# NEW PROTOCOL FOR DIOXINS ANALYSIS OF HUMAN BLOOD

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## Introduction

We are analyzing the PCDFs in the blood of Yusho patients to establish new criteria in Yusho. To treat the many blood samples for PCDFs analysis, we needed to shorten the time frame, the accuracy and increase the level of sensitivity of the analysis. Therefore, we improved the dioxins analytical protocol for human blood, and this new method is ten times more sensitive than the conventional method. This method consists of an extraction of lipids from the blood using an accelerated solvent extraction system (ASE), a reduction of regent blank using a integrated column cleanup system, and a large volume injection of the sample to GC/MS. Using this method, we could determine the dioxins from only 5ml blood, and we could easily make the sample preparation from blood in dioxins analysis. Therefore, we suggest that through using this method it will be possible to analyze the dioxins in many blood samples in a short time.

## Materials and Methods

Native PCDDs, native PCDFs and native polychlorinated biphenyls (PCBs) as authentic standards were purchased from Wellington Laboratories, Ontario, Canada. [$^{13}C_{12}$]-PCDDs, [$^{13}C_{12}$]-PCDFs, and [$^{13}C_{12}$]-PCBs as internal standards were also purchased from Wellington Laboratories, Ontario, Canada. An active carbon column was prepared as follows; active carbon was purchased from Nacalai Tesque, Kyoto, Japan, refluxed 3 times with toluene for 1 hr, and dried *in vacuo*, then 500 mg of the active carbon was mixed with 500 g of anhydrous sodium sulfate (Wako Pure Chemicals Ind. Co. Ltd., Tokyo, Japan. A silver nitrate/ silica gel was purchased from Wako Pure Chemicals Ind. Co. Ltd., Tokyo, Japan. Distilled water used in this experiment was treated with hexane. All other chemicals used were of the analytical grade of dioxins commercially available. We obtained the informed consent from all the Yusho patient's blood samples analyzed in this study. [$^{13}C_{12}$]-Labeled standards related dioxins were added in 5 mL of the Yusho patient's blood, and extracted 2 times for 10 min with ASE-200 (Dionex Corporation, Sunnyvale, CA). The extract conditions were as follows. The temperature was 150 °C, the pressure was 2000 psi, and the solvent used was acetone/hexane (1: 4). The extract was evaporated to dryness, and measured gravimetrically. The column cleanup method reported previously was scaled down a quarter, and applied to the blood dioxin cleanup. PCDDs and related compounds were analyzed using a gas chromatography - mass spectrometry (GC-MS). The analytical conditions were as follows; gas chromatography used an HP-6890 A series II (Hewllet-Packard, Palo Alto, CA), equipped with an Autospec Ultima E, (Micromass Ltd., Manchester, UK) and a solvent cut large volume injection system (SCLV), SGE International., Victoria, Australia; the column used was an BPX-5 fused silica pre-capillary column, 0.25 mm i.d. 6 m, 0.25 mm film thickness (SGE International., Victoria, Australia); the analytical column, 0.15 mm i.d. 30 m, 0.15 mm film thickness (SGE International., Victoria, Australia); the column temperature was heated at 80 °C to 320 ºC at a rate of 20 °C/min, maintained at 320 °C for 5 min, cooled down to 180 °C at the rate of 70 °C/min, maintained at 180 °C for 1 min,

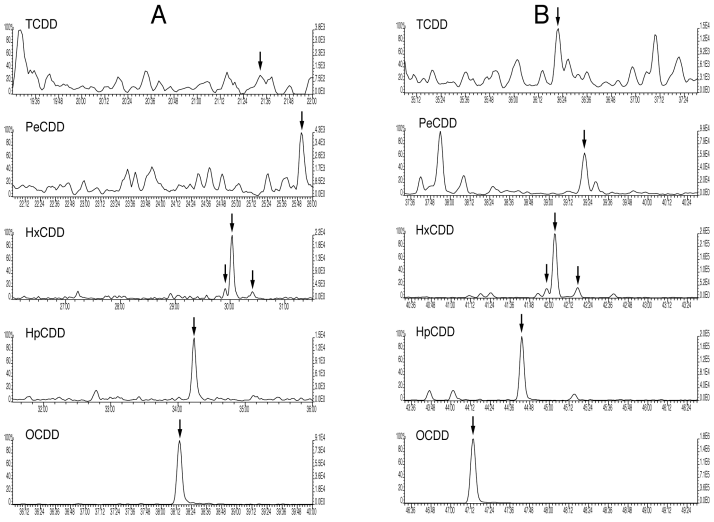


Figure 1 HRGC/HR/MS Chromatograms of PCDDs in Human Blood
A: Normal injection 2 μl/5 μl
B: Solvent cut large volume injection 10 μl/25 μl

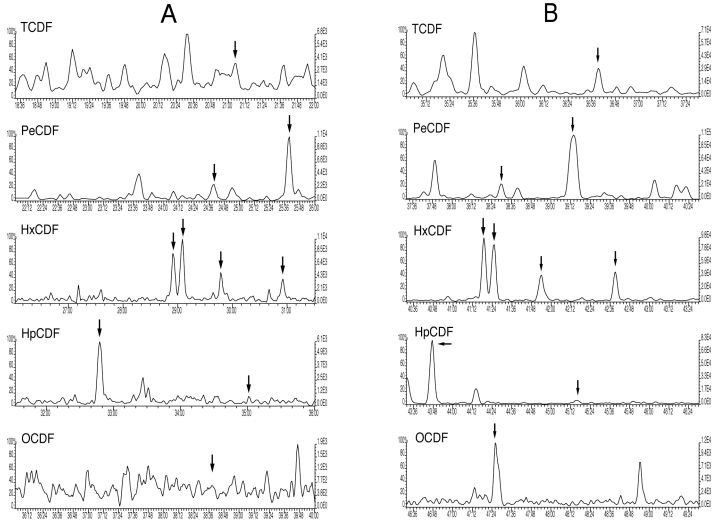


Figure 2 HRGC/HR/MS Chromatograms of PCDFs in Human Blood
A: Normal injection 2 μl/5 μl
B: Solvent cut large volume injection 10 μl/25 μl

Table1 Background level of Dioxin in the new protocol.

| Congeners | Whole basis | | | Lipid basis | | |
|---|---|---|---|---|---|---|
| | Blank 1 | Blank 2 | Mean | Blank 1 | Blank 2 | Mean |
| 2,3,7,8-TCDD | ND | ND | ND | ND | ND | ND |
| 1,2,3,7,8-PeCDD | 0.0018 | ND | 0.0018 | 0.60 | ND | 0.60 |
| 1,2,3,4,7,8-HxCDD | ND | ND | ND | ND | ND | ND |
| 1,2,3,6,7,8-HxCDD | 0.0004 | 0.0022 | 0.0013 | 0.13 | 0.73 | 0.43 |
| 1,2,3,7,8,9-HxCDD | 0.0010 | 0.0014 | 0.0012 | 0.33 | 0.47 | 0.40 |
| 1,2,3,4,6,7,8-HpCDD | 0.0020 | 0.0034 | 0.0027 | 0.67 | 1.1 | 0.90 |
| OCDD | 0.014 | 0.019 | 0.016 | 4.5 | 6.2 | 5.4 |
| 2,3,7,8-TCDF | 0.0034 | 0.0022 | 0.0028 | 1.1 | 0.73 | 0.93 |
| 1,2,3,7,8-PeCDF | 0.0024 | 0.0010 | 0.0017 | 0.80 | 0.33 | 0.57 |
| 2,3,4,7,8-PeCDF | 0.0022 | 0.0048 | 0.0035 | 0.73 | 1.6 | 1.2 |
| 1,2,3,4,7,8-HxCDF | 0.0048 | 0.0080 | 0.0064 | 1.6 | 2.7 | 2.1 |
| 1,2,3,6,7,8-HxCDF | 0.0010 | 0.0012 | 0.0011 | 0.33 | 0.40 | 0.37 |
| 1,2,3,7,8,9-HxCDF | 0.0006 | ND | 0.0006 | 0.20 | ND | 0.20 |
| 2,3,4,6,7,8-HxCDF | 0.0004 | ND | 0.0004 | 0.13 | ND | 0.13 |
| 1,2,3,4,6,7,8-HpCDF | 0.0010 | 0.0018 | 0.0014 | 0.33 | 0.60 | 0.47 |
| 1,2,3,4,7,8,9-HpCDF | 0.0010 | 0.0004 | 0.0007 | 0.33 | 0.13 | 0.23 |
| OCDF | 0.0008 | 0.0010 | 0.0009 | 0.27 | 0.33 | 0.30 |
| 3,4,4',5-TeCB | 0.0038 | 0.0052 | 0.0045 | 1.3 | 1.7 | 1.5 |
| 3,3',4,4'-TeCB | 0.017 | 0.020 | 0.018 | 5.7 | 6.5 | 6.1 |
| 3,3',4,4',5-PeCB | 0.0028 | 0.0070 | 0.0049 | 0.93 | 2.3 | 1.6 |
| 3,3',4,4',5,5'-HxCB | 0.0024 | 0.0006 | 0.0015 | 0.80 | 0.20 | 0.50 |

The data represents pg/g.

The whole basis indicates the level per 5 g of whole blood.

ND: not detected.

heated to 320 °C at the rate of 5 °C/min, and then maintained at 320 °C for 2 min. The injection temperature and ion source temperature were respectively maintained at 280 °C and 270 °C, and the carrier gas (helium) flow rate (Constant flow) was 1.3 mL/min. The ionizing current, ionizing energy, accelerating voltage, and trap current were 550 mA, 40 eV, 8 kV and 750 mA, respectively. PCDDs, PCDFs and coplanar PCBs (Non-ortho-chlorine substituted biphenyls) were analyzed in a single ion recording mode. The resolution was maintained at 10000.

**Results and Discussion**

We improved our usual dioxin analysis method so that dioxins could be determined in only 5 mL of human blood. The SIM chromatograms are shown in Figure 1 – 2. In Table 1, the background levels of dioxins in the new protocol are shown. The sensitivities were relatively up to 10-fold against the usual

analytical method (1). All dioxins congeners were detected in the use of the SCLV system. The reasons for this are due to the use of SCLV and the integration of the cleanup procedure in the dioxin GC/MS analysis. This method in combination with SCLV and the integrated cleanup procedure is suitable for the accurate and rapid identification and quantification of dioxins in human blood. Therefore, an efficient cleanup is necessary to allow the introduction of volumes of up to 4 mL into GC.

**References**

1. S. Takenaka, H. Hirakawa, M. Nakamura, R. Nakagawa, T. Iida and T. Todaka, Follow-up survey of dioxins in the blood of Yusho patients (in 1998 – 1999), Fukuoka Acta Med., 92, 139 – 148, 2001. (in Japanese)