

Comparison of Various Bioassays for Dioxins Measurements in Fuel gas, Fly ash and Bottom ash

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Introduction

In Japan, the control standards for dioxins (PCDDs, PCDFs and Co-PCBs) in the emission gas, fly and bottom ashes from waste incinerators have been defined in the Law Concerning Special Measures against Dioxins (Dioxins Law)¹. Based on the Dioxins law, an installation personnel of waste incinerators of specified facilities shall measure dioxins in the emission gas and fly and bottom ashes more than once every year followed by reporting the results to their prefectural governor. The present regulating procedure has been set to use high-resolution gas chromatography/ high-resolution mass spectrometry (HRGC/HRMS, hereafter GC/MS) systems to determine dioxin-concentrations. However, the GC/MS measurements are often money- and time-consuming, since they need complicated steps for sample preparation, expensive equipments and highly skilled technicians. Therefore, it is of high priority to develop rapid and economical alternative methods to measure dioxins. Recently, various assays using biological reactions have drawn a high degree of attention as a candidate for alternative measurement methods of dioxins²⁻⁴. During the past decade several studies demonstrated the utility of a chemical (GC/MS) and biological (bioassays/biomarkers) control of waste thermal recycling processes like pyrolysis or incineration treatment⁵⁻¹¹. In this paper, we report the results of our recent examinations on the possibility to apply various bioassays to supplementary methods for the present procedure.

Methods and Materials

Eleven out of the 13 bioassay methods (applied from the public) were tested as practical methods. Each applicant carried out their assay methods (classified as described in **Table 1**) to measure concentrations of dioxins in common samples, which include three standards made of dioxins reagents, four samples of flue gas extracts, five fly ash samples (one ash sample and four ash extracts), and four bottom ash extract samples. These samples were analyzed in parallel by the standard GC/MS method. The source and the toxic equivalent (TEQ) value of these samples are listed in **Table 2**. Since the concentration of dioxins in Sample 2E, 2G, and 2H turned out to be below the range of detection for major isomers by the GC/MS method, the data of these samples were eliminated from the evaluation.

Results and Discussion

Eleven methods were classified into four categories shown in **Table 1**- Category 1: Reporter gene assay, Category 2: Immunoassay with anti-aryl hydrocarbon receptor (AhR) complex antibodies (Ah-immunoassay), Category 3: AhR Polymerase Chain Reaction (PCR) Assay, and Category 4: Immunoassay with anti-dioxins antibodies (DXNs-immunoassay). Category 1, 2, and 3 are AhR-based Bioassays. These four categories were evaluated from the point of view of accuracy in comparison with TEQ values by the GC/MS method, the limit of measurements, and the reproducibility.

Accuracy: comparison with the GC/MS measurements

The TEQ-values obtained by the bioassays are shown in **Table 3** and ratios of the bioassays / GC/MS are listed in **Table 3** and **Figure 1**. Reporter gene assays, Ah- immunoassay, and AhR PCR assay gave values within the range of 50 to 300%, ~30 to 200%, ~30 to 300% of the GC/MS value, respectively. Thus, these bioassays exhibit comparable performance in accuracy as compared to the GC/MS method. On the other hand, accuracy of measurements in DXNs-immunoassays depends upon the case: some assays gave ratios within the limits of ~30 to 300 % of the GC/MS value, but others are out of this range. Immunoassays are designed to measure specific congeners, and therefore may not fit well to overall TEQ estimation.

Limits of quantification

The quantification limits of the bioassays are indicated in **Table 4**. Given that 10% of the control standards for new facility should be measured in the bioassay, the quantification limits of the bioassays, except for some methods of DXNs-Immunoassays, generally meet requirements for the small-scale facilities with ~2 tons/h capacity of incineration. For the application to the processing of fly and bottom ash, of which control standard is 3 ng-TEQ/g, all methods are capable to measure dioxins below the 10% value of the control standard.

Reproducibility of the measurements

We evaluated each category of bioassay if their Correlation Variabilities (CV) values are in acceptable range of variation: < 20% in authentic samples and < 30% in actual samples (flue gas and ash samples). The CV values of each bioassay in the present study are indicated in **Table 4**. In the reporter gene assays, Ah-immunoassay, and DXNs-immunoassays, the CV values of the authentic samples and actual samples were generally less than 20% and 30%, respectively. On the other hand, the AhR PCR assay gave more reproducible results: the CV values of the authentic samples and actual samples were less than 10% and 20%, respectively.

Conclusion

The present results indicate that the reporter gene assays, Ah-immunoassay, and AhR PCR assay are already at applicable levels as supplementary methods for the standard GC/MS procedures. On the other hand, some DXNs-immunoassays still have technical problems at this stage for TEQ measurement, although others are supposed to meet the requirements if they are technically improved in the future. Based on the above evaluation, we are planning to introduce some bioassays as supplementary methods for the standard method, considering QA/QC, legal and institutional matters.

References

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Table 1: The Classification of Bioassay Methods

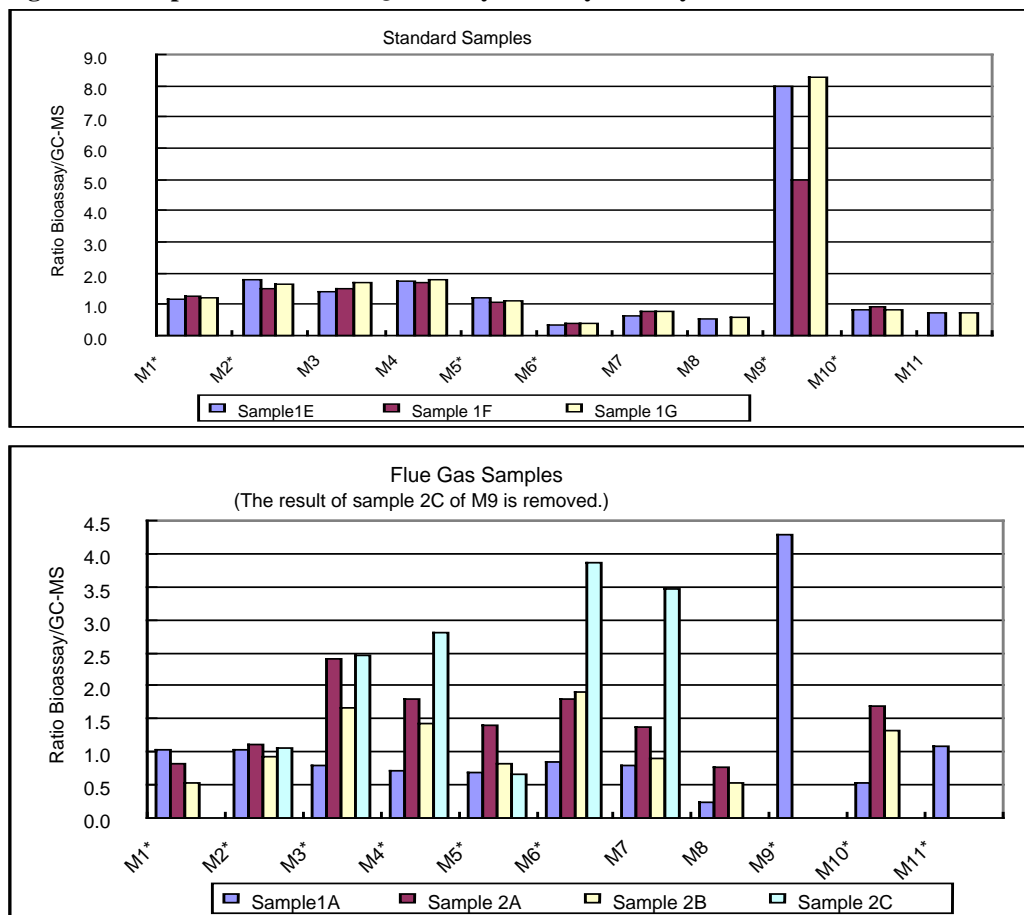
Category		Method	Remarks
AhR – based Bioassay	Reporter Gene Assay (1)	M1	Recombinant cells: 101L cells (human)
		M2	Recombinant cells: Hepa-1L1.6 cells (mouse)
		M3	Recombinant cells: H4IIE-Luc cells (rat)
		M4	Recombinant cells: Hepa-1-2H9-1G4 cells (mouse)
	Ah-Immunoassay (2)	M5	EIA, goat anti-ARNT pAb
	AhR PCR Assay (3)	M6	
DXNs-Immunoassay (4)		M7	EIA, rabbit anti-DXNs pAb
		M8	EIA, mouse anti-DXNs mAb
		M9	FI, mouse anti-DXNs mAb
		M10	EIA, mouse anti-DXNs mAb
		M11	EIA, rabbit anti-DXNs pAb

Notes EIA: Enzyme-linked Immunoassay, FI: Fluorescence Immunoassay, mAb: monoclonal antibody, pAb: polyclonal antibody

Table 2: The Source and TEQ value of Samples

Sample	Media	TEQ-value	Source
1A	Flue gas (extract)	2.8 ng-TEQ/m ³ N	Municipal waste incinerator
1B	Fly ash (extract)	1.9 ng-TEQ/g	Municipal waste incinerator
1C	Bottom ash (extract)	0.018 ng-TEQ/g	Municipal waste incinerator
1D	Fly ash	2.3 ng-TEQ/g	Municipal waste incinerator
1E	PCDD/DF reagent	20 ng-TEQ/ml	Reagent mixture
1F	PCDD/DF reagent	2 ng-TEQ/ml	Reagent mixture
1G	PCDD/DF+Co-PCB reagent	20.56 ng-TEQ/ml	Reagent mixture
2A	Flue gas (extract)	0.10 ng-TEQ/m ³ N	Industrial waste incinerator I
2B	Flue gas (extract)	0.12 ng-TEQ/m ³ N	Industrial waste incinerator II
2C	Flue gas (extract)	0.015 ng-TEQ/m ³ N	Industrial waste incinerator III
2D	Fly ash (extract)	0.0046 ng-TEQ/g	Industrial waste incinerator I
2E	Fly ash (extract)	0.000056 ng-TEQ/g	Industrial waste incinerator II
2F	Fly ash (extract)	0.44 ng-TEQ/g	Industrial waste incinerator III
2G	Bottom ash (extract)	0.00034 ng-TEQ/g	Industrial waste incinerator I
2H	Bottom ash (extract)	0.000035 ng-TEQ/g	Industrial waste incinerator II
2I	Bottom ash (extract)	0.0067 ng-TEQ/g	Industrial waste incinerator III

Figure 1: Comparison of the TEQ value by bioassays and by GC/MS



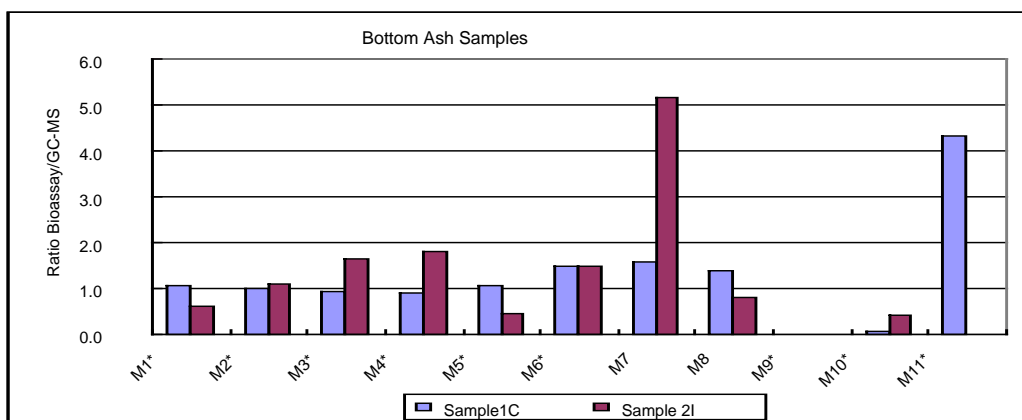
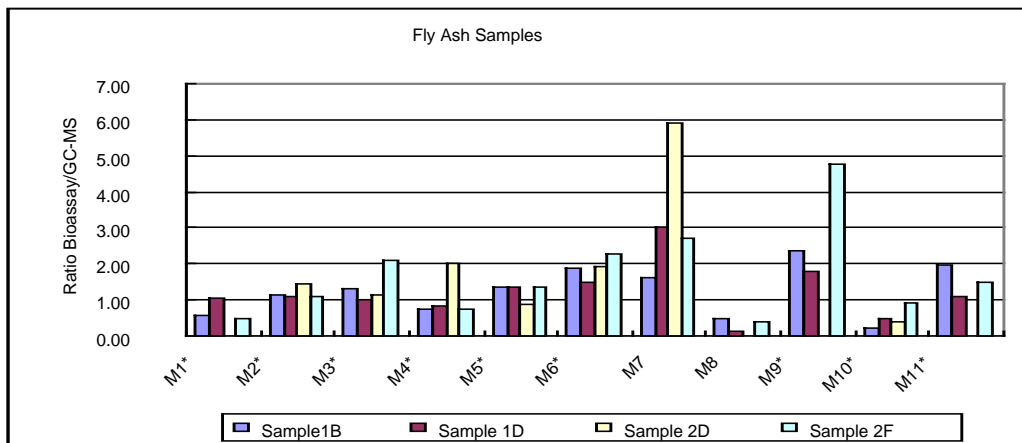


Table 3 : The TEQ-value by Bioassays and Ratio of Bioassays / GC-MS

(Notes *: All samples are converted., **: Real samples are converted., n.d.: not detected, (): value of under the limit of quantification more than the limit of detection)

Category	1						2			3			4			
	Method	M1*	M2*	M3**	M4**	M5*	M6*	M7	M8	M9*	M10*	M11**				
TEQ-value	Standard sample (ng-TEQ/ml)	Sample 1E	23	36	28	35	24	7.1	12	11	160	16	14			
		Sample 1F	2.5	3.0	3.0	3.4	2.1	0.81	1.6	n.d.	(10)	1.8	(1.3)			
		Sample 1G	25	34	35	37	23	7.7	16	12	170	17	15			
		Sample 1A	2.9	2.9	2.2	2.0	1.9	2.4	2.3	0.69	12	1.5	3.0			
		Sample 2A	0.083	0.11	0.24	0.18	0.14	0.18	0.14	0.076	(0.15)	0.17	n.d.			
		Sample 2B	0.062	0.11	0.20	0.17	0.10	0.23	0.11	0.065	(0.49)	0.16	n.d.			
		Sample 2C	n.d.	0.016	0.037	0.042	0.010	0.058	0.052	n.d.	3.1	(0.027)	n.d.			
		Sample 1B	1.1	2.2	2.5	1.4	2.6	3.6	3.1	0.89	4.5	0.45	3.7			
		Sample 1D	2.4	2.5	2.3	1.9	3.1	3.4	6.9	0.32	4.1	1.1	2.5			
		Sample 2D	n.d.	0.0067	0.0053	0.0093	0.0040	0.0089	0.027	n.d.	(0.043)	0.0019	n.d.			
Ratio of Bioassay /GC-MS		Sample 2F	0.22	0.49	0.93	0.33	0.60	1.0	1.2	0.17	2.1	0.40	0.66			
		Sample 1C	0.019	0.018	0.017	0.016	0.019	0.027	0.028	0.025	(0.11)	0.0012	0.078			
		Sample 2I	0.0041	0.0073	0.011	0.012	0.0030	0.010	0.035	(0.0054)	(0.087)	0.0028	n.d.			
		Sample 1E	1.2	1.8	1.4	1.8	1.2	0.36	0.62	0.55	8.0	0.80	0.70			
		Sample 1F	1.3	1.5	1.5	1.7	1.1	0.41	0.79	-	(5.0)	0.90	(0.65)			
		Sample 1G	1.2	1.7	1.7	1.8	1.1	0.37	0.78	0.58	8.3	0.83	0.73			
		Sample 1A	1.0	1.0	0.78	0.71	0.68	0.86	0.80	0.25	4.3	0.54	1.1			
		Sample 2A	0.83	1.1	2.4	1.8	1.4	1.8	1.4	0.76	(1.5)	1.7	-			
		Sample 2B	0.52	0.92	1.7	1.4	0.83	1.9	0.90	0.54	(4.1)	1.3	-			
		Sample 2C	-	1.1	2.5	2.8	0.67	3.9	3.5	-	210	(1.8)	-			
	Sample 1B	0.58	1.2	1.3	0.74	1.4	1.9	1.6	0.47	2.4	0.24	1.9				
	Sample 1D	1.1	1.1	1.0	0.83	1.3	1.5	3.0	0.14	1.8	0.50	1.1				
	Sample 2D	-	1.5	1.2	2.0	0.87	1.9	5.9	-	(9.3)	0.41	-				
	Sample 2F	0.50	1.1	2.1	0.75	1.4	2.3	2.7	0.39	4.8	0.91	1.5				
	Sample 1C	1.1	1.0	0.94	0.89	1.1	1.5	1.6	1.4	(6.1)	0.067	4.3				
	Sample 2I	0.61	1.1	1.6	1.8	0.45	1.5	5.2	(0.81)	(13)	0.42	-				

Table 4: The Limit of Quantification and Correlation Variability of Bioassays

(Notes *: All samples are converted., **: Real samples are converted., -: no data, (): value of under the limit of quantification more than the limit of detection)

Category	1										2			3				4			
	M1*	M2*	M3**	M4**	M5*	M6*	M7	M8	M9*	M10*	M11**										
Flue gas (ng- TEQ/m ³ N)	Sample 1A	0.12	0.0028	0.00035	0.021	0.20	0.0025	0.0046	0.096	0.83	0.075	0.18									
	Sample 2A	0.034	0.0020	0.00072	0.020	0.050	0.0020	0.011	0.071	2.4	0.039	1.1									
	Sample 2B	0.027	0.0016	0.00059	0.017	0.040	0.0015	0.0085	0.056	1.9	0.031	0.89									
	Sample 2C	0.030	0.0018	0.0011	0.018	0.0090	0.0017	0.0094	0.062	2.1	0.034	0.98									
	Sample 1B	0.031	0.0014	0.00014	0.0067	0.20	0.0021	0.0020	0.10	0.30	0.014	0.12									
	Sample 1D	0.013	0.0011	0.000088	0.0055	0.20	0.00052	0.0015	0.027	0.21	0.019	0.090									
	Sample 2D	0.0031	0.00034	0.00017	0.0025	0.0040	0.00024	0.0013	0.012	0.29	0.0015	0.14									
	Sample 2F	0.0031	0.00034	0.0018	0.0025	0.13	0.00024	0.0013	0.012	0.29	0.0073	0.14									
	Sample 1C	0.0031	0.00066	0.000083	0.0037	0.0040	0.00045	0.00091	0.021	0.15	0.00044	0.058									
	Sample 2I	0.0015	0.000082	0.000083	0.0016	0.0020	0.00012	0.00091	0.0082	0.15	0.00088	0.069									
Standard sample	Sample 1E	6.9	11	5.8	4.2	9.5	2.1	1.9	5.4	1.5	10										
	Sample 1F	13	9.9	11	4.3	18	3.7	3.5	-	(24)	(27)										
	Sample 1G	1.8	11	4.3	5.8	7.0	4.0	6.1	4.7	6.8	4.4										
	Sample 1A	1.5	16	2.7	3.1	13	4.1	2.0	4.9	5.1	7.6										
Flue gas	Sample 2A	3.3	21	3.4	1.1	23	10	20	-	(41)	-										
	Sample 2B	1.9	9.1	5.3	2.6	13	12	26	12	(48)	-										
	Sample 2C	-	11	5.3	5.6	29	7.2	25	-	3.8	(13)										
	Sample 1B	3.0	11	5.6	3.6	15	4.0	11	1.6	0.0	4.6										
Fly ash	Sample 1D	5.8	4.8	0.0	8.0	13	2.3	11	0.0	3.7	14										
	Sample 2D	-	6.0	11	4.4	23	6.2	15	-	(48)	-										
	Sample 2F	2.4	3.1	9.0	2.0	17	0.0	7.0	18	0.0	4.0										
	Sample 1C	2.8	4.3	5.5	11	24	1.3	39	-	(3.3)	8.9										
Bottom ash	Sample 2I	3.3	7.9	12	5.7	33	5.6	8.7	(2.0)	(24)	-										