

RAPID ANALYSIS FOR DIOXINS DERIVED FROM AGENT ORANGE IN SOIL I - HRGC/HRMS DETERMINATION METHOD

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Introduction

Defoliants were used in central and southern Vietnam in a program codenamed Operation Ranch Hand between 1961 and 1971 during the Vietnam War by the U.S. military to destroy forest cover and food crops¹. The primary defoliant was "Agent Orange" which was one of the herbicides contaminated with the highly toxic chlorinated dioxins, especially 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TeCDD). Therefore, the ubiquitous environmental pollution by 2,3,7,8-TeCDD might have been existing in the southern and central Vietnam for the past 50 years. In 2011, the JST / JICA (Japan Science and Technology Agency/Japan International Cooperation Agency) have launched a project for bio-diesel production by means of plantation of trees for the regeneration of devastated land that has been contaminated with Agent Orange². Therefore, first of all, an investigation on the current contamination levels of dioxin in the devastated land should be conducted for facilitating the production of oil without much contamination by dioxins. However, conventional methods for dioxin analysis in soil using Soxhlet extraction and column chromatograph cleanup are very complex, labor-intensive, and time-consuming for analyzing large number of soil samples from the devastated land.

In this study, we have developed a simple and rapid analytical method for dioxin derived from "Agent Orange", i.e., 2,3,7,8-TeCDD, in soil using ultrasonic extraction³ and a semi-automated cleanup device^{4,5}. Bioassay detection methods of dioxin such as KinExA (Kinetic Exclusion Assay) and CALUX (Chemical Activated Luciferase Gene Expression) assay in addition to HRGC/HRMS were also tested. We tried to evaluate the consistency of these methods by conducting a cross-check of the analysis of same soil samples in the laboratories in Japan and Vietnam. In this paper, analytical method developed using HRGC-HRMS is described and the results obtained from the two laboratories were cross-checked and discussed.

Materials and methods

1) Standard solution: Native and ¹³C-labeled PCDDs/DFs, calibration standards and internal spike standards were purchased from Wellington Laboratories (Canada).

2) Soil sample:

(1) Pseudo soil: Prepared by mixing nicely the soil containing negligible concentration of 2,3,7,8-TeCDD and Celite (Wako, Japan) adsorbed with 10,000pg/g of 2,3,7,8-TeCDD, in different proportions. 2,3,7,8-TeCDD concentrations in pseudo soil are expected to be 0pg/g, 100pg/g, 250pg/g, 500pg/g and 1000pg/g, respectively.

(2) 2,3,7,8-TeCDD Contaminated soil: Collected at an area polluted by Agent Orange in Vietnam (n=2).

3) Apparatus:

(1) Ultrasonic apparatus: UT-206H (Sharp, Japan)

(2) Semi-automated cleanup device: SZ-DX-PT050 (Seeds Tec, Japan)

(3) HRGC/HRMS: JMS-800D (JEOL, Japan), Auto Spec Premier (Waters-Micromass, USA)

4) Methods

(1) Ultrasonic extraction

Figure 1 shows the flow diagram of dioxin extraction from soil using an ultrasonic extraction apparatus. In GC-MS analysis, internal standard for cleanup spike was added to the acetone extract.

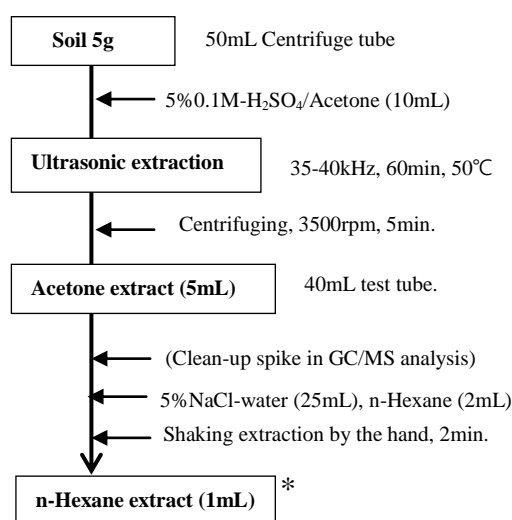


Figure 1. Flow diagram of dioxin extraction from soil based on ultrasonic extraction method.

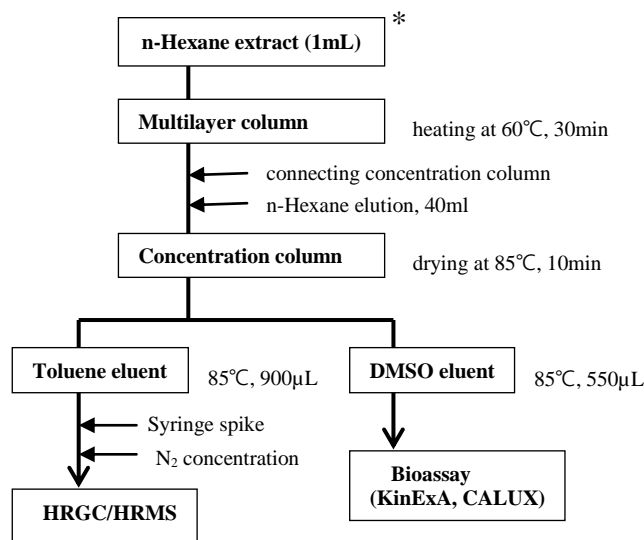


Figure 2. Flow diagram of purification and concentration of the sample extract.

(2) Semi-automated cleanup

One ml of n-hexane extract was used for cleanup using a semi-automated cleanup device as shown in Figure 2. In GC/MS analysis, after an addition of the internal standard for syringe spike, the purified sample solution was analyzed by using HRGC/HRMS.

(3) HRGC/HRMS determination

Identification and determination of dioxins was carried out using a gas chromatograph with a capillary column and a gas chromatograph with a double focusing mass spectrometer. For maintaining the measurement at high resolution of 10,000 or more, the selected ion monitoring (SIM) method was used in a Lock mass mode. After the dioxins were identified using their respective retention times and ionic strength ratios, quantification of their levels was carried out by comparing their peak areas with those of the standards.

(4) Dioxin analysis in Soil samples for evaluation of the method

Each pseudo and contaminated soil sample was extracted for 2,3,7,8-TeCDD with acetone by ultra-sonication (Figure 1). The same acetone extracts were analyzed for dioxins by two laboratories, Ehime University of Japan and CEM of Vietnam, independently. In this study, target compound is only 2,3,7,8-TeCDD because this was found to be a dominant chemical in the environmental and biota samples collected in the areas polluted by “Agent Orange”

Results and discussion

1) Dioxin concentration in soil samples

Table 1 shows the summary of the results reported by the two laboratories. Reported values in each soil sample from two laboratories agreed well within 20% error. Comparison of the reported data to reference data obtained by Soxhlet extraction also showed good agreement within $\pm 30\%$ error, except for pseudo soil-4 sample, the reported values by both laboratories being lower than the reference data which may be due to low extraction efficiency of 2,3,7,8-TeCDD in ultrasonic step as both laboratories obtained low value. In commercially available ultrasonic apparatus, sound pressure varies with positions of ultrasonic irradiation and so the extraction efficiency for 2,3,7,8-TeCDD in ultrasonic extraction step might have been different during the extraction of pseudo soil-4. Therefore, it was recognized that it is necessary to irradiate at the highest position of the sound pressure of ultrasonic apparatus.

Table 1. Summary of the cross-check results of 2,3,7,8-TeCDD in soil sample

Sample	Laboratory						Reference data obtained by Soxhlet extraction
	A			B			Conc. of 2,3,7,8- TeCDD (pg/g sample)
	Concentration of 2,3,7,8-TeCDD (pg/g sample)	LOD (pg/g sample)	Recovery (%)	Concentration of 2,3,7,8-TeCDD (pg/g sample)	LOD (pg/g sample)	Recovery (%)	
Pseudo soil-1	ND	1.3	80	0.2	0.10	68	ND
Pseudo soil-2	91	1.3	73	97	0.12	42	110
Pseudo soil-3	210	1.3	81	240	0.10	42	290
Pseudo soil-4	330	1.3	65	350	0.12	46	560
Pseudo soil-5	990	1.3	61	1200	0.24	<40	1000
Polluted soil-01	1600	1.3	62	1900	0.10	48	1800
Polluted soil-2	760	1.3	58	890	0.10	49	950

Table 2. Recovery rates of ¹³C-2,3,7,8- substituted PCDDs/DFs

Cleanup Spike	Laboratory				Reference*
	A		B		Recovery Ave.(n=2)
	Recovery (n=7)		Recovery (n=7)		
	Average	Range	Average	Range	
¹³ C-2,3,7,8-TeCDD	69%	58 - 81%	46%	<40-68%	93%
¹³ C-1,2,3,7,8-PeCDD	89%	81 - 98%	85%	69-125%	99%
¹³ C-1,2,3,4,7,8-HxCDD	94%	90 - 98%	102%	74-136%	98%
¹³ C-1,2,3,6,7,8-HxCDD	96%	92 - 101%	77%	54-92%	94%
¹³ C-1,2,3,7,8,9-HxCDD	82%	79 - 86%	107%	85-132%	91%
¹³ C-1,2,3,4,6,7,8-HpCDD	94%	90 - 102%	85%	60-123%	97%
¹³ C-OCDD	95%	86 - 102%	75%	59-96%	110%
¹³ C-2,3,7,8-TeCDF	82%	79 - 88%	74%	64-104%	95%
¹³ C-1,2,3,7,8-PeCDF	85%	79 - 91%	75%	59-111%	97%
¹³ C-2,3,4,7,8-PeCDF	92%	88 - 99%	84%	65-130%	99%
¹³ C-1,2,3,4,7,8-HxCDF	94%	91 - 98%	83%	67-107%	91%
¹³ C-1,2,3,6,7,8-HxCDF	95%	92 - 98%	80%	64-100%	94%
¹³ C-1,2,3,7,8,9-HxCDF	89%	83 - 94%	92%	68-114%	96%
¹³ C-2,3,4,6,7,8-HxCDF	91%	88 - 92%	84%	69-100%	99%
¹³ C-1,2,3,4,6,7,8-HpCDF	93%	89 - 99%	78%	59-109%	94%
¹³ C-1,2,3,4,7,8,9-HpCDF	82%	76 - 87%	82%	65-107%	96%
¹³ C-OCDF	84%	77 - 91%	-	-	110%

*Recovery from the purification column under the improvement elution condition.

2) Recovery rates of ¹³C-2,3,7,8-TeCDD

Recovery rate of 2,3,7,8-TeCDD in A and B laboratories were over 58% in all samples and over 40% except pseudo soil-5 sample, respectively. Table 2 shows recovery rates of 2,3,7,8-TeCDD together with the other 2,3,7,8-substituted PCDDs/DFs in this study. It is obvious that the recovery rate of 2,3,7,8-TeCDD was significantly lower than those of the other 2,3,7,8-substituted PCDDs/DFs in both laboratories. It was estimated to be due to low elution efficiency of 2,3,7,8-TeCDD from the purification column in this experiment. Then, the elution condition for 2,3,7,8-TeCDD from the purification column were examined again. As a result, the solvent elution rate and temperature of n-hexane at elution step from the purification column had become clear to be important factors for elution efficiency of 2,3,7,8-TeCDD. Eventually, improved elution conditions were found, that is, lower elution rate of n-hexane than the before and over 20°C of n-hexane temperature. Therefore, recovery of 2,3,7,8-TeCDD will be stable under the improvement conditions at elution step of the purification column, as shown in Table 2.

3) LOD (Limit of detection)

As shown in Table 1, the LODs of 2,3,7,8-TeCDD in this experiment were 1.3pg/g in Laboratory A and 0.10~0.24 pg/g in Laboratory B. Considering the Japanese regulative standard for dioxin (1000pg-TEQ / g) and the index value for investigation (the concentration level requiring a soil investigation) for the soil (250pg-TEQ / g), LODs obtained in this experiment are satisfactory.

4) Analysis time and consumption of organic solvent

Analysis time for extraction and cleanup of soil sample in this simple and rapid method is only about one fourth as compared to conventional methods. Consumptions of organic solvents in the former method is about 1/20th of those in the latter. In addition, this simple and rapid method avoids personal errors of sample preparation, decreases training time for the analyst and accuracy of the experiment was better than the conventional methods.

5) Evaluation

Based on good accuracy, acceptable detection limits, simple and rapid operation, and less time and cost, the method developed this time can be used for screening the soil for dioxin in the areas that have been polluted by Agent Orange

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