PRELIMINARY STUDY ON DIRECT MEASUREMENT OF PCDD/Fs IN EXTRACTS FROM SAMPLES BY COMPREHENSIVE MULTIDIMENSIONAL GC/ HIGH RESOLUTION TOFMS

Hashimoto S[1], Takazawa Y[1], Fushimi A[1], Ito H[1], Tanabe K[1], Noma Y[1], Shibata Y[1], Ubukata M[2], Kusai A[2], Tanaka K[2]
[1]National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba 305-8506, Japan; [2]JEOL Ltd., 3-1-2 Musashino, Akishima, Tokyo 196-8558, Japan

Abstract
Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in crude extracts of fly ash and flue gas from municipal waste incinerators were quantified using a comprehensive multidimensional gas chromatograph coupled to a high-resolution time-of-flight mass spectrometer. For determination and quantification, we developed our own program running on Microsoft Excel. Isolation of all congeners with a TCDD toxicity equivalent factor from the other isomers was confirmed. Quantification of these in the crude extracts was achieved by direct injection to the GC×GC/HR-TOFMS. The results were very similar to the values obtained using generic GC/HRMS systems. It was confirmed that measurement by high-resolution TOFMS effectively reduces interference from other chemicals.

Introduction
A large number of studies on the simplification of analytical methods for polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) have been reported in recent years. Most appear to be designed to obtain only the TCDD toxicity equivalent (TEQ) and do not take into account the levels of individual congeners[1,2]. Congener profiles, however, often give valuable information for identifying their origin and understanding their behavior in the environment. It has proved difficult to achieve simplification by reducing the steps in the clean-up procedure and precise isomer-specific measurement simultaneously, since, without adequate purification, the quantification of the target compounds is interfered with by other chemicals.
In recent years, comprehensive multidimensional gas chromatography (GC×GC), which has extremely high performance in the separation of chemical substances, has been used to qualify hundreds or thousands of petroleum chemicals[3], food components and flavors[4,5]. GC×GC technology has also been applied to the analysis of environmental contaminants with many congeners such as polychlorinated biphenyls (PCBs)[6,7] and PCDD/Fs[8,9]. Its application to these compounds, however, is still in the early stages. For trace contaminants, high sensitivity and selectivity are required on the part of the detector. A GC×GC is usually equipped an electron capture detector (ECD)[10] or a low-resolution mass spectrometer[11]. These detectors are, however, less selective or sensitive to target compounds than a double-focusing mass spectrometer, which is a commonly used analysis instrument for PCDD/Fs and PCBs, but too slow to suit a GC×GC. In addition, although current time-of-flight mass spectrometers (TOFMS) are moderately sensitive if connected to a GC×GC, they have poor mass resolution.
In this study, we investigated coupling a GC×GC with a high resolution (HR-) TOFMS and the application of this combination to environmental samples. We attempted the direct measurement of PCDD/Fs in solutions extracted from environmental samples such as emission gases and fly ash from municipal waste incinerators (MWIs) by GC×GC/HR-TOFMS, utilizing their powerful selectivity. If the whole cleanup procedure can be skipped, the analytical method will be considerably simplified. In addition, if the method can also maintain accuracy, it would represent a major new development in the analysis of environmental samples.

Materials and methods
Samples: The certified reference material (NIES CRM No. 17) used in the experiment was a toluene solution extracted from MWI fly ash. The solution was divided into 3 portions, after which 5 ng/congener of ^{13}C-labeled PCDD/Fs were added to each 100-µl portion. One was measured directly as the "crude sample." One portion was diluted with hexane and cleaned up on a chromatography column containing 5 g of sulfuric acid silica gel (44%-sulfuric acid/silica gel (w/w) for dioxin analysis, Wako Pure Chemical Industries Ltd, Japan). The hexane

Table 1 The GCxGC/HR-TOFMS conditions for PCDD/Fs measurement

GCxGC													
Instrument	Agilent 6890 GC												
GCxGC	Zoex KT2004												
1st Column	GL Science InertCap 5MS/Sil (60m length, 0.25mm i.d., 0.1 µm film thickness)												
2nd Column	GL Science InertCap 17MS/Sil (1.5m length, 0.075mm i.d., 0.1 µm film thickness)												
Oven Program	from	100	°C	holding for	1	min							
	to	270	°C	at rate	2	°C/min	holding for	0	min				
	to	300	°C	at rate	5	°C/min	holding for	9	min				
Injection	volume:	1	µl	temp:	280	°C				method:	split less		
Carrier Gas	type:	He		mode:	constant flow		itial head pressure:	638.5	kPa				
Modulation	period:	3	sec	releasing:	0.3	sec							
HR-TOFMS													
Instrument	JEOL JMS-T100GC												
Ion Source	temp:	250	°C	ionizing voltage:	35	V	ionizing current:	600	µA				
Analyzer	resolution:	5000		recording range:	35 - 550	m/z		cycle:	25	Hz			
Detector	MCP voltage:	2700	V										

solution eluted from the column was concentrated and resolved into 100 µl of toluene as the "rough cleanup sample." The last one was cleaned up on a chromatography column containing 0.5 g of activated carbon silica gel (silica gel-impregnated active carbon for dioxin analysis, Wako Pure Chemical Industries Ltd, Japan) after sulfuric acid column chromatography. The toluene solution eluted from the column after prewashing with 25%-dichloro- methane/hexane (v/v) was concentrated to 100 µl as the "fine cleanup sample ."

Flue gas samples emitted from several MWIs were also measured. The samples were collected and extracted according to the JIS K0311-2005 method[12]. Each sample solution was spiked with 500 pg/congener of ^{13}C-labeled PCDD/Fs and then concentrated to 100 µl. The solution was measured directly.

Instruments: Sample measurement was carried out using a 6890GC (Agilent Technologies, Inc., USA) with a KT2004 GC×GC system (Zoex Corporation, USA) coupled with a JMS-T100GC (JEOL Ltd., Japan) HR-TOFMS. The first GC capillary column was an InertCap 5MS/Sil (60 m length, 0.25 mm i.d., 0.1 µm film thickness, GL Sciences, Inc., Japan) and the second was an InertCap 17MS/Sil (1.5 m length, 0.075 mm i.d., 0.1 µm film thickness, GL Sciences, Inc.). The conditions and parameters of the GC×GC/HR-TOFMS for measurement of PCDD/Fs are shown in Table 1

Data processing: Quantification of PCDD/Fs in samples was processed using our own newly developed macro program, which runs on Microsoft Excel 98 and later versions. Before processing by the macro program, mass profile data acquired by the JEOL JMS-T100GC had to be converted into mass spectra and then finally transformed into text data (.csv) in several steps. As the result of the transformation, the original 15 GB needed for one set of measurements (m/z = 35–550, 25 Hz, 65 min) was resized to less than 100 MB, allowing it to be entered on an ordinary spreadsheet program.

Results and discussion

On optimizing the parameters and conditions of the GC×GC/HR-TOFMS for measurement, we prioritized the separation of PCDD/F isomers on the GC×GC to allow them to be measured in sample extracts directly and accurately. We therefore employed a 60 m length and 0.25 mm internal diameter column as the first GC column, since to obtain a sufficient number of data points, *e.g.*, over 375 points at a 5 s-width peak on the first GC, sufficient to identify an isolated peak on the chromatogram, required a suitable elution time. A very thin column, 0.075 mm i.d., was adopted as the second GC column to enable further separation of the peaks. Each 3 s-cycle modulation was composed of 75 points of data under the conditions described above. As a result, separation of the isomers with TCDD equivalent factor (TEF) from the others was achieved. For example, 2,3,4,7,8-pentachlorinated dibenzofuran (PeCDF), which a conventional GC-equipped single 5MS capillary column is incapable of separating, was isolated from the others on a two-dimensional chromatogram for PeCDFs (m/z = 339.8597), as shown in Figure 1. This means that the TCDD equivalent (TEQ) of PCDD/Fs in a sample must be accurately estimated by only one injection to this instrument. The instrument's lower detection limit of 2,3,7,8-TCDD on the GC×GC/HR-TOFMS was approximately 0.3 pg (S/N = 3).

The mass resolution of this HR-TOFMS is fixed at about 5,000 (m/Δm; at full width at half maximum) at 500 m/z. Measurement with high mass resolution is one of the advantages of this study over other reports that have used GC×GC/LR-TOFMS[7-9] or quadrupole-type MS[11]. It appears likely that target compounds can be isolated

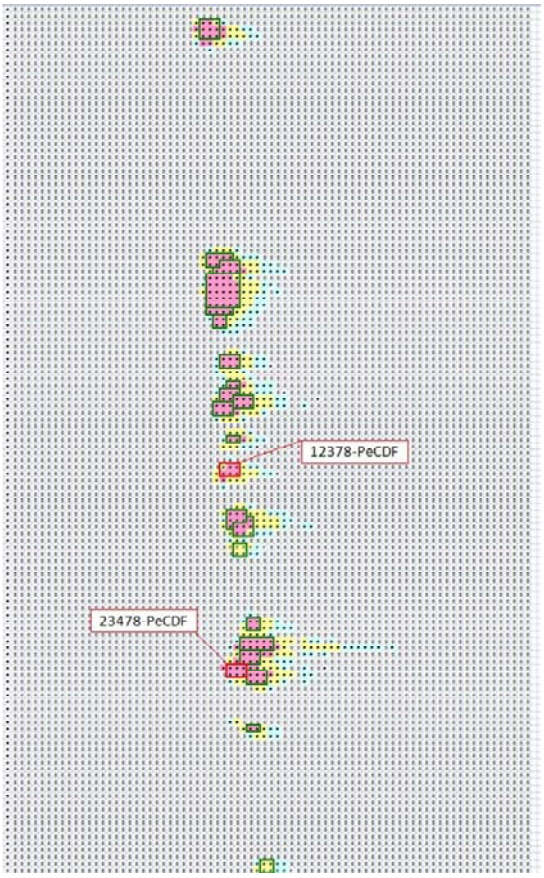

Figure 1 Output after processing the GCxGC/HR-TOFMS data using our own macro program for PeCDFs in the fly ash sample.
This is an example of the display after assignment of the PeCDF isomers on the MS-Excel sheet.

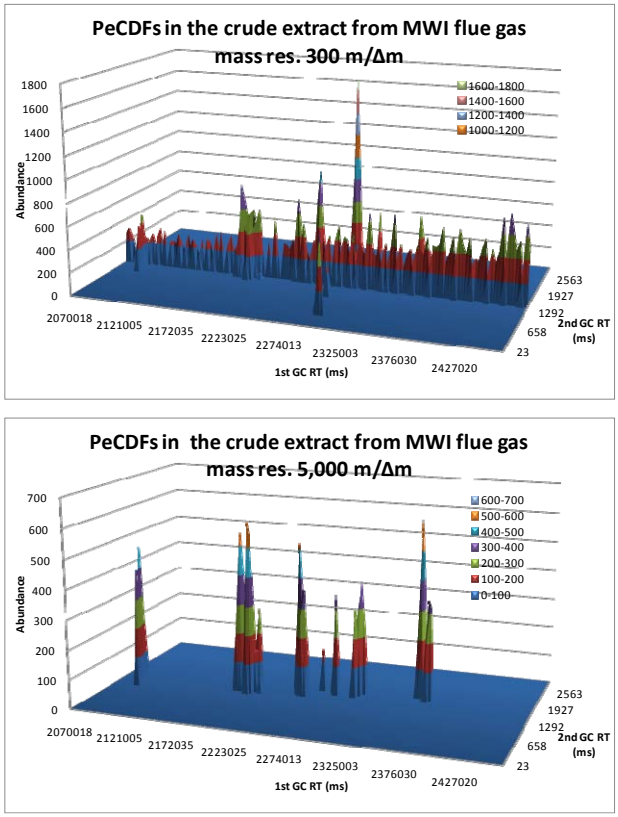

Figure 2 Comparison of the effects of mass resolution on the 3D-Chromatograms for PeCDFs in the crude extract from MWI flue gas.
Upper and lower graphs respectively show the 3D-chromatogram processed data from GCxGC/HR-TOFMS by our own program at mass resolutions of 300 and 5,000 (m/Δm).

from the other compounds, even if eluted at the same time from the GC×GC. In fact, it was confirmed that the high mass resolution contributed to reduction of interference by other compounds on monitored ion channels. In the comparison of the three-dimensional chromatograms for PeCDFs in the MWI flue gas at mass resolution (m/Δm) of 300 and 5,000 in Figure 2, only the peaks of PeCDFs appeared on the 3D-chromatogram at 5,000 m/Δm, whereas numerous interceptive peaks were observed at 300 m/Δm.
We tried direct measurement of PCDD/Fs in the crude extracts (NIES CRM-17) from the fly ash sample using the GC×GC/HR-TOFMS. For comparison, the samples processed with rough and fine cleanups were also measured using the instrument and the fine cleanup sample was measured by the generic GC/HRMS. Figure 3 shows the three-dimensional total ion chromatograms (TICs) of these samples. The PCDD/Fs peaks could be observed from all the TICs, although there was considerable interference in the TICs of the crude and rough cleanup samples. The results for TEF congeners are shown in Table 2. All the congeners could be quantified by the GC×GC/HR-TOFMS, even in the crude extract sample. Moreover, the results of these samples, *i.e.* crude, rough cleanup and fine cleanup, were very similar for each congener. The values of 2,3,7,8-TCDD, for which the monitored channels were substantially intercepted by other chemical ions, were somewhat varied. However, they had not been quantified by low-resolution mass spectrometers without a comprehensive cleanup process. The results from the GC×GC/HR-MS and the GC/HRMS also closely resembled each other, suggesting that the GC /HRMS can be substituted for the GC×GC/HR-TOFMS after some improvements to sensitivity, extension of dynamic range, faster detector response, and better usability of the software.

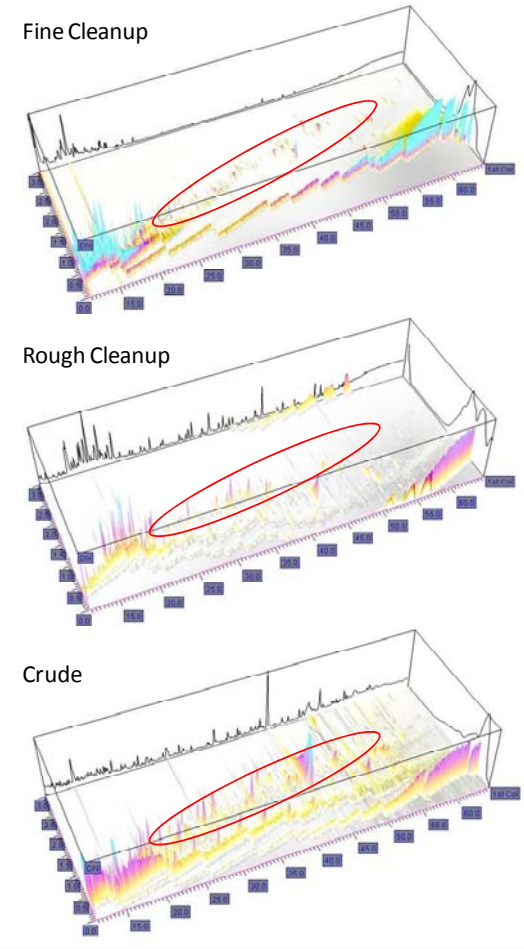

Table 2 Results of measurement of certified reference material (NIES CRM No.17: fly ash extract) by each method

unit: ng ml^{-1}

Measurement Metho	GCxGC/HRTOFMS			HRGC/HRMS	
Apparatus	6890GC-JMST100GC			6890GC-JMS700	
Pre-Treatment	fine cleanup[1]	rough cleanup[2]	crude[3]	fine cleanup Mean[4]	*SD*
2378-TCDD	4.3	1.6	2.4	3.4	*1.3*
12378-PeCDD	42	43	46	61	*17*
123478-HxCDD	70	69	68	96	*28*
123678-HxCDD	125	116	101	231	*51*
123789-HxCDD	112	103	111	174	*35*
1234678-HpCDD	730	759	701	832	*160*
OCDD	439	449	488	564	*100*
2378-TCDF	8.6	11	14	12	*4.8*
12378-PeCDF	20	21	15	36	*4.6*
23478-PeCDF	38	35	20	48	*8.3*
123478-HxCDF	33	31	28	58	*11*
123678-HxCDF	34	33	29	62	*14*
123789-HxCDF	4.1	4.7	5.0	10	*4.3*
234678-HxCDF	61	66	55	85	*17*
1234678-HpCDF	152	142	135	173	*22*
1234789-HpCDF	30	31	27	33	*11*
OCDF	74	75	61	99	*21*

Figure 3(left) 3D-total ion chromatograms of the processed, cleaned-up and unprocessed MWI flue gas samples.
The PCDD/Fs peaks can be observed to follow an elliptic line in each chromatogram. 1 Fine cleanup: the extract was cleaned up with sulfuric acid-silica gel and activated carbon column chromatography. 2 Rough cleanup: the extract was cleaned up by only sulfuric acid-silica gel chromatography.
3 Crude: the extract was not cleaned up. 4 Mean of the results round-robin analysis (*unpublished*)

Acknowledgements
We are grateful to Mr. Otsuka and Dr. Anezaki, at the Hokkaido Institute of Environmental Sciences, and Dr. Hannno at Chiba Prefectural Environmental Research Center, for providing the flue gas extracts from MWIs.

References

1. Masuzaki, Y., Matsumura, T., Ito, H. and Morita, M. (2002) *Organohalogen Compound*, 55, 203-206
2. Nording M Nichkova M Spinnel E Persson Y Gee SJ Hammock BD Haglund P. *Analytical and Bioanalytical Chemistry* 2006; 385: 357
3. Venkatramani CJ Phillips JB. *Journal of Microcolumn Separations* 1993; 5: 511
4. Adahchour M Beens J Vreuls RJJ Batenburg AM Rosing EAE Brinkman UAT. *Chromatographia* 2002; 55: 361
5. Mondello L Casilli A Tranchida PQ Dugo P Dugo G. *Journal of Chromatography A* 2003; 1019: 187
6. Harju M Danielsson C Haglund P. *Journal of Chromatography A* 2003; 1019: 111
7. Focant JF Sjodin A Patterson DG. *Journal of Chromatography A* 2004; 1040: 227
8. Focant JF Eppe G Scippo ML Massart AC Pirard C Maghuin-Rogister G De Pauw E. *Journal of Chromatography A* 2005; 1086: 45
9. Hoh E Mastovska K Lehotay SJ. *Journal of Chromatography A* 2007; 1145: 210
10. Danielsson C Wiberg K Korytar P Bergek S Brinkman UAT Haglund P. *Journal of Chromatography A* 2005; 1086: 61
11. Korytar P Parera J Leonards PEG de Boer J Brinkman UAT. *Journal of Chromatography A* 2005; 1067: 255
12. Japanese Standards Association, *JIS K0311* 2005 (in Japanese)