Comparison between chemical (HRGC/HRMS) and biochemical analysis (Micro-EROD) from thermal processing samples (emission, residues) of municipal waste

Peter Behnisch[1,2], Kazunori Hosoe[1], Ken Shiozaki[3], Tetsuya Kiryu[4], Kenichi Komatsu[4], Karl-Werner Schramm[5] and Shin-ichi Sakai[2]

[1]Takasago Research Laboratories, Kaneka Corporation, Takasago, Japan
[2]Kyoto University, Environment Preservation Center, Kyoto, Japan
[3]Kaneka Techno Research Corporation, Kobe, Japan
[4]Kawasaki Giken Engineering & Construction Co., Ltd, Fukuoka, Japan
[5]GSF, National Research Center for Environment and Health, Neuherberg-Munich, Germany

Introduction

The study was aimed to establish the Micro-EROD bioassay for the determination of 2,3,7,8-TCDD induction equivalents (IEQs) in comparison to those by chemically analysed I-TEQ in samples from different municipal waste incinerators in Germany and Japan. During the past decade several studies demonstrated the utility of a chemical (HRGC/HRMS) and biological (bioassays/biomarkers) control of waste recycling processes like pyrolysis or thermal treatment (Table 1)[1-12]. The here listed literature review of the comparison between by biochemical (EROD, AHH, CALUX, Ah receptor assay) and chemical analysis (HRGC/HRMS) detected dioxin induction/toxic equivalents (IEQ/TEQ) resulted in $R_{b/c}$ values (ratio between IEQ and I-TEQ) for

Table 1: Chemical and bioassay analysis and their ratio $R_{b/c}$ of waste incinerator related samples

Sample	Bioassay	Chemical analysis	Bioassays	Ratio $R_{b/c}$
1) Fly ashes (MWI, n=2)[2]	Micro-EROD/ H4IIE	264/416	450/705	1.7/1.7
2) Emission (PA, n=8)[5]	Micro-EROD (H4IIE)	0.32-14.7	1.6-100	4.7 (1.7-8.2)
3) Filter dust (PA, n=8)[3]	Micro-EROD (H4IIE)	0.23-1.6	0.51-2.1	2.7 (1.7-3.7)
4) Filter dust (MWI, n=3)[2]	Micro-EROD (H4IIE)	651-1645	735-2015	1.1-1.2
5) Emission (WI, n=2)[3]	Micro-EROD (H4IIE)	0.35/5.74	3.2/10.4	9.1/1.8
6) Fly ashes (MWI, n=6)[1]	EROD (H4IIE)	0.45-11.5	0.66-49.5	2.6 (1.1-4.3)
7) Fly ashes (MWI, n=2)[1]	EROD (H4IIE)	0.12/6.4	0.38/24.3	3.2/3.8
8) Fly ash (C, nr)[1]	EROD (H4IIE)	0.48	1.12	2.3
9) Fly ash (WI, n=3)[1]	EROD (H4IIE)	0.28-1.86	10.3-23.3	25 (13-39)
10) Fly ash (NMRF, nr)[1]	EROD (H4IIE)	13-26	35-280	2.2-10.7
11) Emission (IB, n=3)[12]	CALUX (mouse)	3.8/0.78/3.9	13.7/2.8/14.1	3.6
12) Fly ash (MWI, n=2)[11]	Ah receptor assay	0.4/110	3/240	2.2/7.5
13) Fly ash (MWI, n=1)[7]	EROD/EIA	12	22/12	1.8/1
14) Fly ash (MWI, n=1)[8]	AHH (H4IIE)	75	105	1.4

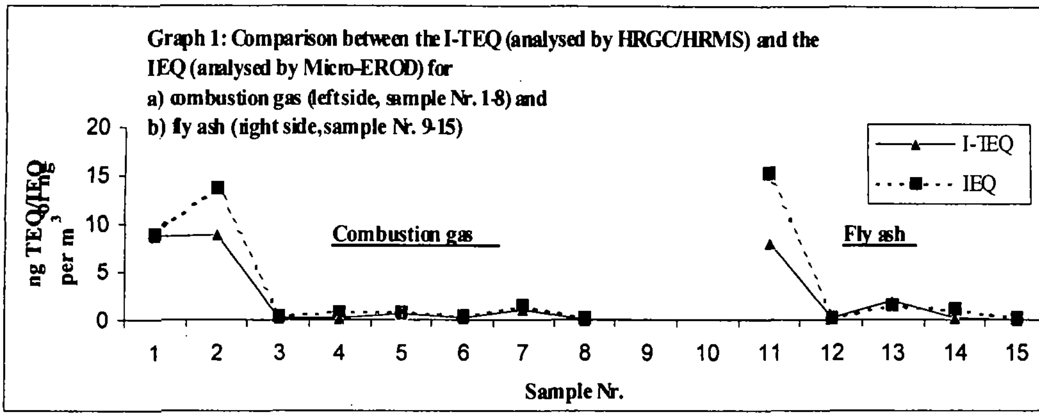
[Municipal waste incinerator (MWI); Plant utilising secondary aluminium (PA); Domestic wood incinerator (WI); Crematorium (C), Noble metal recycling facility (NMRF); Industrial boiler (IB); nr – not reported] fly ashes from municipal waste incinerator samples (1-7.5) and emission samples (1.7-9.1), which indicated that the sum of dioxin-like toxicity (including also unknown dioxin-like compounds) analysed by various bioassays did not exceed an one order of magnitude compared with the I-TEQ.

Methods and Materials

Fifteen samples from two municipal waste incinerators from Kawasaki Giken and one validation flyash from GSF (Neuherberg-Munich, Germany) have been analysed for PCDDs/PCDFs, coplanar PCBs, sum of PAHs and sum of PCNs by HRGC/HRMS (EPA Method 1613 and 23) and Micro-EROD. For the Micro-EROD test 4 g (GSF-sample) and 6.25 g flyash (Kawasaki Giken sample) or 242-825 NL of combustion gas was pre-treated with/without chloride acid and extracted by toluene (16 h). The extracted samples of GSF were cleaned up by a sandwich column (1g Na_2SO_4, 5g SiO_2/10% $AgNO_3$, 5g SiO_2/22% H_2SO_4, 4g SiO_2/44% H_2SO_4, 3g SiO_2/2%KOH, 2.2g SiO_2; elution with 200 ml n-hexane) and an alumina column (14 g, 1^{st}-fraction: 80 ml hexane/dichloromethane 98:2 and 2^{st}-fraction: 150 ml hexane/dichloromethane 1:1). The eluate was evaporated to 5 ml, than 50 µl DMSO/Isopropanol (4:1) was added and reduced to 50 µl by slowly passing nitrogen over the sample and finally diluted into medium (0.05%). The extracted samples from Kawasaki Giken, have been prepared for the Micro-EROD assay, by a simple silica gel column clean up step (activated Silicagel 60 from Merkh, 3 g, elution with 200 ml n-hexane). In this fraction all PCBs, PCDDs/PCDFs, PAHs and PCN were eluted, evaporated, carefully reduced by N_2 stream to the earlier injected 50 µl DMSO/Isopropanol (4:1), diluted into medium (0.05%) and finally analysed by Micro-EROD bioassay. The Micro-EROD bioassay with rat hepatoma H4IIEC3/T cells [principles: a.) TCDD and the sample were simultaneously analysed in min. 5 doses by a 96 well plate-reading spectrofluorometer in comparison to a blank sample; b.) fluorescent-based protein assay; c.) data analysis by comparison of the linear range between TCDD and sample; d.) each concentration was analysed min. n=3 times in at least 3 independent experiments] were performed as described by Schramm and co-workers (1998/1999)[3,4].

Results and Discussion

For a international validation study with the group of GSF (Germany) we analysed at first a fly ash from a municipal waste incinerator in Munich. The flyash was with (A) and without (B) hydrochloric acid pre-treatment extracted and further cleaned up, which resulted in a one magnitude difference in the I-TEQs analysed by GC/MS and in the IEQs determined by Micro-EROD bioassay (all data in ng TEQ/g): GC/MS: Flyash A⇒ PCDD/F: 43.0 (our study) and 47.7 (GSF); PCB: 0.79 (our study) and 0.28 (GSF); Flyash B⇒ PCDD/F: 5.1 (our study) and 4.8 (GSF); PCB: 0.2 (our study). Micro EROD bioassay (n=5): Flyash A⇒ our study 51.3 ; B) Flyash B⇒ our study 5.11. A IEQ/I-TEQ-ratio of 1.2 and 1.0 for the flyash pre-treated with HCl or without HCl, respectively showed that chemical and biochemical analysis data are comparable. After the validation study 15 samples from municipal waste incinerators in Japan were determined by HRGC/HRMS (for PCDD/PCDF, coplanar PCBs, Benzo[b]fluoanthrene, sum of PAHs, hexa-PCN and sum of PCNs) and Micro-EROD bioassay (see Table 2 and Graph 1). The dioxin-like PCB contributed for maximal 14% (gas: mean 3.1%, n=8; ash/slag: mean 4.6%; n=7) of the I-TEQ, indicating the importance of dioxins for the I-TEQ in combustion gas and fly ashes of these municipal waste incinerators. The $R_{b/c}$ and the correlation factor in this study were calculated for the combustion gas (mean 2.6; 1.1-5.6; n=8; r^2= 0.95) and fly ash (mean 2.3; 0.82-4.1; r^2= 0.98) and demonstrated the utility of the Micro-EROD bioassay for monitoring and as strategy for characterising potential environmental dioxin-like compounds



Graph 1: Comparison between the I-TEQ (analysed by HRGC/HRMS) and the IEQ (analysed by Micro-EROD) for
a) combustion gas (leftside, sample Nr. 1-8) and
b) fly ash (right side, sample Nr. 9-15)

The here also reviewed literature study showed similar $R_{b/c}$ values (analysed by EROD/AHH assay with H4IIE cells) for municipal waste incinerator samples (mean 2.0, range 1.1-4.3, n=15). The level of dioxins contained in slag from melting processes and bottom ashes from incineration processes are lower by 1 ~ 2 orders of magnitude than that in fly ash. When the I-TEQ was at ppt level, the IEQ analysed by Micro-EROD was below the lowest detection level. This study indicated a strong relationship between dioxin-TEQ analysed by chemical analysis and the sum of dioxin-IEQ detected by Micro-EROD bioassay. It also leads to the suggestion that the here also analysed PAHs and PCNs didn't contribute more then one magnitude to the sum of dioxin-like toxicity in the here analysed combustion gas and fly ash samples from two municipal waste incinerators.

Reference

1. Till M., Behnisch P., Hagenmaier H., Bock KW. and Schrenk D. (1997) Environ Health Perspect. 105, 1326.
2. Schwirzer SMG., Hofmaier AM., Kettrup A., Nerdinger PE., Schramm KW., Thoma H., Wegenke M. and Wiebel FJ. (1998) Ecotoxicol Environ Safety. 41, 77.
3. Schramm KW., Hofmaier A., Klobasa O., Kaune A. and Kettrup A. (1999) J. Analyt. Appl. Pyrolysis 49, 199.
4. Schramm KW., Klimm C., Hofmaier A. and Kettrup A. (2000) Chemosphere (submitted).
5. Hofmaier AM., Markmann A., Lehnardt R., Schramm KW., Kaune A. and Kettrup A. (1998) Organohalogen Compounds 36, 187.
6. Schramm KW., Kaune A., Lehnardt R., Hofmaier A., Henkelmann B. and Kettrup A. (1998) Organohalogen Compounds 36, 289.
7. Li W., Wu WZ., Barbara RB., Schramm KW. and Kettrup A. (1999) Chemosphere 38, 3313.
8. Safe S., Mason G., Sawyer T., Zacharevski T., Harris M., Yao C., Keys B., Farrell K., Holcomb M., Davis D., Safe L., Piskorska-Pliszczynska J., Leece B., Denomme MA., Hutzinger O., Thoma H., Chittim B. and Madge J. (1989) Toxicol. and Indust. Health 5, 757.
9. Kopponen P., Toerroenen R., Ruuskanen J., Tarhanen J., Vartiainen T. and Kaerenlampi S. (1992) Chemosphere 24, 391.
10. Bunce NL. (1995) Organohalogen Compounds 23, 209.
11. Hu K., Bunce NJ., Chittim BG., Tashiro CHM., Yeo BR., Sharratt BJ., Campbell FJ. and Potter DW. (1995) Environ. Sci. Technol. 29 (10), 2603.
12. Clark GC., Chu M., Touati D., Rayfield B., Stone J. and Cooke M. (1999) Organohalogen Compounds 40, 79.

Table 2: Chemical and biochemical analysis of 15 samples from two different municipal waste incinerators in Japan.

Subcategory	Combustion gas/ fly ash	PCDD/PCDF -TEQ[1] (% TEQ)	Co-PCB-TEQ[1]	ΣTEQ	Bfa[2]	ΣPAHs[2]	H_6CNs[2]	ΣPCNs[2]	IEQ[1]	CV in % (n)	*$R_{b/c}$*
Incineration	a) CG3	0.31 (100)	0.00021	**0.31**	11	1200	0.62	26	**0.93**	47 (4)	*3.0*
Incineration	b) CG4 before BF	0.55 (95)	0.034	**0.58**	23	2400	2.3	790	**0.92**	18 (6)	*1.7*
Melting #1	c) CG1	8.2 (95)	0.46	**8.66**	1500	100000	210	640	**8.89**	29 (5)	*1.1*
Melting #1	d) CG1 particle	8.4 (93)	0.46	**8.86**	1500	100000	210	640	**13.6**	41 (4)	*1.5*
Melting #1	e) CG2 after BF	0.22 (100)	0.00038	**0.22**	38	2200	2.5	180	**0.36**	36 (5)	*1.6*
Melting #2	f) CG 5	0.12 (100)	0.00032	**0.12**	na	2300	na	31	**0.53**	8 (3)	*4.4*
Melting #2	g) CG6 before BF	0.92 (88)	0.13	**1.05**	na	2800	na	800	**1.60**	31 (3)	*1.5*
Melting #2	h) CG6 after BF	0.046 (100)	0.000093	**0.046**	na	740	na	130	**0.26**	31 (3)	*5.6*
Incineration	1) Bottom ash	0.0054 (100)	0.000019	**0.0054**	1.1	110	0.04	4.0	**nd**	nd	*nd*
Incineration	2) Fly ash in BF	0.10 (86)	0.016	**0.116**	2.1	950	0.91	120	**0.16**	40 (4)	*1.4*
Melting #1	3) Fly ash in BF	7.7 (96)	0.33	**8.03**	1100	67000	68	1200	**15.2**	32 (3)	*1.9*
Melting #1	4) Slag	0.0016 (100)	0.00002	**0.0016**	0.94	50	0.05	0.38	**n.d.**	n.d.	*n.d*
Melting #2	5) Slag	0.052 (100)	0.000004	**0.052**	n.a.	18	n.a.	1.2	**0.18**	33 (3)	*3.5*
Melting #2	6) Fly ash in BF	1.80 (97)	0.052	**1.85**	na	320	na	270	**1.51**	33 (3)	*0.8*
Melting #2	7) Dust before BF	0.25 (89)	0.03	**0.28**	n.a.	2800	n.a.	800	**1.16**	18 (4)	*4.1*

[Appreviatons: Bfa Benzo[b]fluoanthrene' BF Bag filter; CG Combustion gas; CV correlation variety; $R_{b/c}$ ratio between IEQ/I-TEQ, nd not detected; na not analysed]; [1] ng-TEQ/m^3N or ng-TEQ/g; [2] ng/m^3N at O_2 or ng/g