# The level and influencing factors of dioxin in breast milk of lactating women around the waste incineration plant.

# Lei Ma<sup>1</sup>, Xie Chen<sup>2</sup>, Min Zhao<sup>2\*</sup>

<sup>1</sup>Business Administration School of Nanjing Audit University, No. 86 Yushan Road (W), Nanjing, 211815, PR China

<sup>2</sup>Business School of Hohai University, 1 Xikang Rd, Gulou Qu, Nanjing Shi, Jiangsu Sheng, PR China

#### Abstract

Objective: To detect the levels of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs) in breast milk of lactating women around the waste incineration plant in Shenzhen city, and analyze the related influencing factors.

Methods: In 2011 4~12 months, 89 breast milk samples within 1~2 month birth from lactating women within the range of 5 km in the waste incineration plant were collected from 2011.04 to 2011.12. After pretreatment, 17 gradients of PCDD/Fs in breast milk samples were quantitatively analyzed with isotope dilution high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS), and the normal concentration of total toxic was calculated. SPSS13.0 was used to analyze the relationship between nurse age, residence time, diet and other factors and PCDD/Fs concentration of breast milk. Results: In breast milk samples, the median of mass concentration of  $\Sigma$ PCDD/Fs was 181.52 pg/g fat, and the median concentration of  $\Sigma$ TEQ-PCDD/Fs was 5.80 pg/g fat. According to the mass concentration, the dominant compounds are OCDD of 84.26%, 1, 2, 3, 4, 6, 7, 8-HpCDD of 5.84% and OCDF of 2.14%. Based on the TEQ concentration, the dominant compounds of pollution compounds were 2, 3, 4, 7, 8-PeCDF of 39.45%, 1, 2, 3, 4, 7, 8-HxCDF of 7.56% and 1, 2, , 3, 7, 8-PeCDD of 24.20%. The statistical analysis found that PCDD/Fs load was related to age and breast milk, PCDD/Fs load in breast milk of high maternal age group was higher than the low age group (7.71 pg/g fat and 6.34 pg/g fat, P=0.04); PCDD/Fs load level in breast milk was associated with family income (r=0.22, P<0.05). Conclusion: The levels of dioxin compounds in breast milk around the waste incineration plant in Shenzhen are equivalent with common population in Shenzhen. The level of PCDD/Fs in breast milk is positively related to maternal age and family income.

Keywords: Dioxin, Waste incineration, Load, Risk analysis.

#### Introduction

Dioxin and dioxin compounds are the most toxic pollutants in the environment, not only have carcinogenic, mutagenic and teratogenic effects, but also reproductive toxicity. neurotoxicity, liver toxicity, immunotoxicity, endocrinedisrupting toxicity and other comprehensive toxic effects [1]. Waste incineration is the most important source of dioxin pollution release into the environment. It is estimated that the dioxin produced by waste incineration accounts for about 80% to 90% of the total dioxin emissions [2]. In recent years, the international community has erupted a number of dioxin pollution incidents, so whether waste incineration has a potential health impact on the surrounding residents and its impact gets great concern from much domestic and foreign scientists. With the rapid progress of urbanization in China and the increasing amount of municipal solid waste, the rapid establishment of a number of domestic waste incineration plant

results in more and more residents exposed to waste incineration emissions [3].

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Waste incineration started in the 80's of last century in China, later than developed countries. Because of the high cost of dioxin detection, there is no routine monitoring of the daily emissions of dioxin from waste incinerators, and no studies on the relationship between dioxins and human health have been conducted. The milk samples have the advantages of convenient sampling and non-invasion, and can be simultaneously widely used to assess the exposure of dioxin to the maternal load and the new-borns during lactation [4]. Based on the ultra-trace POPs detection analysis platform, this study employed the international standard method---isotope dilution---high resolution gas chromatography/high resolution dual focus magnetic mass spectrometry (HRGC/HRMS) for qualitative and quantitative detection of dioxin in breast milk of lactating women around the waste incineration plant, and the effects of waste incineration on the level of dioxin loading and

the related factors were studied. This study will provide basic data and reference for the study of dioxin level in population exposed in waste incineration in China.

# Materials and Methods

#### Materials and instruments

Standard substance for standard curve, <sup>13</sup>C labelled 2, 3, 7, 8polychlorinated dibenzo-p-dioxin substituted and polychlorinated dibenzofurans (PCDD/Fs) standard solution, and quantitative internal standard were purchased from Cambridge Isotope Laboratories Company (USA) with purity ≥ 98%; N-hexane, dichloromethane, nonane, methanol are pesticide residues reagents, and purchased from the Merk (USA); Acetone, ethyl acetate, toluene are pesticide residues reagents from J.T. Baker company (USA); Concentrated sulfuric acid is excellent grade pure from Hengyang Xinkai Chemical Reagent Co., Ltd. (China); Siliceousearth was purchased from States Dai An (USA); Silica gel (70-230 mesh) was purchased from Fluka (Germany).

**Instruments:** Freeze-drying machine (STD, FTS), electronic balance (one ten thousandth) (ME614S, Sartorius), accelerated solvent extraction (ASE300, Daian, USA), automatic purification system (SYNCORE, Buchi Corporation, Switzerland), nitrogen evaporator (N-EVAP TM12, System), and the commercial carbon column (AX-21, Fluid Management System, USA), multi-sample parallel evaporator (SYNCORE, Buchi, Switzerland), nitrogen evaporator (N-EVAP TM12, Fisher Scientific, USA), DFS high resolution double focus magnetic mass spectrometer (American thermoelectric company).

## Methods

Object of study: Two garbage incinerators with running time of 13 and 26 respectively in Shenzhen were determined as exposure points, and the range of 5 km around the incinerator was the exposed area. All lactating women in the 12 districts of the community health service center in the exposed area from April 2011 to December 2011 were enrolled in the study. The subjects who met the requirements were selected from the survey, and the subjects should be satisfied with the following: primiparous women who live in the exposed area for more than 3 y and produced singleton births, no occupational exposure, no exposure to organic pesticides. The subjects were physically healthy and signed informed consent for voluntary participation, meet the ethical standards. Refer to "the fourth WHO survey guidelines for persistent organic contaminant in breast milk", the number of samples in line with the acquisition criteria was 89 [5].

During the April to December of 2011, under the guidance of professionals, breast milk between  $1\sim2$  months postpartum of the objects was collected in the non-organic polluted special sampling glass bottle treated with the dioxin. Collection for 100 ml samples was finished within 72 h to avoid high temperature and pollution and kept at -20°C. The questionnaire

investigation of maternal milk donators was conducted, including age, occupation, education level, family income, residence time, living environment; smoking and drinking, pre pregnancy and pregnancy diet; body mass index (BMI) etc.

## Pre-treatment of breast milk sample

The sample was thoroughly lyophilized with a freeze dryer and grinded into fine powder. The weighed sample and 10 µl of the <sup>13</sup>C labelled standard solution were mixed into diatomaceous earth fully and then extracted by a rapid solvent extractor (ASE). The extract was concentrated by rotary evaporator and dried to constant weight. After weighing the fat, the concentrate was added about 150 ml hexane, and 30% acidic silica gel till the clear upper liquid. The mixture was placed at 70°C in the water bath, shaken for 2 h, and then filtered, and the filtrate was rotated and concentrated. The purification and enrichment of PCDD/Fs were carried out by automatic purification system (FMS), and then rotated and concentrated after separation and purification. The purified PCDD/Fs solution by silica gel column was collected, and the nitrogen gas drying apparatus was used for concentration. Further purification was conducted by alumina column, and then concentrated by nitrogen. The sample in the collection bottle concentration was fractionatedly transferred to micro tube trace for micro concentration in small nitrogen flow. Nonane was employed for constant volume, and then 10 µL of internal standard solution was added for determination.

## Instrumental analysis

Based on the international common EPA1613 method [6], high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) was used to quantitatively and quantitatively analyze 17 kinds of monomer compounds of PCDD/Fs in the samples by means of isotope dilution technique (Table 1). The detection limit was  $10^{-15}$ ~ $10^{-12}$  g. Calculate the concentration C (pg/g) and the Toxic Equivalence Quantity (TEQ) for each monomer compound in the sample. In the process of determination and analysis, regular standard curve correction should be performed; standard sample detection, blank detection, mass spectrometer performance inspection were utilized for quality control to ensure accuracy and precision of testing equipment's. The concentration of each PCDD/Fs monomer compound was calculated according to the following formula:

 $C=(An \times Cl/Al \times RRav) \times v \times (1/M)$ 

Where, C is the concentration of PCDD/Fs in the sample (pg/g).

An is the total area of the two major molecular or ions for each unlabelled PCDD/Fs in the sample extract.

Al is the total area of the two major molecular or ions of each labelled PCDD/Fs in the sample extract.

Cl is the concentration of the labelled PCDD/Fs in the sample extract (pg/g).

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RRav is the average relative response factor of PCDD/Fs.

V is the final constant volume of the sample extract (L).

M is the weight of the extracted sample (g).

If a monomer in the sample is below the detection limit, it will be expressed by the method detection limit. Calculate the toxicity equivalent concentration according to the following formula:  $TEQ=C\times TEF$ 

Where, TEQ is the toxic equivalent concentration of dioxin.

C is the concentration of PCDD/Fs (pg/g) in the sample.

TEF value is based on WHO toxic equivalence factors in 1998.

Table 1.	Contents of 17	kinds of monomer	compounds of PDCC/Fs i	n breast milk samples (pg/g fat).
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	Mass concentration				TEQ concentration			
Compounds	Average	Minimum	Maximum	Median	Average	Minimum	Maximum	Median
2, 3, 7, 8 tetrachloro-dibenzofuran (2, 3, 7, 8- TCDF)	1.364	0.110	5.600	1.180	0.136	0.011	0.560	0.120
1, 2, 3, 7, 8 pentachloro-dibenzofuran (1, 2, 3, 7, 8-PeCDF)	1.456	0.055	7.190	1.150	0.073	0.003	0.360	0.058
2, 3, 4, 7, 8 pentachloro-dibenzofuran (2, 3, 7, 8-PeCDF)	5.654	0.058	18.340	4.970	2.837	0.029	9.170	2.480
1, 2, 3, 4, 7, 8 hexachloro-dibenzofuran (1, 2, 3, 4, 7, 8-HxCDF)	5.421	1.140	29.780	4.610	0.542	0.110	2.980	0.460
1, 2, 3, 6, 7, 8 hexachloro-dibenzofuran (1, 2, 3, 6, 7, 8-HxCDF)	3.228	0.820	16.460	3.000	0.329	0.082	1.650	0.300
2, 3, 4, 6, 7, 8 hexachloro-dibenzofuran (2, 3, 4, 6, 7, 8-HxCDF)	1.510	0.170	9.090	1.260	0.015	0.000*	0.052	0.014
1, 2, 3, 7, 8, 9 hexachloro-dibenzofuran (1, 2, 3, 7, 8, 9-HxCDF)	0.150	0.000a	0.520	0.140	0.015	0.000*	0.052	0.014
1, 2, 3, 4, 6, 7, 8 heptachloro-dibenzofuran (1, 2, 3, 4, 6, 7, 8-HpCDF)	9.758	0.510	57.370	6.950	0.097	0.005	0.570	0.069
1, 2, 3, 4, 7, 8, 9 heptachloro-dibenzofuran (1, 2, 3, 4, 7, 8, 9-HpCDF)	1.065	0.087	6.580	0.760	0.011	0.001	0.066	0.008
heptachloro-dibenzofuran (OCDF)	9.964	0.035	272.160	4.640	0.001	3.5E-6	0.027	4.6-E-4
2, 3, 7, 8 diphenyl tetrachloro-dioxin (2, 3, 7, 8-TCDD)	0.520	0.010	2.270	0.390	0.520	0.010	2.270	0.390
1, 2, 3, 7, 8 pentachloro-diphenyl - dioxin (1, 2, 3, 7, 8-PeCDD)	1.734	0.064	8.000	1.370	1.734	0.064	8.000	1.370
1, 2, 3, 4, 7, 8 hexachloro-diphenyl - dioxins (1, 2, 3, 4, 7, 8-HxCDD)	0.734	0.052	7.020	0.460	0.073	0.005	0.700	0.046
1, 2, 3, 6, 7, 8 hexachloro-diphenyl-dioxins (1, 2, 3, 6, 7, 8-HxCDD)	2.799	0.230	16.180	1.970	0.280	0.023	1.620	0.200
1, 2, 3, 7, 8, 9 Hexachloro-diphenyl-dioxins (1, 2, 3, 7, 8, 9-HxCDD)	0.668	0.075	19.170	0.300	0.067	0.008	1.920	0.030
1, 2, 3, 4, 6, 7, 8 Hexachloro-diphenyl-dioxins (1, 2, 3, 4, 6, 7, 8-HpCDD)	27.193	2.540	642.290	11.040	0.272	0.025	6.420	0.110
Hexachloro-diphenyl-dioxins (OCDD)	392.383	18.200	12792.460	137.330	0.039	0.002	1.280	0.014
Total polychlorinated dibenzofuran	39.631	2.985	423.090	28.660	4.181	0.258	16.345	3.639
Total polychlorinated diphenyl-dioxins	426.032	21.171	13487.390	152.860	2.986	0.137	22.210	2.160
In total	465.663	24.156	13910.480	181.520	7.167	0.394	38.555	5.799

Note: The content of monomer is lower than the limit of detection method, and the concentration of the compound is substituted by the detection limit, and \*means that the data is not detected.

#### Statistical analysis

Epidata3.1 was used to establish the database of breast milk survey, SPSS13.0 was used for statistical analysis. The concentration of PCDD/Fs and the range, mean value, median value, maximum value and minimum value of toxicity equivalent concentration in breast milk were calculated respectively, and the concentration of PCDD/Fs in breast milk was analyzed and compared with the samples in common population in Shenzhen; The monomer compounds with main contribution were analysed. The correlation analysis of breast milk age, family income, educational level, living time, BMI and dietary status with PCDD/Fs concentration and were analyzed.

#### Results

# Analysis of PCDD/Fs concentrations in breast milk samples

In 89 samples of breast milk, fat content range was from 1.45% to 5.92%, with an average of 3.57%. The mass concentration of  $\Sigma$ PCDD/Fs in milk samples was 24.16~13 910.48 pg/g, with the average of 465.66 pg/g and the median of 181.52 pg/g. Calculated as the toxic equivalent concentration, the concentration of TEQ-PCDD /Fs in breast milk samples ranged from 0.09 to 38.56 pg/g, with an average of 7.17 pg/g, and with a median of 5.80 pg/g, 2, 3, 7, 8 -TCDD (2, 3, 7, 8 tetrachlorodibenzo-p-dioxin) is the most toxic PCDD/Fs, known as the century highly toxic.

# Analysis of pollution characteristics of PCDD/Fs in breast milk samples

The toxicity equivalent is used to assess the potential effects of PCDD/Fs on health. The toxicity equivalent factor of the most toxic 2, 3, 7, 8-TCDD is considered as 1, the toxicity of other dioxin isomers was calculated relative to the toxicity of 2, 3, 7, 8-TCDDD [7]. OCDD (octachlorodibenzo-p-dioxin) is the most common compound monomer in PCDD/Fs detection, 1, 2, 3, 7, 8-PeCDD (1, 2, 3, 7, 8 pentachlorodibenzo-p-dioxin) is another of the most toxic homologues. In this study, we found that in 17 kinds of monomer compounds in PCDD/Fs, if calculated according to the mass concentration, the three major compounds contributed to 92.24% of the total concentration, mainly including high chlorine-substituted dioxin and furan, OCDD (octachlorodibenzo-p-dioxin) of 84.26%, 1, 2, 3, 4, 6, 7, 8-HpCDD (1, 2, 3, 4, 6, 7, 8 Chlorodibenzo-p-dioxin) of 5.84% and OCDF (octachlorodibenzofuran) of 2.14%. According to the TEQ concentration, the three compounds with the total contribution rate of 71.21% were 2, 3, 4, 7, 8-PeCDF of 39.45%, 1, 2, 3, 4, 7, 8-HxCDF (1, 2, 3, 4, 7, 8hexachlorodibenzofuran) of 7.56%, 1, 2, 3, 7, 8-PeCDD (1, 2, 3, 7, 8 Pentachlorodibenzo-p-dioxin) of 24.20%. 1, 2, 3, 7, 8, 9-HxCDF (1, 2, 3, 7, 8, 9 hexachlorodibenzofuran) were not detected in the seven samples, and 17 kinds of monomer compounds are all detected in the rest samples.

#### Analysis on the influencing factors of breast milk load level in the surrounding residents of waste incineration plant

The analysis of the questionnaire on breast milk donors showed that 89 respondents had no smoking habits, 21 respondents with occasional drinking behaviour. The lactation age, residence time, BMI, fish consumption, milk and dairy consumption, meat consumption, egg consumption were 26.91  $\pm$  3.30 y old, 7.55  $\pm$  6.51 y, 21.81  $\pm$  2.5, 361.74  $\pm$  371.3 g/ week, 117.21  $\pm$  130.098 g/week, 904.10  $\pm$  583.73 g/week and 216.69  $\pm$  162.10 g/week respectively.

Further grouping was divided into 18~26 and 27~37 groups according to the age, and the difference was analyzed by Wilcoxon rank sum test. The results showed that the concentration of  $\Sigma$ TEQ-PCDD/Fs in maternal breast milk of older mothers was higher than that of younger mothers (Z=-2.08, P<0.05) (Table 2). The level of PCDD/Fs in breast milk was associated with the period of residence, family income, educational level, BMI, fish consumption, consumption of milk and dairy products, consumption of meat type and egg consumption by spearman correlation analysis. The results demonstrated that PCDD/Fs level in breast milk was positively correlated with household income (Table 3).

**Table 2.** The relationship between STEQ-PCDD/Fs load levels (pg/g) in breast milk and age (years old).

Mother age (years old)	range	x	м	S	Z value	P value
18~26	2.17~20.2 3	6.34	4.94	3.96	-2.079	0.038
27~37	1.51~27.5 6	7.71	6.79	4.39		

**Table 3.** Correlation between the factors and the load level (pg/g) of STEQ-PCDD/Fs in breast milk.

Factors	R value	P value
Duration of residence	0.10	>0.05
Family income	0.22	>0.05
Education level	0.04	>0.05
BMI	-0.10	>0.05
Fish consumption	0.07	>0.05
Milk consumption	0.10	>0.05
Meat consumption	0.07	>0.05
Egg consumption	-0.05	>0.05

## Discussion

This study is aimed to investigate the load levels of PCDD/Fs in the population around urban garbage incinerator. Due to the use of waste incineration technology and the extensive establishment of waste incineration plant in the country, whether the incineration of waste will cause changes in the PCDD/Fs load level and its impact on human health has always been a hot spot problem for scientists, governments and the public, but there is lack of the study on the effect of waste incineration on the human dioxin load. The country's first waste incinerator in Shenzhen has been running for 26 years. This study found that the average concentration of  $\Sigma TEQ$ -PCDD/Fs in the breast milk around the incinerator in Shenzhen was 7.17 pg/g, with the median of 5.80 pg/g fat. In 2008, our laboratory collected 60 breast milk samples of common pregnant women who were living in high non-POPs exposed areas in Shenzhen for the analysis of influencing factors of the PCDD/Fs load level. The mean  $\Sigma$ TEQ-PCDD/Fs concentration in the common population was 7.16 pg/g fat, with a median of 6.34 pg/g fat [8]. Compared with the two, the concentration of dioxin in the breast milk of the residents around the waste incinerator is comparable to that of the normal population, and there is no significant increase in dioxin load. Foreign research has been carried out on the dioxin load of residents around the waste incinerator. In Italy, a study showed that the  $\Sigma TEQ$ -PCDD/Fs concentration in the exposed population was 3.78 pg/g fat, and the mean STEQ-PCDD/Fs concentrations in the breast milk of the two control populations were 4.67 pg/g fat and 4.70 pg/g fat, which cannot indicate the cumulative effect of waste incineration on PCDD/Fs content in breast milk [9]. A study in Portugal showed that the  $\Sigma TEQ$ -PCDD/Fs concentration in the exposed area was 9.50 pg/g fat and the  $\Sigma$ TEQ-PCDD/Fs concentration in the control area was 9.10 pg/g fat, the difference was not statistically significant [10]. Another study compared the levels of PCDD/Fs in human breast milk in three different periods (1998, 2002, and 2007), and found that the incinerator had no effect on its surrounding population, and that in 2007, the mean **STEQ-PCDD/Fs** concentration was 7.60 pg/g fat [11].

Compared with the average PCDD/Fs total concentration of 3.73 pg/g of fat and the median of 3.46 pg/g of fat in the fourth global breast milk POPs survey, the total concentration of PCDD/Fs in the exposed and normal populations of Shenzhen was twelve times the country's 12 representatives of the city [12]. Studies in Dalian, China showed that the median  $\Sigma$ TEQ-PCDD/Fs concentration in breast milk is 15.19 pg/g fat [13]. The differences in human dietary habits and living conditions in different regions make the different baselines of dioxin content in different places, and the bias of waste incineration technology equipment and unaffected factors contributed to the different results. This requires more in-depth, detailed and long-term research to further determine the effect of waste incineration on PCDD/Fs levels in exposed populations.

This study found that human dioxin load levels were positively correlated with age and household income. The level of dioxin load in breast milk in Shenzhen population in 2008 showed that the level of PCDD/Fs in breast milk was related to the age of mothers, residence time and fish consumption [8]. Todaka et al. found that the level of PCDD/Fs in young maternal breast milk was lower than that in high age maternal age, and the difference was statistically significant, probably because the exposure time to contaminants of young maternal was short [14]. Although no correlation between human dioxin load levels and diets was found, the analysis showed that household income was positively correlated with human dioxin load and that high-income mothers were ingested more in fish, meat, milk and eggs. The results show that the concentration of dioxins in fish, meat, milk and eggs is higher than that of other foods [15]. In 2009, the survey of POPs in breast milk in 12 cities nationwide showed that PCDD/Fs load level in breast milk was related to fish consumption [12]. While in 2008, the level of PCDD/Fs in breastfeeding in Italy was not related to meat and fish intake [16]. The results of the above studies are not consistent. It is possible that the preferences of personal dietary also have an impact. The study found that most of the nurse did not like to eat fish, the fish consumption was increased after pregnancy, and the use of frequency survey meal was used to recall the estimated intake, so dietary survey data has the accuracy bias.

In summary, this study shows that the level of PCDD/Fs in the breast milk around the Shenzhen municipal waste incinerator is comparable to the PCDD/Fs load level in the breastfeeding of the general population in the city. The high level of PCDD/Fs in the mothers of older mothers is higher than that in the younger ones, and breast milk PCDD/Fs load level and family income was positively correlated.

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#### \*Correspondence to

Min Zhao

Business School of Hohai University

1 Xikang Rd

Gulou Qu

Nanjing Shi

Jiangsu Sheng

PR China