THE RAPID ANALYSIS FOR DIOXIN DERIVED FROM AGENT ORANGE IN SOIL ~Ⅱ ANALYTICAL METHOD WITH FLOW IMMUNOSENSOR ~

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Introduction

In Da Nang airport Middle Vietnam, decontamination of Agent Orange contaminated soil is started by cooperation projects the U.S. Agency for International Development (USAID) and the Ministry of Defence of Vietnam in 2012, Interest in soil survey has increased in Vietnam this opportunity. In Vietnam, the effective use of degraded land of about 9 million ha, spread the cause of soil contamination by Agent Orange during the war and slash-and-burn is required. When the use of degraded land, the analysis of 2,3,7,8-TCDD(D48) which is a byproduct of Agent Orange is a problem[1),2),3)] for a wide range of soil in Vietnam, since the only use of instrumental analysis that requires expensive equipment and advanced technology is not realistic, to adopt more quick, simple, and inexpensive analytical method is desirable.

In recent years, more than 175 countries are working on reducing Persistent Organic Pollutants (POPs) that seem to take immediate steps based on treaty established on the purpose of building the international framework. Regular monitoring is required for the understanding of pollution by POPs in the environment in particular. Technological development of bio-assay has made dizzying development because the need for simple, rapid, inexpensive analytical technique is increasing in Japan.[4)].

By the contamination of substances that cannot assume in the environment, the accuracy of analysis is reduced in bioassays, to ensure the reliability is difficult. In order to solve these problems, we developed a trace PCBs analysis system in insulating oil and dioxin analysis system in the exhaust gas by combining the automatic pretreatment device which has a high refining capacity and flow immunosensor using antibodies were developed strategically. And then, these systems were certified as an analytical device with high reliability[5)]. It is to perform the antibody development and measurement system constructed corresponding as a particularly important point in the development of this sensor. Since it is necessary to calculate toxicity equivalent amount (TEQ) the amount of dioxins, targeting indicators isomer highly contribute to the TEQ of dioxins in incineration samples of insulating oil in the Dioxin in exhaust gas it is to target a wide PCB isomer group having a common structure of the major isomer of 3-6 chlorine contained in the product that had been using a PCB in the PCB analysis.

Similarly, in the development of the dioxin-contaminated soil analysis system derived from Agent Orange, it should be noted that to be calculated TEQ dioxins unlike dioxin problem in the world is not the purpose.
Furthermore, the practical use of analytical techniques, for verification test using actual sample is essential that it be examined by using a soil sample to be analyzed is also an important point of development.

Materials and Methods

1. Samples

The black soil and paddy soil (0.2pg/g or less HRGC/HRMS measured value of the D48) were used for blank test. The standard soil sample (1000pg/g) which made by mixing paddy soil without dioxin and Celite (Kanto Chemical) that was added D48 hexane standard solution to be a 100ng/g in advance, was used for the test of reproducibility confirmation. Simulated samples (100, 250, 500, 1000pg/g) were prepared by the method in similar to standard soil samples were used for the test of accuracy confirmation. the standard soil of were used. As real samples, 10 samples (2–5000pg-TEQ/g) were taken in Japan, 2 samples (about 1000pg-TEQ/g) were taken in Vietnam was used.

2. Reagents and instrument

Acetone and Hexane for pesticide residue and PCBs analysis were supplied by Wako Pure Chemical Industries, Ltd. DMSO and Sulfuric acid of special grade chemicals were supplied by Kanto Chemical Co., Inc. Hybridoma with D48 antibody was given from Food and Drug Safety Center. This antibody reacts with the 2,3,7,8-TeCDD as shown in Table 1. Measurement cell and Buffer were supplied by Kyoto Electronics Manufacturing Co., Ltd. A pretreatment device for Dxn analysis ; SZ-DXN-PT010 (Seeds Tec Co., Ltd.) was used to prepare soil samples. A flow immunosensor DXS-610 (Fig1, Kyoto Electronics Manufacturing Co., Ltd.) on the principle of kinetic exclusion assay was used to measure. D48 hexane or DMSO solution which were exchanged from nonane (CIL's) one by solvent substitution was used as standard solution.

Fig.1 Flow Immunosensor DXS-610

3. Procedure of Sample Pretreatment

In the extraction step, collect soil samples 5g first, and after the operation according to the flow shown in Fig.2, 2ml hexane crude extract was obtained as the result.In the pretreatment step, the separated 1.25g substantial amount of hexane crude extract ware applied to pretreatment column, finaly DMSO samples are obtained by heating multilayer silica gel / alumina column method shown in Fig.3 by using the sample preparation device[6].

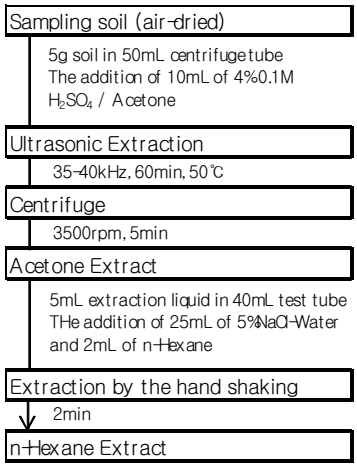

Fig.2 Flow diagram of the extraction

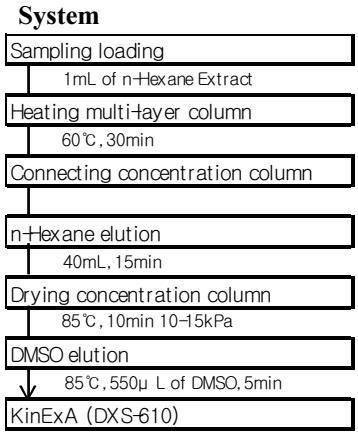

Fig.3 Flow diagram of the pretreatment

4. Immunosensing Methods of D48

A Flow Immunosensor DXS-610 (Kyoto Electronics Manufacturing Co., Ltd.) for the principle of Kinetic Exclusion Assay (Fig.4) was used to measure Dioxins. This cross reactivity of the antibody for 2,3,7,8-TeCDD shown in Table 1. The detection limit of the measurement method shown in Fig.4.

The preparation of the measurement sample was stirred with 20 μ L DMSO in 80 μ L pretreated DMSO samples, and was stirred after added 1.4ml sample preparation buffer. In addition to 0.5ml fluorescent labeled anti-D48 monoclonal antibodies (D48 Ab) prepared to the optimum concentration and gently stirred. 0.2ml measurement sample solution was measured at a flow rate of 0.75mL/min by Flow Immunosensor.

Table 1. Cross Reactivity of D48 A.b.

Dioxin Isomer	Cross-reactivity (%)	Dioxin Isomer	Cross-reactivity (%)
2,3,7,8-TeCDD	100	3,4,4',5-TeCB	<1
1,2,3,7,8-PeCDD	7	3,3',4,4'-TeCB	1
1,2,3,4,7,8-HxCDD	<1	3,3',4,4',5-PeCB	<1
1,2,3,6,7,8-HxCDD	<1	3,3',4,4',5,5'-HxCB	<1
1,2,3,7,8,9-HxCDD	<1	2',3,4,4',5-PeCB	<1
1,2,3,4,6,7,8-HpCDD	<1	2,3',4,4',5-PeCB	<1
OCDD	<1	2,3,3',4,4'-PeCB	<1
2,3,7,8-TeCDF	9	2,3,4,4',5-PeCB	<1
1,2,3,7,8-PeCDF	<1	2,3',4,4',5,5'-HxCB	<1
2,3,4,7,8-PeCDF	4	2,3,3',4,4',5-HxCB	<1
1,2,3,4,7,8-HxCDF	<1	2,3,3',4,4',5'-HxCB	<1
1,2,3,6,7,8-HxCDF	<1	2,3,3',4,4',5,5'-HpCB	<1
1,2,3,7,8,9-HxCDF	<1		
2,3,4,6,7,8-HxCDF	<1		
1,2,3,4,6,7,8-HpCDD	<1		
1,2,3,4,7,8,9-HpCDF	<1		
OCDF	<1		

<1: means concentration of IC50 is 100 times more than D48

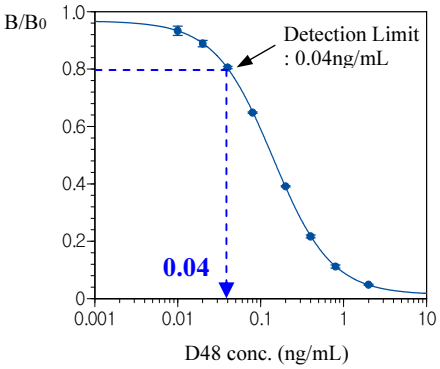

Fig.4 Comparison of Calibration Curve in D48 of antibody.

We used D48 as standard substance

Results and Discussion

1. The blank test and the spike recovery test

In the results of the blank test using the black soils and paddy soils, the measurement values ware 100pg/g or less, lower limit of quantitation of this measurement method.

The samples that was added a final concentration of 40pg/mL, and 80pg/mL to be cleaned-up black soils and paddy soils were used for the recovery test. As the result of the recovery tests, each recovery rates were 100% (paddy soil) and 97% (black soils). And therefore we determined that there is no contamination of matrix components affecting the measurement system in the soil samples which were prepared using the extraction and the pretreatment method.

2. Reproducibility check from the extraction to the measurement

In Table 2, as to the standard soil samples the values and the variation coefficient of the 3 times from extract process to measured process (N=3) were showed. Coefficient of variation is 4.6, the recovery rate was about 80 %. It is confirmed that the present analysis is an analytical system with high **r**eprodusivility.

Table 2 Validation of Analysis system for D48.

Sample	Times	Quantitative value pg/g	Average pg/g	CV %
	1	790		
A	2	738	778	4.6
	3	806		

3. Accuracy check for Flow Immunosensor (DXS-610)

The D48 values by DXS-610 and D48 and TEQ values by HRGC / HRMS was shown in Table 3.

The correlation plot of the D48 values by DXS-610 against those by HRGC / HRMS for 32 soil samples (16 kinds) was shown in Fig.5. For all the samples, DXS-610 measurement results were obtained that is within the range of 82-146% relative to the value of D48 HRGC/HRMS, further, the correlation diagram is good linearity through the origin.

Therefore, this system was found to be an analytical method which can be quantified accurately D48 in real soil samples.

Table 3 Measurement result for soil samples

Sample	Kind	HRGC/HRMS (pg-TEQ/g)	HRGC/HRMS (pg-D48/g)	DXS-610 (pg-D48/g)
No.1-1	Pseudo-sample	91	91	N.D.
No.1-2		91	91	N.D.
No.1-3		91	91	N.D.
No.2-1		210	210	192
No.2-2		210	210	186
No.2-3		210	210	171
No.3-1		330	330	363
No.3-2		330	330	334
No.3-3		330	330	341
No.4-1		990	987	856
No.4-2		990	987	927
No.4-3		990	987	811
No.5-1	Sample in Vietnam	1659	1640	1660
No.5-2		1659	1640	1704
No.5-3		1659	1640	1708
No.6-1		761	760	840
No.6-2		761	760	803
No.6-3		761	760	787
No.7	Sample in Japan	1.9	0.2	N.D.
No.8		11	0.4	N.D.
No.9		23	0.7	N.D.
No.10		40	1.6	N.D.
No.11		97	4	N.D.
No.12		150	7	N.D.
No.13-1		330	14	13
No.13-2		330	14	14
No.13-3		330	14	15
No.14		1200	33	47
No.15-1		3100	100	94
No.15-2		3100	100	101
No.15-3		3100	100	103
No.16		5500	160	182

Conclusion

This analysis system could also be applied to soil samples of different species and pollution from Japan and Vietnam, and was able to accurately quantify the D48 in samples.

Therefore, it can not only take full advantage of the monitoring and screening of dioxin derived Agent Orange, it will contribute to the production of dioxin pollution map in Vietnam.

Acknowledgment

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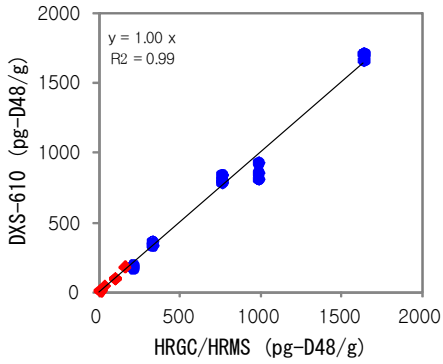

Fig. 5 Correlation diagram
(HRGC/HRMS value vs. DXS-610 value)
●**:Japan,** ●**:** Vietnam