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Demographic and Lifestyle Factors Associated with Dioxin-like Activity (CALUX−TEQ) in Human Breast Milk in Hong Kong

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Maternal exposure to dioxins and related compounds before conception may affect the health of the fetus. To identify factors affecting dioxin body load in Hong Kong, in 2002, total dioxin-like activity was estimated in 250 individual milk samples at 2−6 weeks postpartum, from a representative group of primiparous mothers, aged 18−42 years (mean 29 ± 5 years), by a chemically activated luciferase expression (CALUX) bioassay. Associations between the CALUX−TEQ and 20 socio-demographic and dietary variables were examined separately in mothers younger than 30 years (n = 114) and 30 years or older (n = 119), by multiple linear regression analysis. CALUX−TEQ (mean 14.5 ± 5.8 pg/g fat) significantly increased by 0.4−0.5 pg/g fat for every year of the mother's age. Mothers born in Guangdong province of China had a significantly higher CALUX−TEQ. Higher seafood consumption (older mothers; p = 0.07) and having a female baby (younger mothers; p = 0.002) were associated with a higher maternal CALUX−TEQ level. Age was the strongest factor affecting human dioxin levels in Hong Kong. Birthplace and residence are important indicators of variations in exposure to environmental pollution in the Asia Pacific region. Temporal trends in body loads of dioxins remain to be established and continuous monitoring of dioxins in humans and foods is necessary as a precautionary approach to guide environmental control measures and prevent exposure to infants.

Introduction

Dioxins (PCDDs), furans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs) are structurally related chemicals that persist in the environment and accumulate in animal sources of food and human tissues, including breast milk. In humans, more than 90% of human exposure to PCDD/F congeners is from food (*1*), especially foods of animal origin. Seven PCDDs and ten PCDFs with substitutions at the 2, 3, 7, 8 positions, and twelve coplanar PCBs exert a number of toxic responses similar to those of 2, 3, 7, 8 tetra-chloro-dibenzo-para- dioxin (TCDD), considered to be the most toxic dioxin and is classified as a human carcinogen (Group 1) by the International Agency for Research on Cancer (*2*).

Dioxins that accumulate in fatty tissue are transferred to the fetus during pregnancy (*3*) and to the infant through breast feeding (*4*). There is evidence that the toxic effect of PCBs could induce persistent neuro-behavioral deficits in the developing fetus (*5*, *6*). A prospective study of formula fed children (representing children with mainly in-utero exposure to dioxins and PCBs) and breastfed children (exposed prenatally and postnatally) showed prenatal PCB exposure was related to a poorer neurological condition at birth (*7*) and 18 months of age (*8*), lower psychomotor abilities at 3 months of age (*9*), poorer cognitive function at 42 months of age (*10*), and poorer cognitive and motor abilities at 6.5 years (*11*). Postnatal dioxin and PCB exposure was related to lower psychomotor abilities at 7 months of age (*9*). There was apparent greater sensitivity to damage during the prenatal period, rendering the fetus more vulnerable to small quantities of these neurotoxicants (*12*).

Dioxins and related compounds are lipophilic and levels found in the fat fraction of breast milk will reflect previous exposures of mothers over many years. In multiparous women total body load is lower because of off-loading of dioxins to the infant during gestation and lactation (*13*). Other factors suggested to be associated with dioxin body load include previous accidental or occupational exposure (*14*−*17*), residency area (*13*), dietary habits (*18*−*20*), smoking (*21*), socio-economic status (*22*), infant gender (*23*), and mother's body weight (*21*, *24*). Dioxin levels in human breast milk of primiparous women have been monitored in different countries to determine the degree of dioxin exposure in the population. Since the 1980s the World Health Organization Regional Office for Europe (WHO/EURO) coordinated three dioxin exposure studies to evaluate health risks and to control and prevent exposure (*25*−*27*). These three studies have demonstrated a continued decrease in dioxin level in breast milk in European countries, which has probably resulted from policies that aim to reduce dioxin emissions, and monitoring of feed and food stuffs.

Hong Kong participated in the 2002−2003 WHO/EURO coordinated PCDDs, PCDFs, and PCBs exposure study in which the toxic equivalent (TEQ) concentration of 29 target congeners were determined by gas chromatography with mass spectrometry (GC/MS) in 13 pooled milk samples (*28*). From the 316 milk samples forming the 13 milk pools for the GC/MS analysis, 250 paired samples were separately subjected to a chemically activated luciferase expression (CALUX) bioassay to determine dioxin content. The aim of the present study was to determine factors associated with dioxin levels estimated by CALUX in the individual milk samples collected in Hong Kong.

Materials and Methods

Subject Recruitment and Sample Collection. Milk samples were collected from February to October of 2002 from 250 primiparae, aged 18−42 years (mean 29 ± 5 years), giving birth to a single live-born infant, and recruited by nurses during their postnatal visits to one of 16 Maternal and Child Health Centres distributed over Hong Kong. A 30 mL milk sample from each participant, who exclusively or partially breastfed their babies, was collected using an electronic breast

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pump (Medela) or hand expression when the baby was 2−6 weeks old. All breast-pump attachments that had contact with the milk sample were cleaned with tap water without detergent, rinsed with acetone and autoclaved. No plastic bottles were used in sample collection to avoid the theoretical transfer of dioxins to the milk sample. All glassware was cleaned with tap water without detergent, rinsed with acetone and air-dried before used. Each milk sample was divided into 20 mL and 10 mL portions, stored in individual glass vials with polytetrafluoroethylene lined caps and frozen at −20 °C. The 20 mL-samples were pooled into 11 pools and included in the 2002−2003 WHO/EURO coordinated PCDDs, PCDFs, and PCBs exposure study (*27*), and the Hong Kong results are reported elsewhere (*28*). This paper focuses on the results of a CALUX bioassay which determined the dioxin-like response in each of the 10 mL-samples. All participants gave written consent before taking part in the study. This study was approved by the Ethics Committees of the Hong Kong University, the Chinese University of Hong Kong, and the Department of Health, Hong Kong SAR Government.

Participants were interviewed face-to-face and provided information on area of residence, occupation, obstetric information, smoking, and demographic characteristics in response to a questionnaire based on the one used for the 2002−2003 WHO/EURO coordinated PCDDs, PCDFs, and PCBs exposure study. Usual consumption frequency and amount per intake over the previous five years for potential dioxin rich foods including freshwater fish ($n = 11$), seawater fish ($n = 23$), seafood and other fish products ($n = 21$), dairy products ($n = 21$), eggs ($n = 4$), meat ($n = 14$), and poultry ($n = 8$) were collected using a semiquantitative food frequency questionnaire. Mothers were advised to ignore any dietary change during pregnancy and each food group intake was calculated as grams per month for comparison.

Chemical Analysis. The CALUX bioassay was performed by the BioDetection Systems b.v., The Netherlands (http://www.biodetectionsystems.com/). This bioassay comprises a genetically modified H4IIE rat hepatoma cell-line, incorporating the firefly luciferase gene coupled to dioxin responsive elements as a reporter gene for the presence of dioxins and dioxin-like compounds (including PCDDs and materials acting in a toxicological manner similar to PCDDs such as PCDFs and coplanar dioxin like PCBs). These compounds bind to cytosolic aryl hydrocarbon receptors (Ah-receptor) and the complex is translocated to the cell nucleus where it induces the transcription of the recombinant gene. Luciferase is produced and following addition of the substrate luciferin, light is emitted. The amount of light produced is proportional to the amount of ligand−Ah-receptor binding, which is expected to be directly proportional to the toxicity mediated by the Ah-receptor. The CALUX bioassay reports TEQs benchmarked against TCDD as CALUX−TEQs.

The sample was thawed just before extraction. Fat-soluble compounds, such as poly-aromatic hydrocarbons, and chlorinated and brominated aromatic hydrocarbons, were extracted with hexane:diethyl ether (97:3), and fat content was then determined gravimetrically. All fat extracted from the samples was used for cleanup on an acid silica column (20 and 33% H_2SO_4), topped with sodium sulfate. The cleaned extracts were evaporated and redissolved in 25 μl dimethylsulfoxide (DMSO), and CALUX activity determined on 96-well microtiter plates (24 h exposure; 0.4% DMSO). On each 96-well microtiter plate, a standard TCDD calibration curve was constructed by exposing cells to quantitatively prepared serial dilutions of TCDD in DMSO. The dioxin level of each milk sample was reported as the CALUX activity after multiplying by the relative potency. Like the toxic equivalent factor, the relative potency represents the potency or toxicity of the chemical relative to TCDD. The dioxin level in each milk sample was reported as CALUX−TEQ in picogram per gram of milk fat (pg CALUX−TEQ/g fat). Each sample was analyzed at multiple dilutions and, for each dilution, a triplicate analysis was performed. The relative standard deviation for the triplicate analysis was calculated for each dilution and a standard deviation of the result for each milk sample provided information on the measurement uncertainty of the CALUX bioassay. Where the dioxin activity was below the limit of quantification, estimation of the dioxin level was made according to the limit of detection, being the lowest detectable amount of TCDD in the exposure medium.

Statistical Analysis. Statistical Package for Social Sciences (SPSS for Windows, version 10.1; SPSS Inc., Chicago, U.S.) was used for statistical analyses. Associations between the CALUX−TEQ and 20 socio-demographic and dietary variables were examined separately in two age groups, those younger than 30 years and 30 years or older, by multiple linear regression analysis. Separate statistical analyses were done on younger and older mothers to avoid confounding by possible interactions between ages of mothers and cohort effects due to the worldwide decreasing trend in dioxin levels in the environment and the different levels and types of exposures to dioxins and related compounds. Factors of interest included geographic characteristics (birth place and residence), obstetric characteristics (age, mothers' body mass index [BMI], birthweight, and gestational age of the baby), socio-economic status (household income and educational level), breast-feeding patterns (exclusivity and duration), dietary factors (consumption of seafood, dairy products, beef, pork, poultry, and eggs), and smoking. The correlation coefficients among these 20 factors were examined and none of the pairs showed a coefficient over 0.5. Factors with a p-value less than 0.25 in the simple linear regression were selected (*29*) for inclusion in the subsequent multiple linear regression analysis. Regression coefficients (β) were reported with two-tailed p-values and an association with a p-value less than 0.05 considered statistically significant. To demonstrate trends in dioxin concentrations across groups of interest, means with 95% confidence intervals (95% CI) of age-adjusted TEQ were plotted against different possible determinants separately for the two age groups, using 30 years old as the cutoff. Age adjustment was obtained by multiple regression. All means are stated with standard deviations unless otherwise specified.

Results

CALUX−TEQ in Breast Milk. The majority ($n = 233$) of the 250 milk samples gave quantifiable responses ranging from 3.2 to 33 pg CALUX−TEQ/g fat. The fat content of one sample was reported as missing and TEQ content could not be determined. Sixteen samples showed responses below the limit of quantification and thirteen of these showed responses only just below the limit of quantification so an estimated TEQ was allocated to each of these. CALUX−TEQ values were obtained for 246 milk samples. The standard deviation and the coefficient of variation were reported for each of the milk samples with detectable CALUX−TEQ. The mean coefficient of variation was 5.51% (range 0−20%). The limit of detection and the limit of quantification were, respectively, 0.3 and 1.0 pg TCDD TEQ/g fat. The distribution of the CALUX−TEQ shows an approximate normal distribution (Figure 1). The mean CALUX−TEQ was 14.5 ± 5.8 pg CALUX−TEQ/g fat.

Factors Associated With CALUX−TEQ In Breast Milk. GC/MS analysis undertaken on pooled samples from the same mothers had shown that the congener pattern of the mothers born in overseas countries (Vietnam, Indonesia, Thailand, and Singapore) were different from those born in mainland China or Hong Kong (*28*). Thus the 10 mothers who were either born overseas or had spent an extensive period of time overseas before migration to Hong Kong were excluded from the regression analysis. Three mothers were

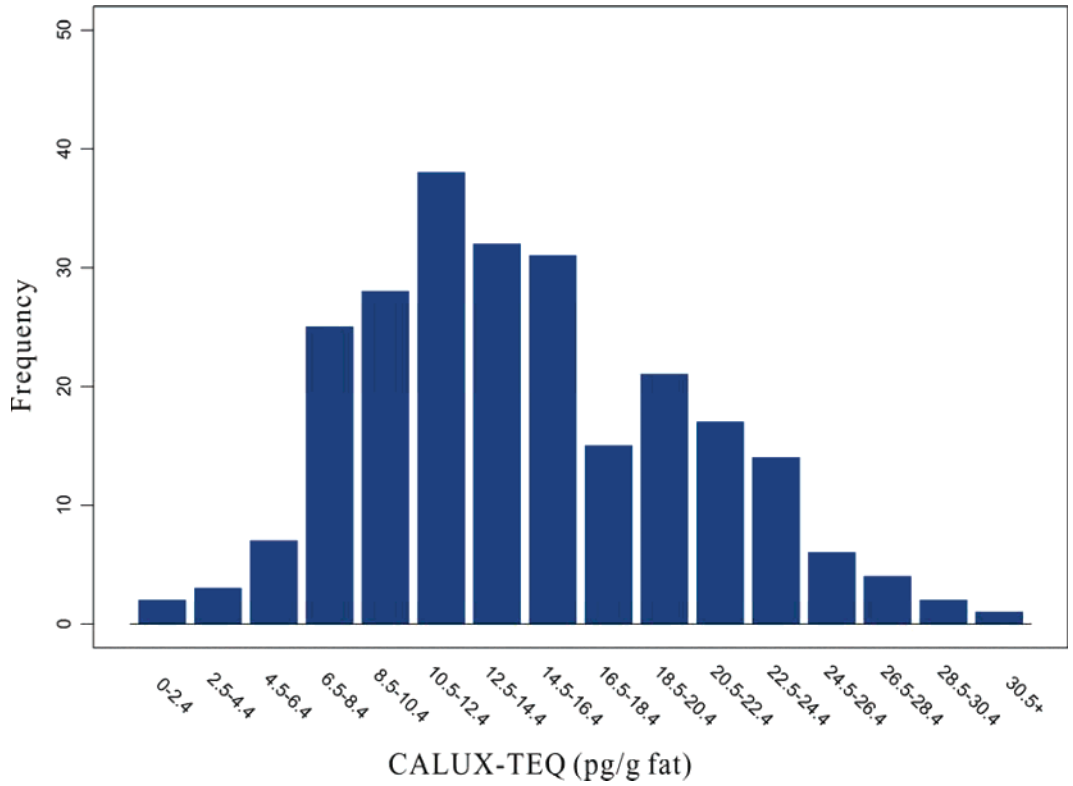

FIGURE 1. Distribution of the CALUX−TEQ ($n = 246$).

TABLE 1. Regression Coefficients, 95% Confidence Intervals and *P*-values in Simple Linear Regressions of CALUX−TEQ Concentration of Mothers, Stratified by Age <30 years old ($n = 114$) and ≥30 years old ($n = 119$)

	age <30 years old ($n = 114$)			age ≥30 years old ($n = 119$)		
independent variable	regression coefficient	[95% CI]	*p*-value[a]	regression coefficient	[95% CI]	*p*-value[a]
age of mother[b]	0.27	[−0.05, 0.60]	*0.096*	0.60	[0.25, 0.96]	*0.001*[b]
place of birth						
other mainland born	1.00			1.00		
Hong Kong born	1.75	[−0.38, 3.89]	*0.106*	2.18	[−0.87, 5.23]	*0.160*
Guangdong born	2.49	[0.09, 0.04]	*0.042*[a]	5.87	[2.26, 9.49]	*0.002*[b]
residential history						
Hong Kong	0.76	[−1.40, 13.82]	0.486	−1.13	[−4.45, 2.19]	*0.501*
mainland China	1.00			1.00		
China immigrant	1.17	[−1.34, 3.69]	0.357	−0.38	[−4.35, 3.59]	0.850
overseas	1.61	[−3.59, 6.80]	0.541	−1.26	[−5.73, 3.21]	0.579
income $25 000+/mth	0.08	[−1.95, 2.11]	0.936	−0.24	[−2.49, 2.01]	0.836
education: senior high +	0.86	[−1.29, 2.99]	0.428	−2.35	[−5.95, 1.24]	0.297
BMI	−0.25	[−0.57, 0.07]	*0.117*	0.62	[0.12, 1.12]	*0.016*[a]
non-primigravidae	−2.00	[−4.35, 0.35]	*0.094*	2.93	[0.17, 5.69]	*0.038*
male baby	−2.24	[−4.04, −0.45]	*0.015*[a]	−0.20	[−2.39, 1.98]	0.855
gestational weeks (weeks)[b]	0.04	[−0.67, 0.75]	0.994	0.53	[−0.24, 1.29]	*0.174*
birthweight (grams)[b]	0.00	[0.00, 0.00]	0.743	0.00	[0.00, 0.00]	0.814
exclusively breast feeding	−0.74	[−2.67, 1.18]	0.447	0.22	[−1.96, 2.40]	0.842
breast feeding duration (weeks)[b]	0.18	[−0.68, 1.04]	0.680	0.17	[−0.81, 1.15]	0.727
ever smoker	−1.41	[−3.62, 0.80]	*0.208*	−0.74	[−4.86, 3.39]	0.724
spouse: ever smoker	−0.24	[−2.08, 1.61]	0.801	−0.25	[−2.61, 2.10]	0.832
seafood consumption 3rd tertile	0.65	[−1.26, 2.55]	0.503	2.44	[0.13, 4.76]	*0.039*[a]
dairy product consumption 3rd tertile	−0.87	[−2.75, 1.01]	0.363	0.27	[−2.15, 2.68]	0.828
beef consumption 3rd tertile	0.77	[−1.32, 2.86]	0.468	−2.62	[−4.79, −0.46]	*0.018*[a]
pork consumption 3rd tertile	−1.02	[−3.03, 0.98]	0.314	−2.56	[−4.80, −0.33]	*0.025*[a]
poultry consumption 3rd tertile	1.29	[−0.68, 3.26]	*0.197*	−0.58	[−2.87, 1.72]	0.620
egg consumption 3rd tertile	−0.24	[−2.14, 1.67]	0.807	0.81	[−1.54, 3.16]	0.496

[a] A variable with $p < 0.25$ (italic) was selected for multiple linear regression. [b] As continuous variables.

excluded from analysis because of improbably high or missing values for meat intake. As a result, 233 subjects (114 under and 119 over 30 years of age) were included in the simple linear regression analysis (Table 1) and multiple linear regression analysis (Table 2).

The regression of CALUX−TEQ on age indicated an increase of 0.44 pg/g fat and 0.47 pg/g fat, respectively, for mothers younger than 30 years and 30 years or older for every year of increase in the mother's age, after adjustment for other variables. Correlation between CALUX−TEQ and

TABLE 2. Regression Coefficients, 95% Confidence Intervals and *P*-values in *Multiple Linear Regression* of CALUX−TEQ Concentration of Mothers, Stratified by Age <30 years old (n = 114) and ≥30 years old (n = 119)

age <30 years old (n = 114)			
independent variable	regression coefficient	[95% CI]	*p*-value
age of mother[a]	0.44	[0.11−0.76]	0.009
place of birth			
other Mainland born	1.00		
Hong Kong born	1.90	[−0.28, 4.08]	0.087
Guangdong born	2.58	[0.27, 4.88]	0.029
BMI[a]	−0.44	[−0.76, −0.12]	0.008
non-primigravidae	−1.32	[−3.61, 0.98]	0.258
male baby	−2.71	[−4.44, −0.99]	0.002
ever smoker	−0.64	[−2.85, 1.58]	0.570
poultry consumption 3rd tertile	1.50	[−0.40, 3.41]	0.121

age ≥30 years old (n = 119)			
	regression coefficient	[95% CI]	*p*-value
age of mother[a]	0.47	[0.11, 0.83]	0.011
place of birth			
other Mainland born	1.00		
Hong Kong born	1.77	[−1.17, 4.71]	0.235
Guangdong born	3.81	[0.10, 7.52]	0.045
BMI[a]	0.57	[0.09, 1.05]	0.020
non-primigravidae	0.53	[−2.17, 3.23]	0.697
gestational weeks (weeks)[a]	0.38	[−0.35, 1.11]	0.300
seafood consumption 3rd Tertile	2.06	[−0.18, 4.30]	0.070
beef consumption 3rd tertile	−2.22	[−4.41, −0.03]	0.288
pork consumption 3rd tertile	−1.15	[−3.27, −0.03]	0.047

[a] As continuous variables.

the mothers' age showed an increasing trend in CALUX−TEQ from younger to older mothers ($p < 0.0005$; $r = 0.40$, $r^2 = 16\%$). The mean CALUX−TEQ concentration increased from 11.0 pg/g fat [95% CI: 8.3, 13.6] in the mothers aged 17−21 years, to 14.3 pg/g fat [13.3, 15.3] in mothers aged 27−31 years, and to 18.5 pg/g fat [15.6, 21.4] in mothers aged 37 years or above. (Figure 2)

In multiple linear regression the CALUX−TEQ was significantly higher in mothers born in Guangdong province (Figure 3A) and this relationship was consistent in both younger ($\beta = 2.58$, $p = 0.029$) and older ($\beta = 3.81$, $p = 0.045$) mothers. There was a significant negative relationship between BMI and CALUX−TEQ in mothers younger than 30 years old ($\beta = -0.44$, $p = 0.008$) but a weak nonlinear positive relationship in the older mothers ($\beta = 0.57$, $p = 0.02$) (Figure 3B). A lower TEQ concentration was observed in mothers with male babies compared to those with female babies in the younger age group ($\beta = -2.71$, $p = 0.002$), but not the older group (Figure 3C). The highest tertile of seafood consumption was associated with higher CALUX−TEQ, at a marginal level of significance in older mothers ($\beta = 2.06$, $p = 0.07$) but not the younger group. The lowest tertile of pork consumption was associated with higher CALUX−TEQ among the older mothers only ($\beta = -1.15$, $p = 0.047$) but there was no consistent trend across the tertiles of consumption. Consumption of poultry, eggs, dairy products, and beef were not significantly associated with CALUX−TEQ in either age group.

A higher CALUX−TEQ was observed in those mothers who had never smoked in the younger age group (Figure 3D), but after adjustment in the multiple linear regression this association disappeared. The variable for history of work exposure to dioxins was not included in the regression model due to the small number of mothers ($n = 8$) with possible occupational exposure. The mean CALUX−TEQ of these mothers was 13.4 pg CALUX−TEQ/g fat (ranging from 1.8 to 28.4). The CALUX−TEQ of four mothers who had been involved in dioxin-related industries (plastic manufacturing and bleaching) were comparable to their counterparts, except one with a level of 28.4 pg CALUX−TEQ/g fat. Household income, education attainment, breast-feeding duration, breast-feeding exclusivity, gestational age, and baby's birthweight were not associated with CALUX−TEQ concentrations in breast milk.

Discussion

The GC/MS measures concentrations of selected dioxin and dioxin-like compounds while CALUX bioassay detects all compounds acting as Ah-receptor agonists. The range of CALUX−TEQ determined in this study was within the range of those detected in 10 Hong Kong breast milk samples in a previous pilot study (*30*). To ensure the CALUX bioassay provided valid TEQ estimates for assessing the factors influencing dioxin body load in this study, milk samples were subjected to both GC/MS chemical analysis (as 11 pools of 12−34 samples) (*28*) and the CALUX bioassay of individual samples, testing the agreement of the bio-analytical determined TEQ (CALUX−TEQ) and the gold standard (WHO-TEQ). The mean value for WHO-TEQ obtained by GC/MS was 12.8 pg/g fat for the pools available for comparison with CALUX−TEQ. When compared to the WHO-TEQ, the CALUX−TEQ (mean: 14.5 pg/g fat) was very similar but with a higher estimate of dioxin activity for all but one of the pools, with 95% of the overestimation lying between 0.68 and 2.43 pg/g fat. The close agreement between CALUX−TEQ and GC/MS-determined WHO-TEQ, and the pattern of systematically higher values in our study, are consistent with the report by Laier et al. (*31*).

The duration of exposure, indicated by the mother's age, had a strong positive association with the dioxin levels estimated by CALUX bioassay. This association has been well-established in previous studies (*22*, *32*−*34*). Although dioxins and furans can be eliminated from the body through bile, urine, and feces (*35*), the half-lives of dioxins and PCBs are usually very long, ranging from 4 to 12 years for PCDDs and PCDFs (*36*) and 7−30 years for PCBs (*37*). Dioxins in the environment have declined since the peaks of the 1960s and so mothers born in the 1970s or earlier (aged 30 or above in this study) might have been exposed to higher concentrations of dioxins than mothers born later. Since primiparae in developed countries are anticipated to be older than those in developing countries, it will be important to consider age in the pooling strategy of future WHO exposure studies.

Birthplace was another important factor affecting CALUX−TEQ in Hong Kong mothers. Significant numbers of mothers in Hong Kong were born in mainland China, and this study was able to demonstrate variation in CALUX−TEQ by area and duration of domicile. The four mainland China immigrant pools in the WHO/EURO dioxin exposure study, which included 93% of the Guangdong mothers who participated in this study, had higher TEQ estimates by CALUX than by GC/MS, suggesting a higher level of unidentified assay-reactive chemicals other than dioxins and dioxin-like PCBs. The cleanup process for the CALUX bioassay using acid silica columns will remove compounds which react with acid, such as polyaromatic hydrocarbons, but not chlorinated or brominated aromatic hydrocarbons. The reason for the higher CALUX−TEQ concentrations in breast milk in Guangdong-born women is unknown but it could be related to exposures to the widespread practice of uncontrolled waste burning in this region (*38*), including computer components treated with flame retardants.

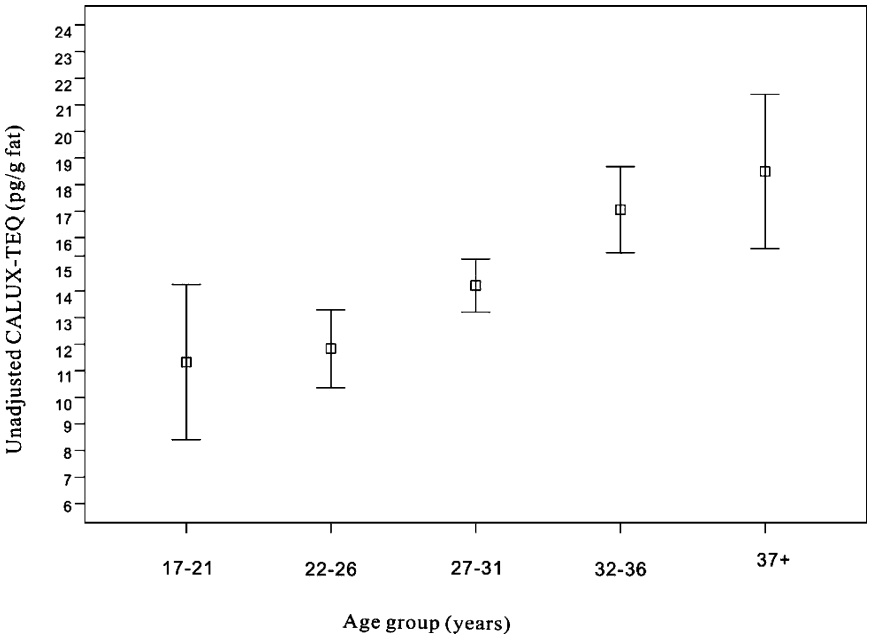

FIGURE 2. Mean unadjusted CALUX−TEQ (95% CI), by age groups ($n = 246$).

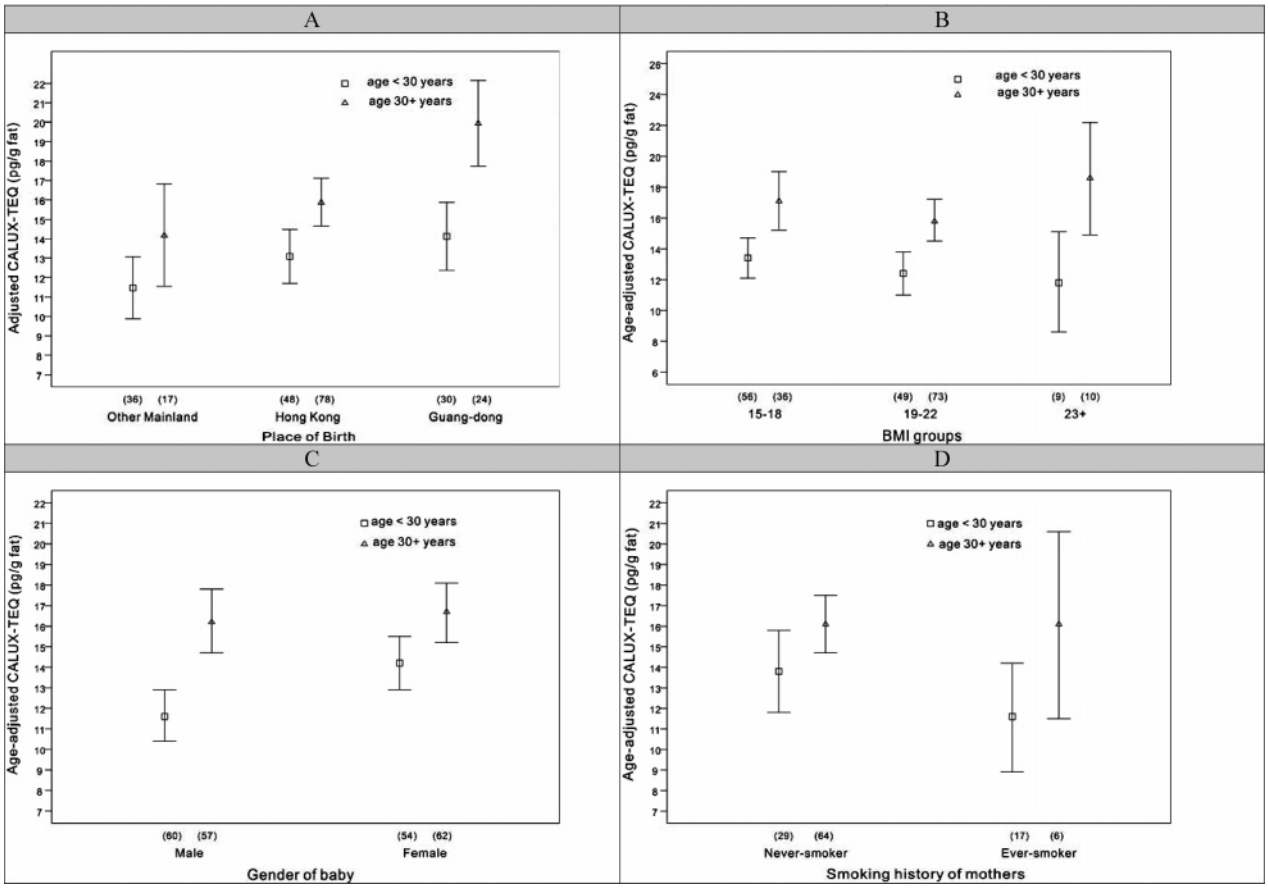

FIGURE 3. Mean age-adjusted CALUX−TEQ ±95% CI by (A) mother's place of birth, (B) mother's BMI, (C) baby's gender and (D) mother's smoking habits, stratified by age groups. (number of mothers stated in brackets).

It is estimated that more than 90% of the dioxin load in the human body is derived from dietary intake, with food of animal origin being the dominant source, but although body dioxin load is probably a result of prolonged dietary preferences, exact relationships are still unclear. A positive association between seafood intake and estimated dioxin body load was observed in this study and in others (*21*, *22*, *32*). In view of the worldwide downward trend of dioxin contamination in the environment, it is possible that current fish and seafood stocks might contain less dioxin, and that could explain the association of higher levels only in the older but not in the younger mothers. The negative relationship between pork consumption and CALUX−TEQ found in the older mothers is more difficult to explain because dioxin concentration in pork has been found to be relatively low compared to that in beef (*39*). The association may have

arisen by chance or as a result of an interaction with other dietary practices, such as increased cereal, fruit, and vegetable intakes, details of which were not included in the food frequency questionnaire. Beef and dairy products are important sources of dioxin in some western countries but were not related to dioxin levels in our study. This may reflect the Chinese diet, which is typically low in these foods.

A strong negative relationship between BMI and dioxin levels was found in younger mothers but a weak nonlinear positive relationship was found in older mothers. Previous studies have shown that the mother's postpartum weight was negatively associated with dioxin load (*22*, *34*). It has been suggested that an increase in body fat could result in a dilution of dioxins stored in fatty tissues, thereby lowering dioxin levels in breast milk (*34*). However, a previous report found concentrations of some congeners were significantly increased and others decreased in overweight mothers (*21*). BMI and body fat may not have a consistent relationship and increased food consumption could result in increases in both BMI and dioxin body load. Since body fat was not measured in the present study, an explanation for the association could not be determined.

Previous studies have reported a relationship between a lower male ratio in the offspring of fathers with previous exposure to dioxin during their teenage years (*23*). This association is consistent with reports of a deficit of male births following dioxin exposure of communities. The mechanism for this effect could be some form of endocrine disruption leading to an increased chance of a female conceptus either forming or surviving. However an association between having a female infant and a higher dioxin body load of the mother, as shown in the younger mothers (<30 years), has not been reported previously. The absence of any association between the sex of the baby and maternal dioxin levels in older mothers who had a higher dioxin body loads may indicate that dioxin levels alone are not the explanation. Another possible explanation for such an association in the younger mothers could reflect a trend in post-conception gender selection in earlier pregnancies in young Guangdong mothers, leading to depletion of dioxins through abortion.

Although dioxins are present in cigarette smoke (*40*), a lower PCDD/Fs level in active smokers (*41*) and a decreasing dioxin level with increasing tobacco consumption (*21*) have been shown previously, possibly due to a faster elimination rate of PCDD/Fs with tobacco smoking (*36*). Smoking mothers may have poorer diets and nutritional intake may be derived from less dioxin-rich foods. In our study, a higher CALUX−TEQ was observed in younger mothers who never smoked but this association disappeared after adjustment for age. It should be noted that only about 4% of all Hong Kong females smoke, and in ante-natal clinics the prevalence of ever smokers since conception is about 5% (*42*). Despite conflicting findings relating to smoking and dioxin body load (*4*), it may be important to control for smoking in future exposure studies, particularly in those populations with a higher prevalence of regular smoking among females.

We found no relationship between the CALUX−TEQ and either the breast-feeding exclusivity or the breast-feeding duration. This lack of association was possibly the result of collecting samples within a narrow range of 2−6 weeks postpartum. Household income and educational attainment of mothers were unrelated to dioxin body load.

This is the first study of dioxin and dioxin-like compounds using a bioassay method which produces comparable data to that obtained from GC/MS analysis, in the breast milk of a representative sample of individual mothers, in the rapidly developing region of the Pearl River Delta. It provides a baseline to determine future trends and potential targets for environmental controls. This survey demonstrates that the CALUX assay provides a potentially convenient and cost-effective method, as an alternative to GC/MS chemical analysis, to study variations in dioxin levels by potential risk factors in individuals and population sub-groups. However, the possibility of false positive results due to the presence of dioxin-like agonists in the bioassay needs to be considered. Our data suggest that, although age was the strongest and the most consistent factor affecting dioxin levels in humans in Hong Kong, there may be important geographic variations in dioxins, dioxin-like compounds, and other persistent organic pollutants as illustrated by the association with birthplace. Future studies will need to focus on the possible health impacts of environmental pollution in different areas of the Pearl River Delta region and the effects of interventions.

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Note Added after ASAP Publication

A production error resulted in omission of some corrections in the version published ASAP on February 1, 2006. The corrected version was published ASAP on February 14, 2006.

Abbreviations

PCDD	Polychlorinated dibenzo-para-dioxins/dioxins
PCDF	Polychlorinated dibenzofurans/furans
PCB	Polychlorinated biphenyls
WHO	World Health Organization
GC/MS	Gas chromatography with mass spectrometry
CALUX	Chemically activated luciferase expression
TEQ	Toxic equivalents
TCDD	2, 3, 7, 8 tetra-chlorodibenzo-para- dioxin
Ah	Aryl hydrocarbon
BMI	Body Mass Index

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