DIOXINS AND COPLANAR PCBS IN DIET SAMPLES BY DUPLICATE SERVICE METHOD

Toru Matsumura [1], Yoshie Seki [1], Masaki Hijiya [1], Hiroji Shamoto [1], Masatoshi Morita [2] and Hiroyasu Ito [2]

Environmental Risk Research Center, Institute of General Science for Environment, METOCEAN Environment Inc., 1334-5 Riemon, Ohigawa, Shida, Shizuoka 421-0212, JAPAN [1]
National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki 305-0053, JAPAN [2]

Introduction

When estimating human exposure to dioxin and dioxin like compounds, it is sufficient to consider "Air (aspiration)", "Soil (oral, skin contact)", "Food (oral)" as routes of intake. Of these intake routes, it was reported (Environmental Agency Japan,1998) that greater than 90% is from "Food (oral)", making it important to obtain accurate information regarding the concentration of dioxins in various foods upon which human exposure can be assessed. Currently, methods for analysis of dioxins in food and food products have been reported but this describes the concentrations in agricultural products, meat products, etc. separately.
In order to shed additional light on dioxin intake from food, the Market Basket Method and Duplicate Service Method can be considered. In the Market Basket Method, it is necessary to obtain data from each food and food materials. However, the number of foods that we consume is expanding and a large amount of labor is required to determine the concentration of dioxins in each food type separately. In addition, in obtaining data on dioxins contained in foods other than those that have already been determined to be high, there are problems such as the sample volume values must be increased in order to assay accurately with current technology. On the other hand, with the Duplicate Service Method, the foods actually consumed are collected and analyzed. This method is superior in that intake data is evaluated based on the foods actually eaten. However, with the Duplicate Service Method other obstacles arise. For example, (A) there are a wide variety of components such as grains, vegetables, and meats; (B) high water content; (C) samples from low concentration areas must be analyzed. Therefore a new pretreatment/sample work-up method becomes necessary. In the present paper, authors present the Duplicate Service Method for dioxin analysis, and compare these results to results obtained using the Market Basket Method.

Objective LOD and Sample Size

In food samples there are many target compounds below LOD (Limit of Detection), and there are cases where the evaluated toxic equivalent is excessively high or low and therefore, lower LOD's is necessary. To date, the reported estimated human intake of dioxins from food is estimated as low pg-TEQ/kg/day level.

For example, "Daily Exposure to Dioxins from Food Intake" was determined to be 2.25pg/kg/day based on 50 kg body weight (Ministry of Health Japan, 1998). Back-calculating from these values, indicates it is necessary to assay in the 0.001pg/g range for TeCDD/F, PeCDD/F. Considering dioxin concentration in samples for GC-MS analysis, it is estimated that a starting sample weight of 1000-1500g would be needed.

Method

Food samples were collected in containers washed with organic solvents. Samples of each meal were homogenized, and after mixing according to proportions, spiked with internal standards (cleanup-spike) and re-homogenized. For samples where lack of water made homogenization difficult, water was added during homogenization. Mixed samples were separated to supernatant and precipitant in a refrigerated centrifuged (4500rpm, 30min., 20 degree C) The appropriate amount of 2N-NaOH-ethanol was added and allowed to stand over night. After alkali was added samples are allowed to stand, but it has been shown that de-chlorination of OCDF can occur if allowed to stand for long periods, so samples were not allowed to stand for more than one day. Precipitant was transferred to a glass petry dish and the centrifuge tube rinsed thoroughly with dichloromethane. This was also included with the sample. The petry dish was covered with aluminum foil, and sealed into two polyethylene bags. After freezing, samples were freeze dried in a large desiccator. After centrifugation, liquid/liquid extraction was performed on the supernatant using hexane (3 times for 10 min.) The freeze-dried precipitant was mixed with sodium sulfate anhydride, and soxhlet extraction performed with toluene over 24 hours (124mm ID extraction column. After extraction, soxhlet elute was reduced in a vacuum with a rotary evaporator. This extract and the supernatant extract were combined. After adding hexane, sample clean up was performed with sodium sulfate pre-treatment, multilayer silica gel, and activated carbon columns. Obtained eluant was concentrated using a rotary evaporator. Finally, internal standards (syringe-spike), were added, a final volume of 30 μL obtained, and assayed on HRGC/HRMS (HRGC ; HP model 6890 series GC system, HRMS; Micromass, AutoSpec-Ultima). Because high sensitivity and low S/N ratio are required BPX5 (SGE), BPX50 (SGE), HT8 (SGE), RH-5ms (INVENTX), and RH-17ms (INVENTX) were used. All pre-analysis sample workup was performed in a clean room and all solvents purified (sub-boiling) in a clean room prior to use.

Results

Examples of analytical results are shown in Table 1. In the present study a comparison of results from the analysis a 1500 g sample and results based on limits of detection for an assumed sample weight of 100 g sample are shown. In comparing both methods, detected compounds were lower in the case of the 100 g sample because of higher detection limits. Comparing the daily intake calculated from only the concentration of compounds and the calculated maximum toxicity using 1/2 the limit of detection value, it is difficult to say there is a large difference or that accurate quantitation was achieved. On one hand, with the 1500 g sample analysis of low-level samples was possible and there was little difference between maximum estimated exposure, so it can be said that accurate data was obtained.

Results from samples of a mixture of more than 3-days of meals (food and drink included) are shown in A and B of Table 2. Before mixing samples, foods were divided into 14 groups by type and weighed. As a reference, based on the weight ratio for each food group and intake using existing dioxin concentration data for each group, the intake from each food group was calculated. In the Table, there is a difference between the Market Basket and Duplicate Service results for total food, but this is because Group 14 (drinking water) sample intake was not reported in the Market Basket Method. Body weights in both A and B were calculated as 60 kg, WHO-TEF's (1998) were used for both PCDD's/PCDF's and Co-PCB's.

There is a large difference in between the (1)-(3) of A and B in the calculated intake using the Market Basket Method. Values were decided based on limits of detection, so cases where intake was large from food groups with lower dioxin concentration, the difference increased. In the Duplicate Service Method it was possible to quatitate at a low concentration, so there was not a large difference in (1)-(3), and no intake quantities were judged to be too small or too large.

Table2. Comparison of Market Basket (MB) and Duplicate Service (DS) method.

Group No.	Name of Food Groups	Dioxin Concentration by the Food Group (pg-TEQ/g)			A							B							
					Intake (g/day)	MB Method Conversion Conc. (pg-TEQ/day)			DS Method Measured Conc. (pg-TEQ/day)			Intake (g/day)	MB Method Conversion Conc. (pg-TEQ/day)			DS Method Measured Conc. (pg-TEQ/day)		
		(1)	(2)	(3)	-	(1)	(2)	(3)	(1)	(2)	(3)	-	(1)	(2)	(3)	(1)	(2)	(3)
1	Rices,Processed rices	0.0000050	0.025	0.051	472.0	0.0024	12	24				466.8	0.0023	12	24			
2	Cereals,Potatos	0.00014	0.026	0.051	176.8	0.25	4.6	9.0				158.5	0.22	4.1	8.1			
3	Sugars,Confectionery	0.029	0.029	0.029	12.2	0.35	0.35	0.35				6.2	0.18	0.18	0.18			
4	Oils and Fats	0.0040	0.14	0.27	11.7	0.047	1.6	3.2				1.0	0.004	0.14	0.27			
5	Beans	0.00013	0.025	0.051	82.6	0.011	2.1	4.2				115.9	0.015	2.9	5.9			
6	Fruits	0.00011	0.025	0.051	218.4	0.024	5.5	11				60.0	0.0066	1.5	3.1			
7	Highly-pigmented vegetabl	0.0013	0.026	0.051	48.6	0.063	1.3	2.5	0.030	0.031	0.031	150.5	0.2	3.9	7.7	0.093	0.091	0.091
8	Other vegetables *	0.00062	0.026	0.051	172.2	0.11	4.5	8.8				135.7	0.084	3.5	6.9			
9	Seasoning,Taste drinks	0.000001	0.025	0.051	688.1	0.00069	17	35				267.7	0.00027	6.7	14			
10	Fish and Selffishes	0.79	0.79	0.80	88.3	70	70	71				90.9	72	72	73			
11	Meats,Eggs	0.12	0.13	0.14	99.9	12	13	14				40.7	4.9	5.3	5.7			
12	Milks,Daily products	0.022	0.042	0.062	84.8	1.9	3.6	5.3				0	0	0	0			
13	Other foods	0.023	0.044	0.066	24.3	0.56	1.1	1.6				15.9	0.46	0.87	1.3			
14	Drinking water	1.2E-07	0.00027	0.00054	-							-						
The amount of meals(g)					2179.9				2663.2			1514.0				2019.7		
weight(kg)					60.0				60.0			60.0				60.0		
per day(pg-TEQ/day)						85	137	190	79.5	81.2	82.8		78	113	150	167.6	168.6	168.6
per weight of 1kg(pg-TEQ/kg/day)						1.4	2.3	3.2	1.3	1.4	1.4		1.3	1.9	2.5	2.8	2.8	2.8

Note (1) In the case of "N.D.",TEQ is zero. * : including Mushrooms, Seawoods

(2) In the case of "N.D.",TEQ is multiplied by half of LOD

(3) In the case of "N.D.",TEQ is multiplied by LOD

Table1. An example of results.

	Compounds	100g (estimated from 1500g measured data) LOD (pg/g)	Conc. (pg/g)	TEQ (pg-TEQ/g)		1500g (measured) LOD (pg/g)	Conc. (pg/g)	TEQ (pg-TEQ/g)	
	2,3,7,8-TeCDD	0.03	N.D.	0	(0.01500)	0.0002	0.0011	0.00110	
	TeCDDs	-	0.04	-	-	-	0.045	-	-
	1,2,3,7,8-PeCDD	0.02	N.D.	0	(0.0100)	0.0008	0.0025	0.00250	
	PeCDDs	-	N.D.	-	-	-	0.017	-	-
	1,2,3,4,7,8-HxCDD	0.01	N.D.	0	(0.000500)	0.0007	0.0017	0.000170	
P	1,2,3,6,7,8-HxCDD	0.01	N.D.	0	(0.000500)	0.0007	0.0030	0.000300	
C	1,2,3,7,8,9-HxCDD	0.01	N.D.	0	(0.000500)	0.0007	0.0010	0.000100	
D	HxCDDs	-	N.D.	-	-	-	0.018	-	-
D	1,2,3,4,6,7,8-HpCDD	0.02	N.D.	0	(0.000100)	0.0009	0.017	0.000170	
s	HpCDDs	-	N.D.	-	-	-	0.032	-	-
	OCDD	0.03	0.12	0.0000120		0.002	0.12	0.0000120	
	Total PCDDs	-	0.16	0.0000120	(0.0266)	-	0.23	0.00435	
	2,3,7,8-TeCDF	0.003	0.023	0.00230		0.0002	0.023	0.00230	
	TeCDFs	-	0.065	-	-	-	0.082	-	-
	1,2,3,7,8-PeCDF	0.008	N.D.	0	(0.000200)	0.0006	0.0021	0.000105	
	2,3,4,7,8-PeCDF	0.01	N.D.	0	(0.00250)	0.0007	0.0085	0.00425	
	PeCDFs	-	0.005	-	-	-	0.038	-	-
P	1,2,3,4,7,8-HxCDF	0.005	N.D.	0	(0.000250)	0.0004	0.0048	0.000480	
C	1,2,3,6,7,8-HxCDF	0.005	N.D.	0	(0.000250)	0.0004	0.0037	0.000370	
D	1,2,3,7,8,9-HxCDF	0.02	N.D.	0	(0.00100)	0.002	N.D.	0	(0.000100)
F	2,3,4,6,7,8-HxCDF	0.006	N.D.	0	(0.000300)	0.0004	0.0045	0.000450	
s	HxCDFs	-	0.006	-	-	-	0.027	-	-
	1,2,3,4,6,7,8-HpCDF	0.02	N.D.	0	(0.000100)	0.0008	0.014	0.000140	
	1,2,3,4,7,8,9-HpCDF	0.02	N.D.	0	(0.000100)	0.002	N.D.	0	(0.0000100)
	HpCDFs	—	N.D.	-	-	—	0.021	-	-
	OCDF	0.03	N.D.	0	(0.00000150)	0.002	0.012	0.00000120	
	Total PCDFs	—	0.076	0.00230	(0.00700)	—	0.18	0.00810	(0.00821)
	Total PCDDs/PCDFs	—	0.23	0.00231	(0.0336)	—	0.41	0.0124	(0.0126)
	3,3',4,4'-TeCB (#77)	0.008	0.88	0.0000880		0.0006	0.88	0.0000880	
	3,4,4',5-TeCB (#81)	0.008	0.091	########		0.0005	0.091	0.00000910	
	3,3',4,4',5-PeCB (#126)	0.02	0.11	0.0110		0.002	0.11	0.0110	
	3,3',4,4',5,5'-HxCB (#169)	0.03	0.03	0.000300		0.002	0.031	0.000310	
Co	non-*ortho* PCBs	—	1.1	0.0114		—	1.1	0.0114	
	2,3,3',4,4'-PeCB (#105)	0.02	7.7	0.000770		0.001	7.7	0.000770	
P	2,3,4,4',5-PeCB (#114)	0.02	0.51	0.000255		0.0009	0.51	0.000255	
C	2,3',4,4',5-PeCB (#118)	0.02	29	0.00290		0.0009	29	0.00290	
B	2',3,4,4',5-PeCB (#123)	0.02	0.29	0.0000290		0.0008	0.29	0.0000290	
s	2,3,3',4,4',5-HxCB (#156)	0.02	2.4	0.00120		0.002	2.4	0.00120	
	2,3,3',4,4',5'-HxCB (#157)	0.02	1.0	0.000500		0.002	1.0	0.000500	
	2,3',4,4',5,5'-HxCB (#167)	0.02	1.8	0.0000180		0.002	1.8	0.0000180	
	2,3,3',4,4',5,5'-HpCB (#189)	0.03	0.25	0.0000250		0.002	0.25	0.0000250	
	mono-*ortho* PCBs	—	43	0.00570		—	43	0.00570	
	Total Co-PCBs	—	44	0.0171		—	44	0.0171	
	Total Dioxind	—	-	0.0194	(0.0507)	—	-	0.0296	(0.0297)
	3 days gross value of diet (g)			6649.2				6649.2	
	weight (kg)			60.0				60.0	
	Total PCDDs/PCDFs			0.085	(1.24)			0.46	(0.46)
	Intake per day Total Co-PCBs			0.63				0.63	
	(pg-TEQ/kg/day) Total Dioxins			0.72	(1.9)			1.1	(1.1)