Determination of Polychlorinated Dibenzo-p-Dioxins and Dibenzo-Furans in the common Fishes in Zhujiang area of China by Isotope Dilution HRGC/HRMS

jianqing Zhang[1], zhixiong Zhuang[1], yousheng jiang[1], jian zhou[1], jie jiang[5]

[1]Center for disease control & Prevention of Shenzhen,PRChina

1. Introduction

Polychlorinated Dibenzo-p-Dioxins and Dibenzo-Furans(PCDDs /PCDFs) are important two kinds of persistent organic pollutants(POPs) in environment since their persistent, toxic, and bioaccumulative properties. The primary route of human exposure to POPs for the general population is through consumption of food. The dietary intake of PCDDs, PCDFs for human exposure is estimated to account for greater than 90%[1]. Levels of PCDDs and PCDFs in foods have been widely reported in the largely international literature[2]. Where sufficient historical data are available in many nations and tighter regulatory controls on them, the general trend internationally has been for a decline in the levels of PCDDs and PCDFs in foods. Although the research work on POPs has been processed in China currently, less information especially for food or environment background is available on PCDDs and PCDFs level in China.

The HRGC/HRMS method has been constructed in our laboratory reference US EPA 1613 method[3], and the method has been validated by participated the international laboratory proficiency test for fish. The paper reports the results of a survey to determine the level of PCDDs/PCDFs in fishes commonly consumed by zhujiang river area people.

2. Experimental

2.1 Chemicals and Reagents

EPA Method 1613 standards solutions(CS1 to CS5, window defining and isomer specificity, Labeled compound Stock solution(IS), Clean up standard, Internal standard spiking solution(ISS)) were purchased from Cambridge Isotope

Laboratories Co., solvents: acetone, n-hexane, dichloromethane, ethyl acetate, methanol, toluene for trace determinatin analysis were purchased from Merck.

2.2 Samples

Proficiency test sample------ fish muscle test material 0613 was obtained from Central Science Laboratory(CSL). 22 fish samples were collected in Zhujiang delta area, which was sampled from fish breeding locate, the sampling strategy for the current survey was commonly eaten by local people. Total 13 species were analyzed in the survey. The scientific name by Latin was LutJanus sanguineus, Pampus argenteus, Nemipterus virgatus, Sparus macrocephalus, Parargyrops edita, Mujil cephalus, Decapterus maruadsi, Clupea harengus pallasi, Clu panodon punctatus, Tilapia nilotica, Carassius auratus, Mugil cephalus, Cyprinus carpio etc.. All of the fishes were mature for sale. All the edible parts of fishes were removed and ground then frozen dry by frozen dryer.

2.3 Extraction

Soxhlet extraction was used for the sample extract , glass fiber thimbles and soxhlet system were pre-extracted with hexane/dichloromethane (1:1) for 4 hours, fish samples and sodium sulfate were extracted for 18 hours at least. The extrtaction solvent was removed using a rotary evaporator.

2.4 Clean up

Power prep automated system was used sample clean up. Briefly, hexane extracts were loaded on to a set of disposable columns consisting of a multi-layer silica column , a basic alumina column and a carbon column. Purified extracts were concentrated to approximately 500 µL. and transferred to conical vials containing 10 µL of nonane used as keeper, and concentrated to incipient dryness before addition of the recovery standards.

2.5 HRGC/HRMS analysis

Seven PCDDs and ten PCDFs ware analyzed by HRGC/HRMS using MAT95XL high-resolution mass spectrometer(Finnigan, Bremen, Germany) .DB5-MS (J&W Scientific, USA) fused silica capillary column (60m×0.32mm id., 0.25µm film thickness)was used with helium as carrier gas. The temperature program was from 120 (held for 1 min) to 220 (held for 15 min) at 43 min^{-1}, to 250 at 2.3 min^{-1},and then to 310 (held for 10 min^{-1})at 50 min^{-1} using the splitless injection mode. The HRGC/HRMS operating conditions were :ion source and interface tempetatures, 260 and 280 , respectively; ionization energy 60 eV

(electron ionization mode), and trap current 0.9 mA. The resolving power was kept at 10000(10% valley definition), using selected ion monitoring(SIM) in isotopic dilution. Isotope ratio ,MS sensitivity and relative response factor of each congener were monitored to ensure that the system was permanently under control.

3. Results and Discussion

3.1 Analysis of certified reference material

The accuracy and precision of the analytical methods were evaluated by use of a fish muscle test material 0613 obtained from Central Science Laborotary of British (CSL).This material was analyzed in triplicate. The data obtained for 15 ^{13}C-PCDD/Fs internal standards recovery and the seven certified PCDDs, two PCDFs as well as the assigned concentrations with the associated uncertainties are summarized in Table 1.The concentration levels obtained were comparable with the certified values reported by the CSL.

The mean recovery of PCDD/Fs ranged from 85 to 105 %(Including extraction, clean up and concentration process). RSD of recovery was less than 20%.Similar to those reported in the literature for fish matrices using the similar system[1].The concentration of the 9 PCDD/Fs and WHO-dioxin TEQ lower and upper value are also consistent with the assigned values, The justness ranged from 90 to 129% except 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF. Z-score was less than 2 for 7 PCDD/Fs and WHO-dioxin TEQ lower and upper except 1,2,3,4,7,8-HxCDF which was in 2 to 3.

3.2 Analysis of fish samples

Table 2 summarizes the mean recoveries for 15 ^{13}C-PCDD/Fs used as internal standards and the PCDD/Fs levels in the 22 fish samples. Recovery for PCDD/Fs were between 72 to 86%. RSD(the Relative standard deviations) of Recovery was less than 12%,that shows us the sample preparation system was more stable.

The concentration in wet weight basis for 22 fish samples was found nd to 0.26 ng/kg wet weight, 1,2,3,7,8-HxCDF was only found in one sample while wasn't found at detectable levels in any of other 21 fishes. The total concentration in wet weight basis was 0.99 ng/kg, The PCDD/Fs profile was also analyzed in the 22 samples, OCDD, 2,3,7,8-TCDF, 1,2,3,4,6,7,8-HpCDD was the main contributors to the total PCDD/Fs detected, average account for 26%,21%,18%, respectively. Fig1 shows the main distribution pattern in PCDFs, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDF were the main contamination pattern in PCDFs, while OCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,7,8-PeCDD were the main distribution pattern

in PCDDs(Fig2). The average total TEQ concentration in 22 fish samples was 0.226ng/kg wet weight. The main contributors was 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, 2,3,7,8-TCDD by WHO-TEF, account for 43%,29%,15% respectively, the main contributors difference in total PCDD/Fs concentration and total TEQ was TEF values. The total TEQ for 22 fishes ranged from 0.03 ng/kg wet weight to 0.878 ng/kg wet weight, the distrubution was largely different from sample to samples.

4.Conclusion

PCDD/Fs detection method according to US EPA 1613 has been constructed in our lab. The accuracy , repeatability, reproducibility of the method has been detected. The analysis system is repeatability and accurate. The average of concentration in wet weight is 0.99 ng/kg,, the average total WTO-TEQ for 22 samples is 0.226ng/kg wet weight. The level of fish samples in the zhujiang area is not distinct comparing with other countries, eg Japanese seafood reported a range of 0.32 to 2.07 ng I-TEQ/kg fresh weight for 6 fish species. Finally the detect system will be expected for other food matrices, eg pork, milk, egg and so on, so the human in the local exposure of PCDD/Fs can be evaluated in the future.

Acknowledgement

Wu yongning, who is Institute of Food safety of China CDC, support for the grant and my research work. Dr. Ting tailun, Dr. Chung waicheung, Dr. Sin waimei,who are Government Laboratory, HKSAR, support for the research work.Dr. Dr. Zhuang zhixiong who is my boss support for the finance .

Reference

[1]Eljarrat E, Saulo J, Monjonell A, Caixach JR. Evaluation of an automated clean up system for the isotope-dilution high-resolution mass spectrometric analysis of PCB, PCDD and PCDF in food. Fresenius J Anal Chem, 2001(371) :983-988

[2]Pirard C.,Focant JF,Pauw ED. An improved clean up strategy for simultaneous analysis of polychlorinated dibenzo-p-dixoins(PCDD),polychlorinated dibenzofurans(PCDFs),and polychlorinated biphenyls(PCB)in fatty food samples. Anal Bioanal Chem,2002(372):373-381

[3]United States Environmental Protection Agency. Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS (Method 1613). 1994, October.

Table1 Mean recovery and concentrations in the analysis of CSL Proficiency Test fish n=3

Compounds	Label spiking level pg	Recovery(%) n=3	Our Concentration ng/kg [1]	assigned value (ng/kg)[2]	Z-score	Justness (%)	Recovery RSD(%)
2,3,7,8-TCDF	1000	87±6	7.13±0.34	6.02±2.65	Z<2	118	6.92
1,2,3,7,8-PeCDF	1000	97±4	0.79±0.28	0.703±0.309	Z<2	113	3.89
2,3,4,7,8-PeCDF	1000	97±7	1.8±0.32	1.84±0.81	Z<2	98	7.71
1,2,3,4,7,8-HxCDF	1000	86±8	0.25±0.04	0.136±0.06	2<Z<3	184	8.7
1,2,3,6,7,8-HxCDF	1000	85±5	0.18±0.025	0.126±0.056	Z=2	143	5.5
2,3,4,6,7,8-HxCDF	1000	86±5	0.18±0.10	0.141±0.062	Z<2	129	5.92
*1,2,3,7,8,9-HxCDF	1000	88±2	nd	NA			2.53
*1,2,3,4,6,7,8-HpCDF	1000	94±11	0.26±0.04	0.145			10.37
*1,2,3,4,7,8,9-HpCDF	1000	99±18	0.05±0.02	0.0473			18.38
*OCDF	-		0.06	0.0837			
2,3,7,8-TCDD	1000	88±6	0.31±0.098	0.28±0.123	Z<2	111	6.72
1,2,3,7,8-PeCDD	1000	105±9	0.4±0.106	0.446±0.196	Z<2	90	8.37
*1,2,3,4,7,8-HxCDD	1000	92±5	nd	0.0553±0.0243			4.98
*1,2,3,6,7,8-HxCDD	1000	92±3	0.23±0.20	0.234±0.103			3.29
*1,2,3,7,8,9-HxCDD	1000	100±0	nd	0.0637±0.028			0
*1,2,3,4,6,7,8-HpCDD	1000	103±18	0.17	0.175±0.077			17
*OCDD	2000	99±9	0.7	0.569			9.24
^{37}Cl-2,3,7,8-TCDD	20	86±4	-	-			3.95
WHO-dioxin TEQ lower			2.45±0.03	2.36±0.52	Z<2	104	
WHO-dioxin TEQ upper			2.73±0.02	2.4±0.53	Z<2	114	

Note:1, * The value of the 9 compounds :1,2,3,7,8,9-HxCDF 1,2,3,4,6,7,8-HpCDF 1,2,3,4,7,8,9-HpCDF OCDF 1,2,3,4,7,8-HxCDD , 1,2,3,6,7,8-HxCDD , 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD OCDD weren't be provided the certified value by CSL.

Table 2 PCDD/Fs contents & TEQ on wet weight basis of 22 fish samples

Compounds	Recovery [1]	RSD of recovery (%)	Average of Concentration ng/kg (wet weight basis)	MDL (ng/kg)	WHO-TEF	TEQ ng/kg (wet weight basis)
2,3,7,8-TCDF	78.7	7.5	0.21	0.016	0.1	0.021
1,2,3,7,8-PeCDF	82	10.6	nd	0.050	0.05	-
2,3,4,7,8-PeCDF	84.1	10.3	0.13	0.057	0.5	0.065
1,2,3,4,7,8-HxCDF	78.2	6.1	nd	0.060	0.1	-
1,2,3,6,7,8-HxCDF	77.4	7.8	nd	0.088	0.1	-
2,3,4,6,7,8-HxCDF	79.2	6.9	nd	0.050	0.1	-
1,2,3,7,8,9-HxCDF	82.3	7.5	nd	0.075	0.1	-
1,2,3,4,6,7,8-HpCDF	79.1	8.0	nd	0.063	0.01	-
1,2,3,4,7,8,9-HpCDF	76.4	7.6	nd	0.050	0.01	-
OCDF			nd	0.15	0.0001	-
2,3,7,8-TCDD	79.2	8.6	0.03	0.016	1	0.030
1,2,3,7,8-PeCDD	85.5	11.3	0.10	0.050	1	0.1
1,2,3,4,7,8-HxCDD	80.7	8.8	0.08	0.072	0.1	0.008
1,2,3,6,7,8-HxCDD	81.2	8.9	nd	0.069	0.1	-
1,2,3,7,8,9-HxCDD			nd	0.082	0.1	-
1,2,3,4,6,7,8-HpCDD	75.3	9.3	0.18	0.094	0.01	0.0018
OCDD	72.2	11.5	0.26	0.19	0.0001	0.0000
Total Concentration			0.99			0.2258

Fig1 PCDFs distribution pattern in 22 fishes

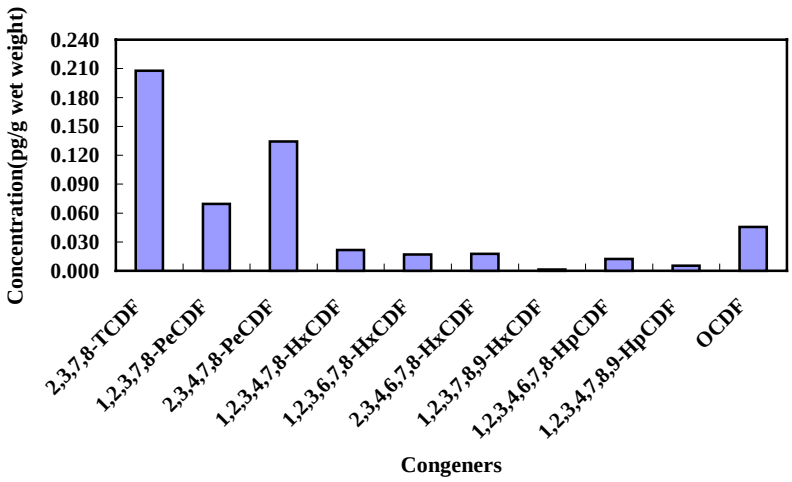

Fig2 PCDDs distribution pattern in 22 fishes

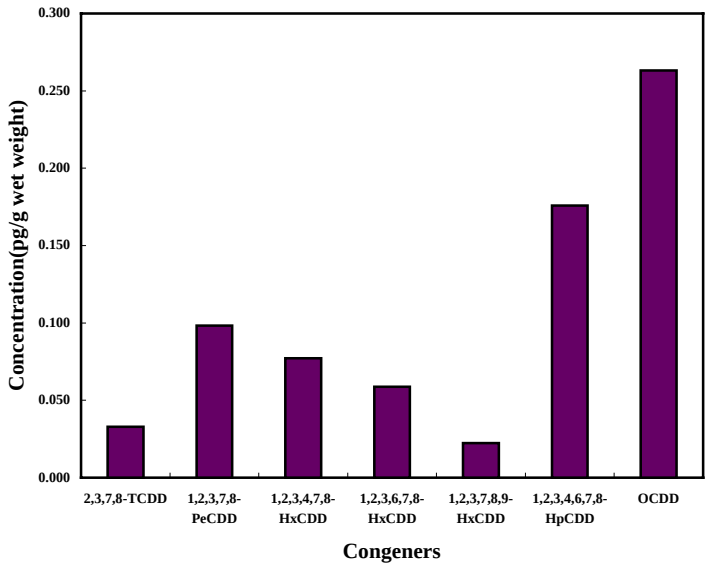

Fig.3 Concentration in WHO-TEQ calculated in 22 fishes