THE PCDD/PCDF LEVELS IN BREAST MILK SAMPLES COLLECTED IN TAINAN CITY OF SOUTHERN TAIWAN

Pao-Chi Liao, Chun-Hu Liu, Ching-Chang Lee, Yueliang L. Guo

Department of Environmental and Occupational Health, Medical College, National Cheng Kung University, Tainan 70101, Taiwan, R.O.C.

Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are broadly distributed environmental contaminants. PCDDs/PCDFs are highly stable in the environment. They are unwanted by-products in a variety of industrial and thermal processes. Daily consumption of contaminated food is the main route of environmental exposure to PCDDs/PCDFs. Food consumption normally comprises 90% of the total daily intake of dioxins. They are lipophilic and can be bioaccumulated *via* food chain into human bodies with long half-lives of elimination[1].

Measurement of PCDD/PCDF levels in human tissue is useful to assess long-term dioxin exposure. The PCDD/PCDF concentrations, based on lipid weight, in human adipose tissue, blood, and milk are very similar[2]. The adipose tissue, blood, and breast milk are usually analyzed for the assessment of human exposure to PCDDs/PCDFs. Among these three types of specimen, analysis of breast milk for exposure assessment provides two specific advantages. Firstly, the collection of breast milk is noninvasive. Secondly, the analytical data can be used to assess the exposure of both the mother and the infant.

Concerns over contamination from dioxins from waste incinerators and other sources have risen in recent years in Taiwan. The objectives of the study are (1) to measure the PCDDs/PCDFs levels in breast milk samples collected in Tainan area of the sourthern Taiwan, and (2) to utilize these data to evaluate infant's exposure *via* breast-feeding.

Methods and Materials

Study Subjects and Breast Milk Sample Collection

The breast milk samples were collected from three hospitals in Tainan city, National Cheng Kung University (NCKU), Sin-Lau, and Chi-Mei hospitals. Breast milk samples were collected from 37 mother volunteers two to eight weeks after delivery in these hospitals in years 2000 and 2001. Each study participant provided 30 mL of breast milk sample. The samples were stored at –80 °C until analysis.

Sample Preparation and HRGC/HRMS Analysis of PCDDs and PCDFs

Breast milk lipids were extracted by ethanol and n-hexane, followed by sulfuric acid digestion. For the analysis of seventeen PCDDs/PCDFs, the extracts were fractionated with ion exchange (SCX), silica (Si), and Florisil solid phase extraction columns, and the fraction containing dioxins and furans were analyzed. The concentrations were determined using high resolution gas chromatography - high resolution mass spectrometry (HRGC-HRMS) combined with isotope dilution method. A Fison 8060 GC and a Micromass AutoSpec Ultima EBE tri-sector mass spectrometer were used for the HRGC/HRMS analysis. Samples were chromatographed on an 60-m Rtx-5MS capillary fused-silica column. The HRMS was operated in electron impact ionization mode. Isotope dilution HRGC/HRMS method

was used to determine quantitatively the seventeen PCDD/PCDF congeners. Selected ion monitoring (SIM) was used to acquire M/(M+2) or (M+2)/(M+4) PCDD/PCDF ions for identification. Quality assurance/quality control protocols were established, according to those defined in USEPA method 1613, in our laboratory to ensure positive identification and the quality of the measurements.

Results and Discussion

The average age of the subjects in this study was 30 years (N = 37, SD = 3.5 yrs). Table 1 lists the levels of seventeen PCDD/PCDF congeners in breast milk samples collected from the volunteers. The values are expressed on a lipid-adjusted basis. The total concentration of 17 dioxin congeners in the breast milk was 213 ± 141 pg/g-lipid. The concentration was expressed as 12.7 ± 8.40 pg-I-TEQ/g-lipid when the international toxic equivalences (I-TEQs) of 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (TCDD) is calculated, or 14.6 ± 9.26 pg-WHO-TEQ/g-lipid using WHO toxic equivalences (WHO-TEQs). The data represent these mothers' dioxin/furan exposure. These concentrations can be regarded as an indicator of background human exposure to PCDDs/PCDFs in the area.

The infants' exposure through breast-feeding given by these nursing mothers can also be estimated. Several assumptions were made to facilitate the calculation of the infants' exposure: (1) the breast milk intakes per day for an infant are 630 mL in the first two months after birth, 660 mL in the third month, and 850 mL from the 4th to the 6th months respectively; (2) an absorption efficiency of 95 %[3] was assumed; (3) a 1.7% per week decrease in a mother's breast milk dioxin/furan levels due to breast-feeding.

Table 2 summarizes the estimation of infants' dioxin/furan exposure reported in the literature and the assumptions made to facilitate the calculation of the infants' exposure[4-6]. The average infant's dioxin/furan exposure was estimated to be 183 ± 109 pg-I-TEQ/day (or 213 ± 125 pg-WHO-TEQ/day). Due to the weight difference between boys and girls, these values translate into 29.6 ± 17.6 pg-I-TEQ/kg/day (34.3 ± 20.2 pg-WHO-TEQ/kg/ day) for boys, and 34.0 ± 20.2 pg-I-TEQ/kg/day (39.4 ± 23.2 pg-WHO-TEQ/kg/ day) for girls.

Table 3 summarizes the dioxin and furan congener levels in human milk obtained in this study and those reported in the literature [7-11]. OCDD comprised 62 % of the total dioxin/furan level in our study, and this congener comprised more than 50 % of the total dioxin/furan level in the literature data. The values obtained in this study were similar to those reported in the literature. The percentage of each congener's contribution relative to the total dioxin/furan concentration was also calculated in Table 3. It is noted that the percentage of 2,3,7,8-TCDF was higher than those reported in these literature. By converting these congener concentrations into TCDD toxic equivalences, 2,3,4,7,8-PeCDF, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-HxCDD were the major congeners contributing to toxicity in the milk samples collected in this study.

References

1. Ryan, J. J., Levesque, D., Panopio, L. G., Sun, W. F., Masuda, Y., and Kuroki, H. (1993) Elimination of polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) from human blood in the Yusho and Yu-Cheng rice oil poisonings. Archives of Environmental Contamination and Toxicology *24*, 504-512.
2. Papke O. (1998) PCDD/PCDF: Human background data for Germany, a 10-year experience. Environmental Health Perspectives *106 (Supplement2)*, 723-731.
3. Dahl P., Lindstrom G., Wiberg K., Rappe C. (1996) Chemosphere 30, 2297-2306
4. Hoover S. M. (1999) Risk Anal. 19, 527-546
5. Patandin S, Dagnelie PC, Mulder PG, Op de Coul E, van der Veen JE, Weisglas-Kuperus N, Sauer PJ. (1999) Environ. Health Persp. 107:45-51

6. Iida Y., Hirakawa H., Matsueda T., Takenaka S. and Nahayama J. (1999) Chemosphere 38, 2461-2466
7. Hashimato S., Yamamoto T., Yasuhara A. and Morita M. (1995) Chemosphere 31, 4067-4075
8. Frery N., Deloraine A., Dor A., Zeghnoun A. and Rouviere F. (2000) Organohalogen Compounds 48, 47-50
9. Schuhmacher M., Domingo J.L., Lobet J.M., Kiviranta H. and Vartiainen T. (1999) Chemosphere 38, 995-1004
10. Schecter A. and Paepke O. Organohalogen Compounds 48, 68-71
11. Koopman-Esseboom C., Huisman M., Weisglas-Kuperus N., Van der Paauw C.G., Tuinstra L.G.M.Th., Boersma E.R. and Sauer P.J.J (1994) Chomosphere 28, 1721-1732

Table 1. Levels of PCDD/PCDF congeners in breast milk samples (N = 37)

congener	pg/g-lipid			pg-I-TEQ/g-lipid			pg-WHO-TEQ/g-lipid		
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median
2,3,7,8-TCDF	2,70	2,64	2,97	0,270	0,264	0,459	0,265	0,262	0,404
1,2,3,7,8-PeCDF	1,40	1,35	1,05	0,070	0,067	0,073	0,069	0,067	0,072
2,3,4,7,8-PeCDF	9,50	4,80	3,98	4,75	2,40	0,459	4,74	2,37	0,404
1,2,3,4,7,8-HxCDF	4,77	4,37	2,95	0,477	0,437	0,274	0,475	0,431	0,274
1,2,3,6,7,8-HxCDF	3,30	3,22	3,29	0,330	0,322	0,322	0,330	0,318	0,327
2,3,4,6,7,8-HxCDF	2,22	3,25	3,41	0,222	0,325	0,353	0,220	0,321	0,353
1,2,3,7,8,9-HxCDF	1,41	3,35	1,52	0,141	0,335	0,152	0,138	0,331	0,152
1,2,3,4,6,7,8-HpCDF	5,53	3,57	1,73	0,055	0,036	0,090	0,055	0,035	0,090
1,2,3,4,7,8,9-HpCDF	1,18	1,89	1,09	0,012	0,019	0,056	0,012	0,019	0,054
OCDF	5,89	6,57	1,61	0,006	0,007	0,017	0,001	0,001	0,015
2,3,7,8-TCDD	1,91	1,06	1,53	1,91	1,06	0,010	1,88	1,06	0,007
1,2,3,7,8-PeCDD	4,17	2,43	2,68	2,09	1,21	1,13	4,14	2,40	1,40
1,2,3,4,7,8-HxCDD	4,46	13,5	2,23	0,446	1,347	1,25	0,438	1,33	1,45
1,2,3,6,7,8-HxCDD	12,3	13,2	3,04	1,23	1,32	1,08	1,22	1,30	1,24
1,2,3,7,8,9-HxCDD	4,51	12,9	3,09	0,451	1,294	0,483	0,444	1,28	0,499
1,2,3,4,6,7,8-HpCDD	15,1	9,53	4,69	0,151	0,095	0,225	0,150	0,094	0,210
OCDD	133	90,3	11,1	0,133	0,090	0,177	0,013	0,009	0,148
Total	213	142	176	12,7	8,40	0,177	14,6	9,26	0,148

Table 2. The estimation of infants' dioxin/furan exposure reported in the literature and the assumptions made to facilitate the calculation of the infants' exposure

Level of PCDD/PCDF in human milk (pg-I-TEQ/g-lipid)	Exposure (pg-I-TEQ/kg/day)	Assumptions
12.7±8.40 (our study)	34.9±20.7 (boy) 40.1±23.8 (girl)	1.PCDD/PCDF decrease of 1.7% weekly 2.duration of breast-feeding was 6 months 3.infant intaked 630 mL in the first two months after birth, 660 mL in the third month, and 850 mL from the 4th to the 6th months per day
23.3[4]	71.6±36.7	1.duration of breast-feeding was 1 year
30.6[5]	112	1.PCDD/PCDF decrease of 1.7% weekly 2.duration of breast-feeding was 6 months
12.8 (primiparas)[6]	97,2	1.infant intaked 120 g breast milk/kg/day
17.6 (multiparas)[6]	121	

Table 3. The dioxin and furan congener levels in human milk obtained in this study and those reported in the literature (unit: pg/g-lipid)

	Japan(1993)		France(1998)		Spain(1996)		Netherlands(1991)		USA(1995)		German(1995)		Our study(2000)	
	N = 26	%	N = 244	%	N = 15	%	N = 209	%	N = 5	%	N = 38	%	N = 37	%
2,3,7,8-TCDF	1,7	0,50	0,97	0,49	0,68	0,28	0,8	0,08	0,91	0,48	0,60	0,29	2,70	1,27
1,2,3,7,8-PeCDF	1,6	0,47	0,38	0,19	0,31	0,13	0,3	0,03	0,51	0,27	0,30	0,14	1,40	0,66
2,3,4,7,8-PeCDF	38	11,19	14,71	7,46	7,95	3,28	22,7	2,29	2,81	1,49	13,50	6,45	9,50	4,45
1,2,3,4,7,8-HxCDF	6,5	1,91	3,99	2,02	3,06	1,26	6,6	0,67	3,88	2,05	4,90	2,34	4,77	2,24
1,2,3,6,7,8-HxCDF	6,8	2,00	3,58	1,82	2,49	1,03	5,7	0,58	2,40	1,27	3,70	1,77	3,30	1,55
2,3,4,6,7,8-HxCDF	4,0	1,18	1,83	0,93	0,04	0,02	3,6	0,36	1,41	0,75	1,70	0,81	2,22	1,04
1,2,3,7,8,9-HxCDF	1,2	0,35	0,12	0,06	1,00	0,41	0,3	0,03	0,15	0,08	-		1,41	0,66
1,2,3,4,6,7,8-HpCDF	4,2	1,24	3,05	1,55	2,00	0,83	7,9	0,80	5,43	2,87	2,80	1,34	5,53	2,59
1,2,3,4,7,8,9-HpCDF	5,9	1,74	0,21	0,11	0,14	0,06	0,2	0,02	0,53	0,28	-		1,18	0,55
OCDF	3,6	1,06	1,1	0,56	0,34	0,14	2,2	0,22	2,46	1,30	0,7	0,33	5,89	2,76
2,3,7,8-TCDD	2,7	0,80	1,72	0,87	1,04	0,43	4,0	0,40	1,45	0,77	2,10	1,00	1,91	0,89
1,2,3,7,8-PeCDD	12	3,53	6,37	3,23	4,02	1,66	10,6	1,07	2,48	1,31	5,70	2,72	4,17	1,95
1,2,3,4,7,8-HxCDD	3,5	1,03	3,2	1,62	2,84	1,17	8,7	0,88	3,01	1,59	4,80	2,29	4,46	2,09
1,2,3,6,7,8-HxCDD	57	16,78	20,27	10,28	27,88	11,51	47,4	4,79	20,10	10,63	21,80	10,42	12,3	5,75
1,2,3,7,8,9-HxCDD	9,9	2,92	4,09	2,07	4,55	1,88	6,7	0,68	3,50	1,85	2,90	1,39	4,51	2,12
1,2,3,4,6,7,8-HpCDD	21	6,18	26,68	13,53	38,26	15,79	63,2	6,38	34,0	17,99	21,90	10,47	15,1	7,10
OCDD	160	47,11	104,93	53,21	145,67	60,13	799,6	80,73	104,00	55,02	121,80	58,22	133	62,33
Total	339,6	100	197,2	100	242,3	100	990,5	100	189,0	100	209,2	100	213,4	100