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A SIMPLIFIED METHOD FOR ANALYZING DIOXINS IN FOODSTUFF IN HONG KONG

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Introduction

The Government Laboratory first adopted a method based on a combination of gel permeation chromatography (GPC) and column chromatography (CC) including acidic silica gel, basic silica gel, alumina and/or florisil for cleanup, and confirmation and quantitation by high resolution gas chromatography / high resolution mass spectrometry (HRGC/HRMS) for determination of dioxins. The cleanup strategy was found to be effective in removing both polar and apolar lipoproteins with good recoveries of carbon-13 labelled internal standards for a number of food and environmental matrices including sediments, ambient air and some biota and food samples. The method was, however, found ineffective in removing low molecular weight saturated lipids and has critical dependence on the activity of basic alumina and polarity of the eluting solvents in separation of dioxins and polychlorinated biphenyls (PCBs). Take for an example, the method is ineffective in removing squalene from health supplement such as shark liver oils and resulting in severe interference in HRGC/HRMS analysis. To overcome the inadequacies, a simplified Smith's method[1] that employs only silica gel and activated carbon for column chromatographic cleanup of food samples with high lipid contents was developed. The method was validated by analysis of reference materials and was found to have good recoveries of carbon-13 labelled internal standards. The validated method was applied in a survey undertook by the Hong Kong Special Administrative Region (HKSAR) during the period of 2000 to April 2001. A total of 105 samples including 13 meat and meat products, 26 poultry and poultry products, 10 milk and milk products, 28 seafood, 11 eggs and egg products and 17 items of food belonging to other food groups were examined in the survey. The survey results will be briefly discussed.

Method and Materials

Survey samples were collected by the Food and Environmental Hygiene Department, HKSAR throughout the territory and submitted to the Government Laboratory for analysis of seventeen World Health Organisation listed 2,3,7,8- chlorine substituted dioxins (PCDD) and furans (PCDF). The purpose of this survey was to assess the regional prevalence and concentrations of PCDD and PCDF in the general food supply of Hong Kong and to estimate the total dietary exposure to dioxins among secondary school students. The lipid content of each sample was analyzed according to AOAC methods.

Each of the samples was cut into small pieces, homogenized and freeze dried. About 10 grams of a freeze dried sample was spiked with carbon-13 labelled internal standards and extracted in a Soxhlet extractor using 20% acetone/hexane as solvent for 18 hours. The solvent should cycle completely

through the system 5-10 times per hour. The solvent is removed under a gentle stream of nitrogen and the residue was dissolved in a 1:1 mixture of hexanes and dichloromethane and eluted through a silica gel column and a 5% Amoco PX21 activated carbon on glass fiber column for cleanup and enrichment. Dioxins was back eluted from the carbon column with toluene. Upon removal of excess toluene, the extract was redissolved in hexanes and subjected to a final cleanup step using an acidified silica gel column and an alumina column. Dioxins fraction was eluted from the alumina column with dichloromethane. The excess solvent was removed under a gentle stream of nitrogen and the sample was spiked with injection standards and reconstituted in 20 μL of nonane. HRGC/HRMS was carried out using SIM with a Finnigan MAT95S HRMS equipped with a Hewlett-Packard 5890 GC and a 0.25 mm i.d. 60 m DB-5 column (0.25 μm stationary phase).

Results and Discussions

Both the simplified Smith's method and the previous cleanup method using a combination of GPC and other chromatography were able to remove impurities from the reference milk powder BCR RM533 for the measurement of dioxins at sub ppt level (Table 1). The results also indicated the there is less co-eluted interference if the former method is used. Recoveries of fortified dioxins in four different food matrix (10 g freeze dried sample spiked with 5 μL of 10 times diluted PAR standard of USEPA Method 1613), which were cleaned up using the simplified Smith's method, ranged from 66 to 123%, with means greater than 85% for most of the 17 WHO listed PCDD/PCDFs (Table 2). The results demonstrated that the method could be adapted for the analysis of trace levels of PCDD/PCDF in foodstuff. The simplified Smith's method was also found to be much more effective in the removal of squalene. Furthermore, at least about 1 to 2 days of intensive labour work could be saved by adopting the new method for sample cleanup. The method was validated by obtaining satisfactory spike recovery of ^{13}C labelled dioxin congeners in many different real samples (chickens, eggs, fish, pork, etc.) and some examples are presented in Table 3. Mean recoveries of the PCDD/Fs were between 75 and 97%.

The limits of quantitation (LOQ) on whole weight basis is defined as signal-to-noise > 3. The Limits of Detection (LODs) are defined as one half of LOQs. Thus, the LODs for the PCDD/PCDFs were, on whole weight basis: 0.04 pg/g for the tetra to penta congeners, 0.1 pg/g for the hexa through hepta congeners, and 0.2 pg/g for the octa congeners.

To evaluate the dietary exposure to dioxins for secondary school students in HKSAR, a value of 1/2 LOD was assigned to all results below LOD.[2] Using the average body weight of secondary school students of 52.0 kg, the average dioxin exposure of secondary school students was calculated as **0.85 pg WHO-TEQ (PCDD/F)/ kg bw/day** for an average secondary school student in HKSAR which is comparable with those for adults in some other countries. The dietary exposure to dioxins of high consumers was **2.07 pg WHO-TEQ (PCDD/F)/ kg bw/ day**. This level was about 2.5 times that of average eaters. Using the upper and lower bound estimates, the dioxins exposure of an average secondary school student would be anywhere between 0.31 (lower bound estimate) and 1.39 (upper bound estimate) pg WHO-TEQ (PCDD/F)/ kg bw/ day while that of high consumers could be anywhere between 0.78 (lower bound estimate) to 3.41 (upper bound estimate) pg WHO-TEQ (PCDD/F)/ kg bw/day.

Table 1. The concentrations of dioxins in milk powder (BCR RM533) determined by different

cleanup methods.

Analyte	Assigned value (μg/kg)	Acceptable range	Simplified Smith's method	Combination of GPC and CC
2,3,7,8-TCDD	0.39	0.31 - 0.47	0.36	0.48
1,2,3,7,8-PeCDD	0.1	0.00 - 0.20	0.06	0.07
1,2,3,4,7,8-HxCDD	1.96	1.56 - 2.36	1.95	2.40
1,2,3,6,7,8-HxCDD	1.06	0.84 - 1.28	1.09	1.23
1,2,3,7,8,9-HxCDD	1.03	0.82 - 1.24	1.04	1.24
1,2,3,4,6,7,8-HpCDD	1.22	0.97 - 1.45	1.48	1.55
OCDD		<0.05	0.05	
2,3,7,8-TCDF		0.15 - 0.3	0.18	0.16
1,2,3,7,8-PeCDF		0.02 - 0.05	0.04	ND
2,3,4,7,8-PeCDF			0.08	
1,2,3,4,7,8-HxCDF	0.42	0.33 - 0.51	0.44	0.50
1,2,3,6,7,8-HxCDF	0.86	0.78 - 1.04	1.03	0.99
1,2,3,7,8,9-HxCDF	0.56	0.44 - 0.68	0.46	0.53
2,3,4,6,7,8-HxCDF	1.27	1.01 - 1.53	1.33	1.70
1,2,3,4,6,7,8-HpCDF	0.47	0.37 - 0.57	0.45	0.51
1,2,3,4,7,8,9-HpCDF		~0.7	0.46	0.54
OCDF		1 - 2	1.60	ND

I=Interference ND=Not determined

Table 2. Recoveries of fortified native dioxins in different matrices.

Matrix	Chicken	Peanut oil	Fish	Fish ball
Recovery / Analyte	Mean (n=7)	Mean (n=4)	Mean (n=3)	Mean (n=2)
2,3,7,8-TCDD	95	89	93	80
1,2,3,7,8-PeCDD	93	98	89	86
1,2,3,4,7,8-HxCDD	92	91	88	80
1,2,3,6,7,8-HxCDD	92	86	86	77
1,2,3,7,8,9-HxCDD	89	99	83	85
1,2,3,4,6,7,8-HpCDD	94	92	90	84
OCDD	91	96	85	84
2,3,7,8-TCDF	93	89	86	83
1,2,3,7,8-PeCDF	89	85	82	77
2,3,4,7,8-PeCDF	89	86	83	77
1,2,3,4,7,8-HxCDF	93	86	90	80
1,2,3,6,7,8-HxCDF	99	82	92	77
1,2,3,7,8,9-HxCDF	94	89	88	85
2,3,4,6,7,8-HxCDF	93	85	91	81
1,2,3,4,6,7,8-HpCDF	96	85	93	77
1,2,3,4,7,8,9-HpCDF	94	83	93	78
OCDF	96	81	98	79

Table 3. Mean surrogate recoveries and standard deviations for fortified carbon-13 labelled

standards in chicken, pork, egg and shellfish.

Matrix	Chicken (n = 19)		Pork (n = 8)		Egg (n = 13)		Shellfish (n = 13)	
Recovery / ^{13}C labelled std.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
2,3,7,8-TCDD	84	11	85	19	79	10	75	19
1,2,3,7,8-PeCDD	93	8.8	88	14	86	8.6	89	11
1,2,3,4,7,8-HxCDD	93	11	89	15	85	11	88	12
1,2,3,6,7,8-HxCDD	88	8.1	90	8.7	82	5.7	85	8.6
1,2,3,7,8,9-HxCDD	93	8.3	95	8.0	86	5.2	89	8.7
1,2,3,4,6,7,8-HpCDD	91	8.6	92	9.9	82	7.9	87	7.2
OCDD	92	13	90	13	87	10	91	8.1
2,3,7,8-TCDF	92	10	93	12	86	8.3	88	10
1,2,3,7,8-PeCDF	91	14	85	16	88	12	89	16
2,3,4,7,8-PeCDF								
1,2,3,4,7,8-HxCDF	94	11	92	16	89	9.8	91	12
1,2,3,6,7,8-HxCDF	85	9.7	80	13	77	9.5	82	11
1,2,3,7,8,9-HxCDF	89	8.3	89	8.4	82	4.6	86	7.2
2,3,4,6,7,8-HxCDF	95	9.9	97	8.6	87	7.7	90	6.7
1,2,3,4,6,7,8-HpCDF	100	0.0	100	0.0	100	0.0	100	0.0
1,2,3,4,7,8,9-HpCDF	90	11	88	13	85	8.5	88	11
OCDF	80	13	77	19	76	16	80	18

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The contents of this paper do not necessarily reflect the views of the Government of the Hong Kong Special Administrative Region, nor does mention of trade names or commercial products constitute endorsement or recommendations of use.

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