

# EVALUATION OF THE RAPID CLEANUP METHOD FOR THE ANALYSIS OF DIOXINS DERIVED FROM HERBICIDE “AGENT ORANGE” IN SOILS

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## 1. Introduction

Dioxins (PCDDs: polychlorinated dibenzo-*p*-dioxins, PCDFs: polychlorinated dibenzofurans, and DL-PCBs: dioxin like polychlorinated biphenyls) are persistent in the environment, and can accumulate in the fat tissue of animals and humans and have hormone disrupting properties<sup>1)</sup>. Especially, 2,3,7,8-TCDD has been described as “perhaps the most toxic molecule ever synthesized by man”<sup>2)</sup>. During the 1960s, large quantities of herbicides were used in the armed conflict in Vietnam in order to defoliate forest and mangroves in the southern part of the country. Large quantity of “Agent Orange” was used and became the popular name to indicate the use of all such herbicide mixtures. The herbicides contained 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxy-acetic acid (2,4,5-T), picloram and cacodylic acid. However, during the manufacture of 2,4,5-T, accidental overheating of the reaction mixture easily caused the product to condense into the toxic self-condensation product 2,3,7,8-TCDD<sup>3)</sup>. Thus, the 2,3,7,8-TCDD sprayed during the war in Vietnam entered water bodies and settled on soil and plants. The hydrophobic characteristic of 2,3,7,8-TCDD means that only very little dissolves in pure water and most was absorbed by soils. Previous monitoring of 2,3,7,8-TCDD in soils of Vietnam mainly focused on the dioxin “hotspots”<sup>3)</sup>. In addition, similar environmental problems on the “Agent Orange” contaminated soil have been lately realized in locations near several airbases of Japan and Korea. Thus, it is necessary to investigate levels and spatial distribution of 2,3,7,8-TCDD in areas surrounding dioxin “hotspots”. Since these sites are widespread in Vietnam and a lot number of monitoring sites in “hotspots” are there in Vietnam, it is important to have an inexpensive, fast and simple method to monitor 2,3,7,8-TCDD in soils. Conventional methods, such as the JIS (Japanese Industrial Standard) method and the U.S. EPA (Environmental Protection Agency) method for dioxins are complex, time-consuming, and use a lot amount of solvents and reagents<sup>4,5,6,7)</sup>.

In this work, for developing a screening method for 2,3,7,8-TCDD in “Agent Orange” contaminated soils, first of all we have evaluated a rapid cleanup method for the GC/MS analysis of 2,3,7,8-TCDD, and measured and described the results in ten types of 2,3,7,8-TCDD contaminated soils collected in Japan.

## 2. Materials and methods

### 2.1. Standards solution

<sup>13</sup>C<sub>12</sub>-labeled PCDDs/DFs were used as internal standards that were purchased from Wellington Laboratories (Canada). Each standard solution of PCDDs/DFs in nonane was diluted to 10 pg/μl by adding appropriate amounts of decane.

### 2.2. Sample pretreatment

We have collected soils near incinerators in Japan. Among these, 10 types of 2,3,7,8-TCDD contaminated soils were selected. About 14g of each sample was used in pretreatment for dioxins analysis. After HCl treatment, 40 μl of the internal standards (400 pg of <sup>13</sup>C<sub>12</sub>-labeled 2,3,7,8 positions chlorinated PCDDs/DFs) were spiked, and dioxins were extracted by Soxhlet extraction method following the JIS method. Soxhlet extraction was performed on each sample with 400 ml of toluene for about 16h, and the extracts were then concentrated to 20 ml.

### 2.3. Semi-automated cleanup device

The semi-automated cleanup device was used in this study. The programmable logic controller (PLC, OMRON) was used for the control unit, and its unit contains the PLC, a power supply, user-selectable pane switches, etc. The PTC (Positive Temperature Coefficient) heater, which provide low operating costs (electric consumption: 102W) was used for the heating unit of columns because the wattage constantly varies from minimum to maximum based on the temperature required. The entire semi-automated system was performed by the PLC

through the touch-panel.

#### **2.4. Multilayer silica gel column/activated alumina column cartridge**

The multilayer silica gel column/activated alumina column cartridge for the semi-automated rapid cleanup method were made by our laboratory. It consists of the purification column (multilayer silica gel column) and the concentration column (activated alumina column). The multilayer silica gel column was filled with 1.4g of silica gel impregnated with silver nitrate (5% mass fraction) at the bottom, with 3.8g of silicagel impregnated with sulfuric acid (44% mass fraction) at the upper end of the column. The activated alumina column was filled with 0.1g of activated alumina.

#### **2.5. Conventional cleanup method**

In this study, the JIS method was used for 2,3,7,8-TCDD analysis as one of the conventional methods. Firstly, the 4ml aliquot of the extract in toluene was substituted by hexane to approximately 2ml and cleaned up with a multilayer silica gel column (0.5g of 2% KOH silica gel, 0.5g of silica gel, 3g of 44% sulfuric acid silica gel, 0.5g silica gel, 6g of 10% silver nitrate silica gel) and activated carbon column. After the treated samples were concentrated to approximately 2ml, 20µl of recovery standard (200pg of <sup>13</sup>C<sub>12</sub>-labeled 1,2,7,8-TCDD) were added, and finally, concentrated to approximately 20µl by nitrogen flow.

#### **2.6. Semi-automated rapid cleanup method**

The 4ml aliquot of the extract in toluene was substituted by decane to approximately 500µl. After the sample has been applied onto the multilayer silica gel column cartridge, followed by setting on the PTC heater, which was preheated to 60°C. Then, the multilayer silica gel column was heated for 30min. After the multilayer silica gel column has cooled to approximately 40°C, an activated alumina column cartridge was connected to the multilayer silica gel column cartridge. 2,3,7,8-TCDD was eluted with 40ml of hexane at a flow rate of 2mlmin<sup>-1</sup>. Then, 2,3,7,8-TCDD was trapped in the activated alumina column cartridge and it was separated from the multilayer silica gel column, and reversibly set on the heater, which was maintained at 85°C. The activated alumina cartridge was then dried on the heater, with a rate of flow of fresh air or nitrogen gas at a flow rate of 1.0mlmin<sup>-1</sup>. 2,3,7,8-TCDD was eluted by the addition of 900µl of toluene at 1.0mlmin<sup>-1</sup> onto the activated alumina which was kept at 85°C on the heater. 2,3,7,8-TCDD was collected in same vial. 20µl of recovery standard (200pg of <sup>13</sup>C<sub>12</sub>-labeled 1,2,7,8-TCDD) were added and finally concentrated to approximately 20µl by nitrogen flow. The entire cleanup procedure was completed in about 2h.

#### **2.7. Measurement**

The identification and quantification of 2,3,7,8-TCDD was performed by GC/HRMS (JMS-800D, JEOL). In this study, we used DB-5ms (60m×0.25mm i.d., 0.20µm film, J&W) capillary columns. The column oven temperature was programmed at a rate of 20°Cmin<sup>-1</sup> from an initial temperature of 150°C (1min hold) to 220°C, and then at a rate of 2°Cmin<sup>-1</sup> to 260°C, and finally at a rate of 5°Cmin<sup>-1</sup> to 320°C (3.5min hold). 2µl of sample was injected in the splitless injection mode. The HRMS was operated in the electron ionization mode, using selected ion monitoring at an electron energy of 38eV and an ionizing current of 500µA. The resolution of the instrument was normally more than 10,000 (10% valleys). Verification of the resolution in the working range was obtained by the measuring PFK.

### **3. Results and discussion**

#### **3.1. Recovery rates of spiked <sup>13</sup>C<sub>12</sub>-labelled internal standard**

The recovery rates of <sup>13</sup>C<sub>12</sub>-labelled 2,3,7,8-TCDD added as internal standards for soils in the conventional and semi-automated rapid cleanup methods were measured, and the results were summarized in Table 1. The recoveries of <sup>13</sup>C<sub>12</sub>-labelled 2,3,7,8-TCDD for all the soils by the rapid cleanup method were between 82.5% and 101%, being in the same range (82.1-101%) as those of the conventional method. These results agreed with the JIS and the U.S. EPA limits (permitted limit of recovery rate: 50-120%). It could be considered that the main reason for high <sup>13</sup>C<sub>12</sub>-labelled 2,3,7,8-TCDD recovery rates by the rapid cleanup method was that the heating of the multilayer silica gel column/activated alumina column was a highly efficient adsorption and desorption process for elution of <sup>13</sup>C<sub>12</sub>-labelled 2,3,7,8-TCDD using different solvents. The use of higher temperatures increases the capacity of solvents to solubilize and mass transfer effects<sup>8)</sup> and some reports have also reported higher solubility

of heated solvent and higher permeation of pressured solvent<sup>9,10,11</sup>).

Table 1. Recovery rates of <sup>13</sup>C<sub>12</sub>-labeled 2,3,7,8-TeCDD by the conventional method and the rapid clean up method

	Recovery rate (%)			Recovery rate (%)	
	Conventional	Rapid cleanup		Conventional	Rapid cleanup
Soil-1	99.0	90.0	Soil-6	89.0	87.5
Soil-2	95.0	87.1	Soil-7	95.1	87.5
Soil-3	101	91.2	Soil-8	82.1	89.0
Soil-4	84.1	101	Soil-9	98.1	92.2
Soil-5	96.0	91.1	Soil-10	90.0	82.5

Table 2. Concentration of 2,3,7,8-TeCDD and recovery rates of <sup>13</sup>C<sub>12</sub>-labeled 2,3,7,8-TeCDD by the rapid cleanup method (n=3)

	Concentration of 2,3,7,8-TeCDD (pg/dry g)	RSD (%)	Recovery rate of <sup>13</sup> C <sub>12</sub> -labeled 2,3,7,8-TeCDD (%)	RSD (%)
Soil-3	0.70	7.5	91.2	3.3
Soil-6	7.30	6.8	87.5	0.8

n: number of sample replicates

RSD: relative standard deviation

Table 3. Comparison of 2,3,7,8-TeCDD concentration in soil samples found in the conventional method and the rapid cleanup method

	Concentration (pg/dry g)		Ratio (%)		Concentration (pg/dry g)		Ratio (%)
	Conventional	Rapid cleanup			Conventional	Rapid cleanup	
Soil-1	0.20	0.22	110	Soil-6	7.00	7.30	104
Soil-2	0.40	0.43	108	Soil-7	14.0	13.1	93.6
Soil-3	0.70	0.70	100	Soil-8	38.0	36.1	95.0
Soil-4	1.60	1.51	94.4	Soil-9	106	113	107
Soil-5	4.30	4.50	105	Soil-10	160	166	104

### 3.2. Reproducibility

Reproducibility experiments for the rapid cleanup method were performed by using “soil-3” and “soil-6”, and the results are shown in Table 2. The RSDs (relative standard deviations) of recoveries were within 3.3% and RSDs of concentrations were within 7.5% for both of them, indicating the high reproducibility of the semi-automated cleanup method. Additionally, there was no sharp drop in the lock mass monitor and no interference peak in chromatograms of all samples during the measurement in GC/HRMS. The main reasons may be the fast progress in chemical reaction by heating the multilayer silica gel column which is useful for purification; and the semi-automated cleanup method has low possibility of cross contamination between samples by using separate disposables and lower solvent usage than the conventional cleanup method.

### 3.3. Correlation between the conventional method and the rapid cleanup method.

The concentrations of 2,3,7,8-TCDD obtained by the conventional and rapid cleanup methods are shown in Table 3. The concentration ratios of the rapid cleanup method/conventional method were in the range from 93.6% and 110%. The correlation factor and correlation formula of 2,3,7,8-TCDD concentrations between the rapid cleanup method (*Y*) and the conventional method (*X*) were  $r^2 = 0.999$  and  $Y = 1.12X - 0.23$ , respectively, indicating that the results from the two methods were significantly correlated, and agreed precisely.

### 3.4. Minimum limit of determination

As the determination limit of the sample is liable to vary depending on the sample weight, the value can be

obtained for every sample by the following formula.

$$C_{QL} = QL \times v/v_i \times V_E/V'_E \times 1/W$$

Where,  $C_{QL}$ : the minimum limit of determination for the sample (pg/g)

$QL$ : the minimum limit of determination for analytical method (pg)

$v$ : volume of the sample solution ( $\mu$ l)

$v_i$ : injection volume into GC/HRMS ( $\mu$ l)

$V_E$ : volume of extracted solution (ml)

$V'_E$ : aliquot volume of extracted solution (ml)

$W$ : sample weight (g)

When the detection limit and lower limit of determination for the analytical method were checked in accordance with the JIS method, the minimum limit of determination for analytical method ( $QL$ ) was 0.028pg on the average (variable range: 0.020-0.050pg) for 2,3,7,8-TCDD with GC/HRMS (JMS-800D, JEOL). In this study, the minimum limit of determination for the sample ( $C_{QL}$ ) was calculated from the  $QL$  (0.028pg) and the other items: volume of the sample solution ( $v$ , 20 $\mu$ l), injection volume into GC/HRMS ( $v_i$ , 2 $\mu$ l), volume of extracted solution ( $V_E$ , 20ml), aliquot volume of extracted solution ( $V'_E$ , 4ml) and sample weight ( $W$ , 14g), and the result was 0.1pg/g for each soil sample. If the regulative (1000pg-TEQ/g) and monitorial (250pg-TEQ/g) soil standards in Japan were compared as an example, the minimum limit (0.1 pg/g for 2,3,7,8-TCDD) of determination obtained in this study are satisfactory, indicating that this rapid cleanup method is useful for screening 2,3,7,8-TCDD in "Agent Orange" contaminated soils.

### 3.5. Comparative study

The analysis using the conventional method was performed as follows: multilayer silica gel column cleanup, concentration by evaporator, activated carbon column cleanup, concentration by evaporator, concentration by nitrogen, which took over 8h and used over 300ml of solvents for each sample. The analysis using the rapid cleanup method was performed as follows: heating and cooling of the multilayer silica gel column, activated alumina column cleanup, concentration by nitrogen. The cleanup procedure by the rapid cleanup method was completed within 2h and used only about 40ml of solvents for each sample. The results show that the rapid cleanup method could reduce time and solvent usage by 4 times and 7-8 times, respectively, compared with those of the conventional method.

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