3,4,7,8-PeCDF except for in the liver sample. In the mesentery fat sample, the mean TEQ concentration was 108 pg-TEQ/g lipid (except for Mono-Co-PCBs). This value was 1.9 times higher than that of our previous data<sup>1</sup> of 56 pg/g lipid for mean age of 56 years old. Age-related increases in dioxin concentrations in human samples have been reported<sup>4,5</sup>; however, whether our observations have a pathophysiological significance or show the effects of age cannot be decided at present.

**Contribution of each isomer to the total TEQ:** Figure 1 shows the toxic contribution of each congener for 8 organs to the total TEQ.

The average toxic contribution of PCDDs, PCDFs, Non -Co-PCBs, and Mono-Co-PCBs in tissue samples was  $32 \pm 6\%$ ,  $25 \pm 7\%$ ,  $24 \pm 6\%$  and  $19 \pm 4\%$  to the total TEQ value, respectively. In the liver and spleen sample, the contribution rate of PCDFs was higher (30%) than in other organs (20%). The contribution rate varied slightly among organs, with the rate of Non -Co-PCBs in spleen falling as low as 13%.

**Correlations of concentration of dioxins in various organs:** We estimated concentrations of the correlations of toxic congeners among 8 tissues determined in this study using linear regression analysis. The data on TEQ are summarized in Table 2. The TEQ concentration in the liver correlated well with that in mesentery fat, blood, kidney, spleen, pancreas and lung; however, the bile did not correlate with the liver. A positive correlation among almost all the congeners was observed in tissues; however, levels of highly chlorinated PCDDs and PCDFs such as HpCDDs/DFs and OCDD/OCDF did not correlate with the levels of the other congeners.

Table 1 Mean concentrations of dioxins and related compounds in various human samples

| Congeners                            | Blood<br>(n=18) | Lung<br>(n=18) | Liver<br>(n=20) | Bile<br>(n=15) | Spleen<br>(n=18) | Pancreas<br>(n=18) | Kidney<br>(n=20) | Mesentary fat<br>(n=20) |
|--------------------------------------|-----------------|----------------|-----------------|----------------|------------------|--------------------|------------------|-------------------------|
| 2,3,7,8-TCDD                         | 4.9             | 8.2            | 7.8             | 2.7            | 5.0              | 5.8                | 5.7              | 5.4                     |
| 1,2,3,7,8-PeCDD                      | 21              | 38             | 35              | 12             | 27               | 25                 | 26               | 23                      |
| 1,2,3,4,7,8-HxCDD                    | 8.6             | 18             | 17              | 2.9            | 21               | 12                 | 18               | 8.5                     |
| 1,2,3,6,7,8-HxCDD                    | 67              | 112            | 96              | 30             | 92               | 90                 | 97               | 73                      |
| 1,2,3,7,8,9-HxCDD                    | 12              | 15             | 20              | 7.3            | 12               | 10                 | 13               | 10                      |
| 1,2,3,4,6,7,8-HpCDD                  | 48              | 101            | 167             | 28             | 145              | 55                 | 129              | 32                      |
| OCDD                                 | 1344            | 2173           | 6837            | 1128           | 1961             | 1158               | 2152             | 923                     |
| 2,3,7,8-TCDF                         | 1.9             | 3.0            | 10              | ND             | ND               | 2.9                | 2.0              | 2.6                     |
| 1,2,3,7,8-PeCDF                      | 1.8             | 2.3            | 9.2             | 1.2            | 1.3              | 2.1                | 2.3              | 2.1                     |
| 2,3,4,7,8-PeCDF                      | 43              | 67             | 119             | 19             | 58               | 51                 | 55               | 42                      |
| 1,2,3,4,7,8-HxCDF                    | 15              | 23             | 81              | 7.9            | 24               | 13                 | 21               | 12                      |
| 1,2,3,6,7,8-HxCDF                    | 21              | 27             | 128             | 8.7            | 22               | 16                 | 21               | 15                      |
| 2,3,4,6,7,8-HxCDF                    | 6.8             | 14             | 30              | 2.2            | 22               | 6.1                | 16               | 4.7                     |
| 1,2,3,7,8,9-HxCDF                    | 5.3             | ND             | 3.6             | ND             | ND               | ND                 | ND               | ND                      |
| 1,2,3,4,6,7,8-HpCDF                  | 14              | 13             | 119             | 4.6            | 20               | 7.9                | 16               | 8.6                     |
| 1,2,3,4,7,8,9-HpCDF                  | ND              | ND             | 11              | ND             | 2.4              | ND                 | ND               | ND                      |
| OCDF                                 | ND              | 4.3            | 14              | ND             | ND               | ND                 | ND               | ND                      |
| 344'5'-TCB (#81)                     | 20              | 19             | 24              | 6.1            | ND               | 24                 | 14               | 21                      |
| 33'4'4'-TCB (#77)                    | 109             | 29             | 50              | 33             | 8.4              | 25                 | 19               | 20                      |
| 33'44'5'-PnC <sub>10</sub> B (#126)  | 309             | 367            | 545             | 91             | 133              | 472                | 276              | 422                     |
| 33'44'55'-HxC <sub>10</sub> B (#169) | 217             | 249            | 242             | 76             | 162              | 317                | 189              | 301                     |
| 2'344'5'-PnC <sub>10</sub> B (#123)  | 955             | 931            | 1211            | 361            | 373              | 1203               | 626              | 1458                    |
| 23'44'5'-PnC <sub>10</sub> B (#118)  | 52116           | 42611          | 58955           | 20505          | 18215            | 44238              | 33899            | 64235                   |
| 2344'5'-PnC <sub>10</sub> B (#114)   | 3997            | 3425           | 4546            | 1840           | 1606             | 4509               | 2817             | 5289                    |
| 233'44'-PnC <sub>10</sub> B (#105)   | 11022           | 9472           | 12743           | 4987           | 4671             | 10901              | 7649             | 14986                   |
| 23'44'55'-HexCB (#167)               | 7715            | 6309           | 10458           | 2660           | 2729             | 8089               | 4469             | 11644                   |
| 233'44'5'-HexCB (#156)               | 24300           | 19446          | 27367           | 10077          | 10068            | 23902              | 14509            | 33700                   |
| 233'44'5'-HexCB (#157)               | 5349            | 4431           | 5662            | 2550           | 2223             | 5367               | 3219             | 6784                    |
| 233'44'55'-HpCB (#189)               | 2486            | 2359           | 3067            | 991            | 1336             | 2779               | 1659             | 3766                    |
| Total PCDD                           | 1505            | 2465           | 7180            | 1210           | 2262             | 1357               | 2441             | 1074                    |
| Total PCDF                           | 113             | 157            | 524             | 49             | 154              | 103                | 139              | 92                      |
| Total Non-ortho PCBs                 | 654             | 663            | 862             | 206            | 308              | 838                | 498              | 764                     |
| Total Mono-ortho PCBs                | 107939          | 88984          | 124009          | 43971          | 41220            | 100989             | 68846            | 141861                  |
| PCDDs-TEQ                            | 35              | 62             | 58              | 19             | 46               | 43                 | 46               | 38                      |
| PCDFs-TEQ                            | 27              | 41             | 86              | 12             | 36               | 29                 | 34               | 25                      |
| Non-ortho PCBs-TEQ                   | 33              | 39             | 57              | 10             | 15               | 50                 | 30               | 45                      |
| Mono-ortho PCBs-TEQ                  | 24              | 36             | 26              | 10             | 16               | 40                 | 28               | 31                      |
| Total TEQ                            | 119             | 178            | 228             | 50             | 113              | 163                | 138              | 139                     |

Table 2 Correlations of concentration of TEQ in various human samples

|               | Blood   | Lung    | Liver   | Bile    | Spleen  | Pancreas | Kidney  | Mesentary fat |
|---------------|---------|---------|---------|---------|---------|----------|---------|---------------|
| Blood         |         |         |         |         |         |          |         |               |
| Lung          | 0.8190* |         |         |         |         |          |         |               |
| Liver         | 0.6328  | 0.8505* |         |         |         |          |         |               |
| Bile          | 0.4137  | 0.8344* | 0.3287  |         |         |          |         |               |
| Spleen        | 0.4816  | 0.8707* | 0.8396* | 0.5868  |         |          |         |               |
| Pancreas      | 0.8183* | 0.9938* | 0.8117* | 0.7727* | 0.9015* |          |         |               |
| Kidney        | 0.7818* | 0.9788* | 0.8854* | 0.4579  | 0.9252* | 0.9678*  |         |               |
| Mesentary fat | 0.8133* | 0.9781* | 0.7969* | 0.8337* | 0.8209* | 0.9934*  | 0.9468* |               |

\*p<0.01

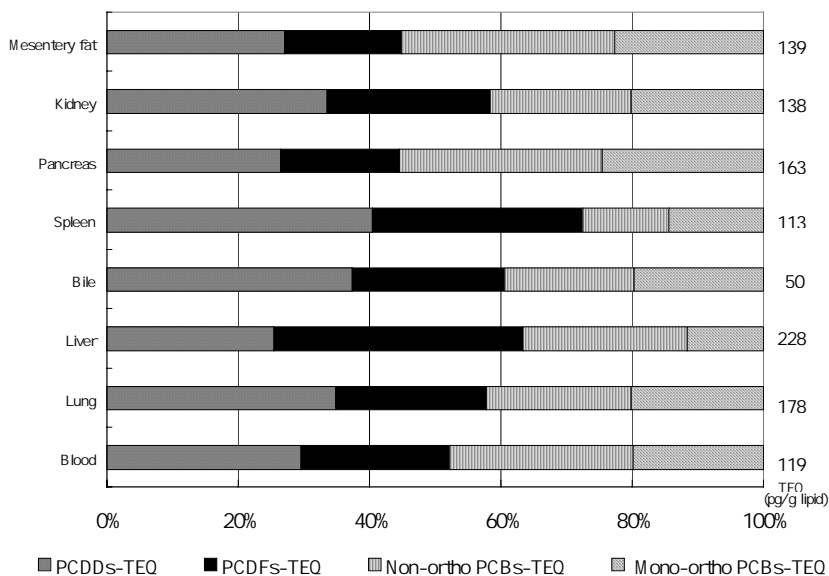


Fig.1 Percent distribution of TEQs in various human

### Conclusions

We determined the concentrations of dioxin-like isomers in 8 human organs from 20 donors. The lipid-adjusted mean TEQ levels were from 50 to 228 pg/g. Positive correlations were observed among concentrations of dioxins in various tissues. We have a plan to evaluate the relationship between the accumulation levels of dioxins and their pathophysiological significance. However, it is needed to collect data from a more extensive group of human samples.

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