EXPOSURE ASSESSMENT AND ESTABLISHMENT OF THE ANALYTIC METHOD OF FOOD IN THE KOREA TO PCDD/Fs AND DIOXIN-LIKE PCBs

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Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans(PCDD/Fs) as well as dioxin-like polychlorinated biphenyls(DL-PCBs) are ubiquitous highly toxic environmental pollutants which exhibit a potential risk for human health.[11] Because of physicochemical properties, PCDD/Fs and DL-PCBs tend to concentrate and magnify in the food chain. Consumption of food is considered as the major source of non-occupational human exposure to PCDD/Fs with foodstuffs from animal origin accounting for more than 90% of the human body burden. In spite of the great concern of evaluating the presence of these chemicals in food, only limited sets of comparable data are available concerning the concentration of dioxins in various foodstuffs.[10] Thus, it is important to gather as much information as possible about PCDD/Fs and DL-PCBs concentration in food and human exposure.[6] The purpose of this study was two folds: to evaluate dietary exposure of Korean populations to dioxins using data collected from national surveys conducted by the Ministry of Food and Drug Safety in 2000-2011, and to establish the analytic method of dioxins for foods. The best ways of extraction, purification and analysis were established based on USEPA 1613 Method. It was verified to reliability and reproducibility by being applied to CRM. This paper presents the results of a congener-specific analysis of PCDD/Fs and DL-PCBs performed on a number of samples of food. Thus, we have assessed the health risk derived from the PCDD/Fs and DL-PCBs exposure of the human population taking into account the results of this analysis together with the estimation of the dietary intake of these pollutants through the consumption of food.

Materials and methods

(1) Sampling and preparation of samples

All organic solvents were ultra-residue grade for dioxin analysis (Wako, Japan). Calibration standard solutions, ^{13}C-labeled surrogate standards, cleanup standards and injection standards specified in USEPA Method 1613 for PCDD/Fs and USEPA Method 1668A for DL-PCBs analysis were purchased from Wellington Laboratories Inc.[10] Samples were acquired from local markets, big supermarkets and traditional markets. All the composite samples were homogenized. Food samples were stored frozen and lyophilized prior to chemical analysis.

(2) Chemical analysis

The methodology used for PCDD/Fs analysis based on the USEPA method 1613 has been described in detail elsewhere. There are few ways to extract the fat depending on the phase of the sample. It uses soxhlet or ASE in case of a solid phase, and LLE(liquid-liquid extraction) or SPE(solid phase extraction) in case of liquid phase. The methodology was examined to extract fat after comparing between soxhlet and ASE on the phase of the solid and, on the phase of the liquid, comparing between LLE and SPE.

Soxhlet extraction. About 20 g of the analytical samples were mixed with anhydrous sodium sulfate and extracted using n-hexane : dichloromethane(1:3,v/v) as solvents in soxhlet extractor during 18-24h.

ASE(Accelerated Solvent Extraction). About 20 g for each sample that was mixed with anhydrous sodium sulfate extracted in 100 ml stainless steel extraction cell with an ASE 350 Accelerated Solvent Extractor (Dionex Sunnyvale, California). The extraction solvent was hexane:dichloromethane(1:1, v/v) and 2 x 5 min extraction cycles, 100 ℃ temperature, 1500 psi pressure, and 60% flush volume were used.[8]

LLE(Liquid-Liquid extraction). About 50 ml of the analytical samples was used. Milk fat globules membranes were disrupted by Sodium oxalate (0.5 g) and ethanol that were added to milk (1:1) as well as 50 ml of ethyl ether:hexane(1:1,v/v). The homogenized sample was extracted with ethyl ether:hexane(1:1,v/v). The previous process was repeated two or three times.[9]

SPE(Solid-phase extraction). About 50 ml of the analytical samples were processed after pre-treatment based

on a modified version of the AOAC method (Williams, 1991). Milk fat globules membranes were disrupted by potassium oxalate (0.5 g) and acetonitrile that were added to milk (1:1) as well as water (1:1). Extractions were carried out on disposable solid phase extraction (SPE) 25 g C_{18} Flash^{TM} cartridges from International Sorbent Technology (IST, Hengoed, UK) using a manifold. After conditioning the cartridge with methanol and water, the treated samples were applied on C_{18} bed and eluted with hexane after the C_{18} was dried under vaccum. [10]
For identification and quantification, appropriate ^{13}C -labelled internal standard were added to sample prior to extraction. The extracts were concentrated to determine the fat contents. Each extract was then purified in a sequence that comprises purification on column with sodium sulphate and sulfuric acid impregnated silica gel. The obtained extract was then transferred to multilayer chromatography clean-up column in order to further remove the interference. Finally the organic extract was subjected to a chromatographic filtration on activated chacoal to separate the PCDD/Fs from the DL-PCBs and from interfering components. The Fraction containing PCDD/Fs was eluted with toluene. The quantification of PCDD/Fs was carried out by the isotopic dilution method and methodology was validated according to US EPA Method 1613 by performing an initial, ongoing precision and recovery studies. Qualitative and quantitative determination of PCDD/Fs and DL-PCBs was done by HRGC/HRMS. HRGC/HRMS analysis were performed with Thermo trace Ultra gas chromatography interfaced to a Finnigan DFS mass spectrometer which were in MID mode operating positive electron ionization at a resolving power of >10,000 at *m/z* 314 of FC43. The detection limits were 0.01ppt for TCDD/Fs, 0.02ppt for PeCDD/Fs, HxCDD/Fs and HpCDD/Fs and 0.04ppt for OCDD/Fs and 0.02ppt for dioxin-like PCBs at S/N >3. To assess the reliability of our results, we have participated in interlaboratory studies related to dioxins and PCBs (Interlaboratory Comparison on Dioxins in Food, 2007~2010, Division of Environmental Medicine, Norwegian Institute of Public Health, Folkehelse, Norway). As for PCDD/Fs global concentrations, toxic equivalents (TEQ) were calculated using the toxic equivalent factors (TEFs) reported by the World Health Organization in 1998 and 2005. The total concentrations of PCDD/Fs and DL-PCBs have been calculated assuming that non-detected congener concentration is equal to zero.

(3) Dietary intake estimation
PCDD/Fs and DL-PCBs daily dietary exposure through the consumption of food has been calculated multiplying the concentrations of the pollutants found in the food item analyzed by the consumed amount of that item. The average consumption figures taken from the 2008 National Health and Nutrition Examination Survey.[7] The average bodyweight were also obtained from the same survey.

Table 1. Concentration of PCDD/Fs and DL-PCBs in Food samples.

Food	No.	Mean	Min	Max	Stdev
		(pg TEQ/g ww)			(%)
Cereals	70	0.013	0.000	0.064	0.010
Tuber	16	0.016	0.000	0.159	0.039
Sugar	5	0.000	0.000	0.000	0.000
Meats	290	0.371	0.000	1.519	0.123
Eggs	45	0.157	0.000	2.606	0.395
Fish and shellfish	455	8.428	0.000	19.441	1.503
Crustacea and mollusks	88	0.375	0.000	1.022	0.212
Seaweed	10	0.017	0.000	0.099	0.032
Dairy products	123	0.072	0.000	0.240	0.054
Legume	23	0.012	0.000	0.026	0.006
Vegetables	35	0.017	0.000	0.019	0.004
Fruits	29	0.010	0.000	0.020	0.004

Oil	27	0.105	0.000	0.267	0.080
Sesame	3	0.020	0.001	0.055	0.031
Beverage and alcohol	26	0.090	0.000	0.330	0.085
Spices	117	0.208	0.000	1.212	0.151
Total	1,362	0.275	0.000	19.441	0.923

Results and discussion

(1) Establishment of the analytic method

This paper compares the extraction effectiveness of two different commonly applied extraction techniques for the determination of Dioxin and PCBs in food. ASE was initially performed at 100℃ using n-hexane/dichloromethane (1:1,v/v) with a single 5 min extraction step. This resulted in extraction rate of fat, which were close to Soxhlet, or in some cases even below extraction rate of Soxhlet. However Soxhlet usually requires large amounts of solvent and is often carried out for 18 h or more. As the demands for minimizing solvent consumption and time has decreased, extraction conditions of ASE were modified. Thus, extraction conditions of ASE by comparing temperature and cycle Were determined 100℃/ 2cycle.[12] (Fig.1.).

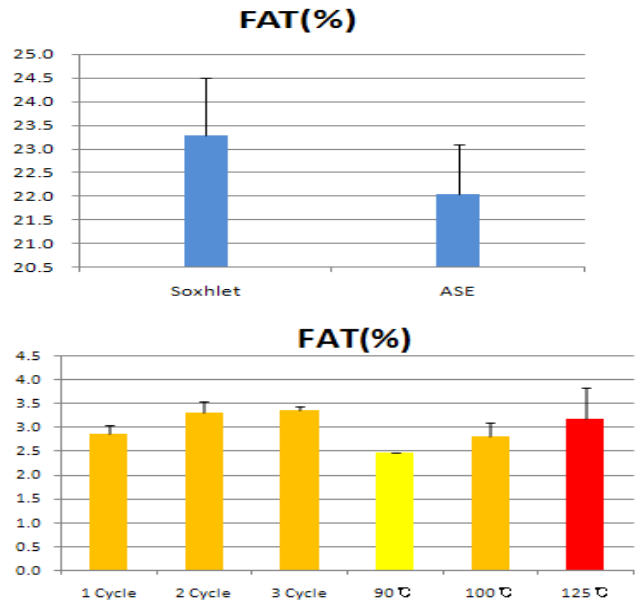

Fig.1. Comparison of Soxhlet and ASE and Temperature, cycle-specific extraction

(2) Analysis of CRM (WMF-01)

The best ways of extraction, purification and analysis were established based on USEPA 1613 Method. It was applied to CRM WMF-01 to verify reliability and reproducibility.(Fig.2.).

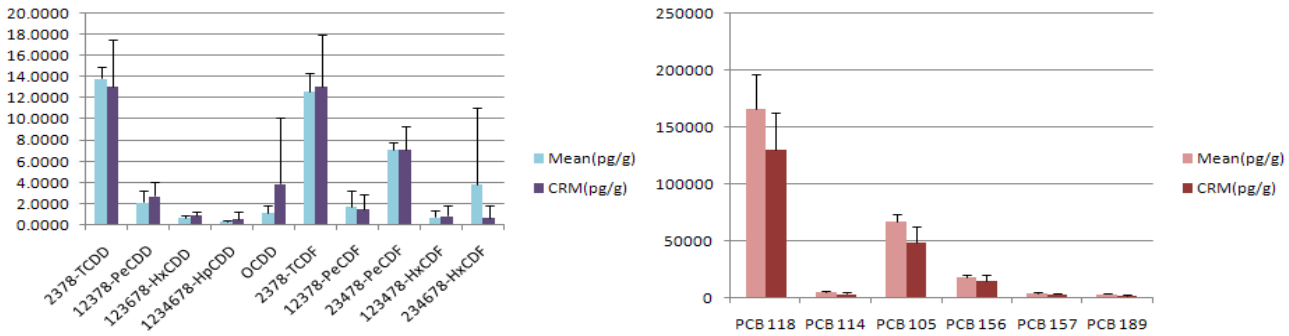

Fig.2. concentration of PCDD/Fs and DL-PCBs in CRM (WMF-01)

(3) PCDD/Fs and DL-PCBs levels in food

The results of the chemical analysis of PCDD/Fs for the food samples accumulated for 12 years from 2000 to 2011 in Korea were summarized in Table 1. The content of PCDD / Fs and DL-PCBs in fish and seafood was 49.2% and the largest of several foods.(Fig.3.).

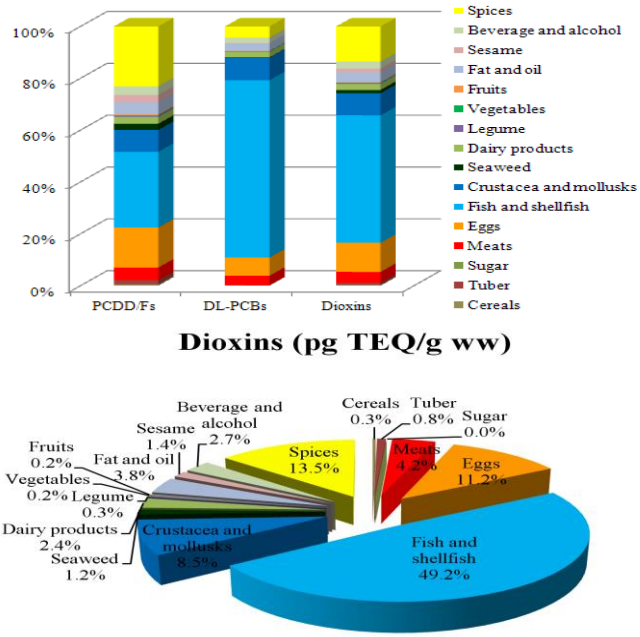

Fig.3. Distribution of the PCDD/Fs & DL-PCBs levels and Relative ratio in food.

(4) Dietary intake to PCDD/Fs and DL-PCBs

The estimated dietary intakes were blow the tolerable daily intake(TDI) proposed by Ministry of Food and Drug Safety of 4 pg TEQ/kg bw/day. Contribution by intake of fish and shellfish to EDI of PCDD/Fs and DL-PCBs was 63.1% and the largest of several foods.(Fig.4) As a conclusion, the dietary consumption of food does not represent a risk for Korean population health concern, although the ingestion of some food may be of significance importance for consumer health. That fact implies that continuous monitoring of these contaminants is still strongly recommended.

Table 2. Estimated daily intake and % TDI and contribution of dioxins exposure in food group

Food	No.	EDI (pg TEQ/kg bw/day)	% TDI	Contrbution (%)
Cereals	70	0.014	0.3	3.4
Tuber	16	0.005	0.1	1.1

Sugar	5	0.000	0.0	0.0
Meats	290	0.040	1.0	11.3
Eggs	45	0.019	0.5	5.7
Fish and shellfish	455	0.220	5.6	63.9
Crustacea and mollusks	88	0.004	0.1	1.1
Seaweed	10	0.000	0.0	0.0
Dairy products	123	0.022	0.5	5.7
Legume	23	0.001	0.0	0.0
Vegetables	35	0.007	0.2	2.2
Fruits	29	0.005	0.2	2.2
Fat and oil	27	0.000	0.0	0.0
Sesame	3	0.000	0.0	0.0
Beverage and alcohol	26	0.001	0.0	0.0
Spices	117	0.008	0.2	2.3
Sum	1,362	**0.346**	**8.7**	**100**

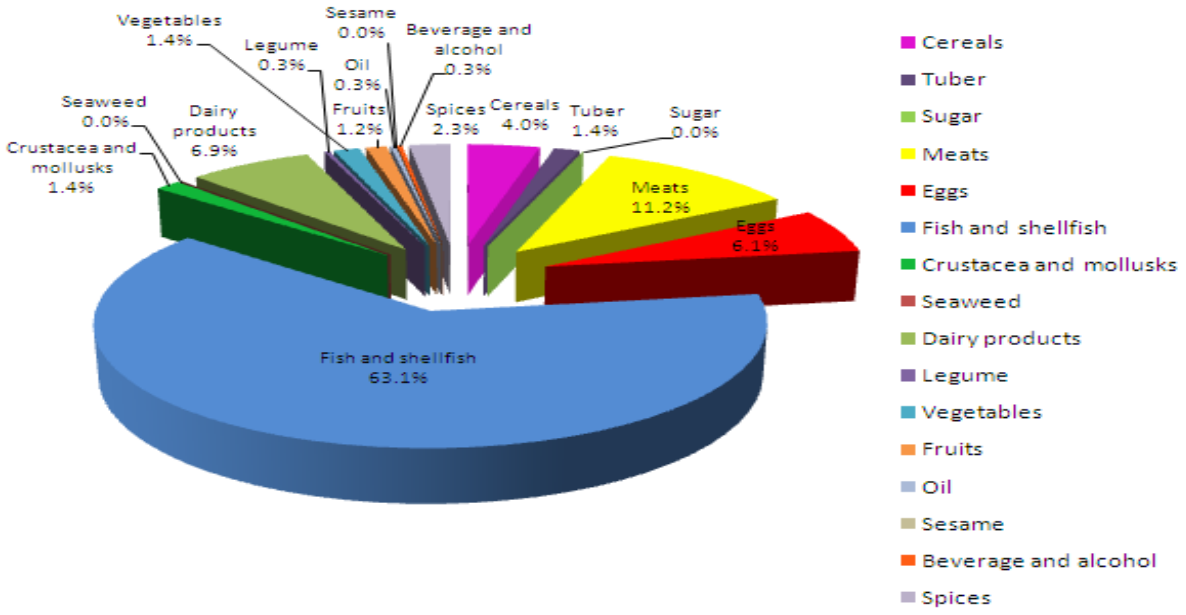

Fig.4. Contribution of individual food to EDI.

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