

## **Possible additional exposure to dioxin and dioxin-like compounds from waste incineration. Biomonitoring using human milk and animal samples.**

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### **Introduction**

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), collectively referred to as dioxins, are unwanted by-products in ultra trace amounts from many industrial and thermal processes<sup>1</sup>. Among the thermal sources, particularly municipal solid waste (MSW) incinerators, has been identified as a major source of dioxins during the 1980s and early 1990s<sup>2</sup>. In contrast to dioxins, polychlorinated biphenyls (PCBs) have been manufactured and widely used in industry from the early 1930s<sup>3</sup>. Although these compounds have been banned in most industrialized countries since the early 1980s, they are still entering the environment, mainly from recycling of old materials and volatilisation and leakage from landfills and sewage sludge<sup>4</sup>. MSW incineration has also been identified as producing PCBs<sup>5</sup>.

Owing to the diversity of sources, these halogenated aromatic compounds (some extremely toxic) are ubiquitous in the environment and have been found in almost every compartment of the global ecosystem, including human samples<sup>6, 7</sup>. Moreover, because PCDDs, PCDFs and PCBs are resistant to both biological and chemical degradation and have a lipophilic nature, these compounds bioaccumulate and sometimes biomagnify in the food chain, mainly in the lipids, increasing the potential hazard for human and animal health.

Human exposure to dioxins and dioxin-like PCBs for more than 90% occurs via the food chain, with eggs, milk, meat, fish and their products being the predominant sources<sup>8</sup>. Specific population groups, namely individuals with higher consumption of animal lipids and breast-fed children, can be highly exposed and therefore at higher risk to the adverse health effects associated with dioxins.

Relating dioxin exposure with health effects requires quantitative data relative to the “internal dose” of these compounds or, if possible, their “biologically effective dose”. For PCDDs, PCDFs and PCBs, human blood or easier to collect breast milk (shown to be significant biomarkers of exposure to these compounds) contain enough lipids to quantify internal dose with analytical procedures<sup>9-12</sup>. Levels of dioxins and dioxin-like compounds in breast milk reflect the cumulative exposure of a specific demographic segment (women at reproductive age and breastfeeding) for a short period within individual lifespan (breastfeeding period). They can be used to provide information on dioxin exposure of the general population, when in biomonitoring programs, to evaluate temporal and spatial trends.

In the ambit of an Environmental Health Survey Program relative to a MSW facility, which has been operating near to Lisbon since 1999<sup>13</sup>, a biomonitoring study using human breast milk has been performed. Specific aims of this study were: 1) determine whether living in the vicinity of the incinerator increases dioxin maternal body burden and accordingly perinatal (intra-uterus and lactacional) exposure; 2) to investigate the possibility of increased human exposure to dioxins and dioxin-like compounds via locally produced food items from animal origin. Therefore, levels of dioxins and dioxin-like compounds have been determined in human milk samples collected in the vicinity of the incinerator and in a control area, for comparison. From the same areas, cow and sheep milk and eggs from free-range chickens have also been collected to get an indication of possible local additional exposure to air-borne dioxins via the food chain.

Analyses of TCDD-equivalents (TEQs) were mainly performed with a reporter gene assay for dioxin-like activity, the DR-CALUX bioassay (Dioxin Responsive Chemical Activated LUciferase gene eXpression)<sup>14</sup>. To determine congeners profile, some human milk samples have also been analysed for PCDD/Fs and relevant dioxin-like PCBs, by using high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS). Both the Ethics Committees of the Faculty of Medicine, University of Lisbon, and of the Maternity Dr. Alfredo da Costa have approved the study protocol.

## Materials and methods

**Study group:** Apparently healthy pregnant women, non-occupationally exposed to dioxins, primiparous and/or breast-feeding first child or, at least, 3 years after breast-feeding the last child, living at residing area for more than 1 year, volunteered to participate in the biomonitoring study giving written informed consent. Study group included 49 volunteers, 31 living at a distance less than 5 km from the incineration facility and, as controls, 18 living far from the plant for more than 5 km, but as much as possible similar to those from the exposed area in relevant socio-demographic characteristics, in order to avoid between-group bias.

**Sample and data collection:** From the study group, all women who were still breast-feeding 30 days after delivery, gave breast milk samples, which have been collected during a visit to the women residence four weeks after delivery. For gathering relevant information not only on study participants (for example, age, residence, parity, smoking habits, use of medicines, dietary information on recent fat intake, occupational exposure), but also on their newborns, a questionnaire was applied. Complementary information was obtained from the mothers and newborns records at the Maternity Dr. Alfredo da Costa in Lisbon, also after informed consent given by the women.

From both the exposed and control areas, samples of locally produced food items of animal origin were collected, in total 6 mixed milk samples (corresponding 3 to more than 300 cows and another 3 to almost 500 sheep) and 8 sets of eggs from adult free-range chickens. Relevant information for animal samples, such as type of animal diet, was registered during collection time.

**Analytical procedures:** After lipid extraction and clean-up procedures, aliquots of about 2 ml from each sample have been analysed by the DR-CALUX bioassay, performed at the Toxicology Department of Wageningen University. Details of the procedures are described elsewhere<sup>14</sup>.

Analysis via HRGC/HRMS was performed by ERGO, Hamburg, Germany<sup>15</sup>, for the determination of PCDD/PCDFs and the non-ortho PCBs 81, 77, 126 and 169 in 41 human milk samples (27 and 14 from the exposed and control groups, respectively). Only 12 samples could be analysed for the mono-ortho PCBs 105, 114, 118, 123, 156, 157, 167 and 189. PCDD/F and PCB-levels were converted into TEQ-levels using WHO-TEFs<sup>16</sup>.

**Statistical analysis:** Data base management was performed using Microsoft Access 2000 (9.0.3821 SR-1) and, for the statistical analyses, SPSS software version 12.0 for Windows was used. Significance level was generally fixed at  $\alpha=0,05$ . Numerical variables were described by their arithmetic means and 95% confidence intervals or medians, percentage of results above them and variation intervals. Appropriate tests (t-Student, Mann-Whitney, Chi-square and Fisher exact) were used to compare means, medians and proportions across the two areas of residence and between age and other relevant related groups. Single and subsequent multiple linear regression analyses were used to identify determinants of dioxins body burden among individual characteristics and environmental factors.

## Results and discussion

**Study group:** In relation to the specific living area of the 49 participants, differences in the studied variables such as age, main professional activity, relevant dietary and smoking habits were not statistically significant. Mean age was about  $30\pm6$  years within a range of variation from 18 to 42 years. These findings led to the conclusion that the results to be obtained from the study are not likely to be confounded by a selection bias.

**Dioxin-like compounds in human milk:** CALUX-TEQ values in breast milk in the exposed-group ranged from 6 to 76 pg TEQs/g fat (mean  $35\pm19$  pg TEQs/g fat), which was slightly but significantly ( $p = 0,029$ ) higher than that from the control-area group ( $25\pm22$  pg TEQs/g fat, within a range from 6 to 81 pg TEQs/g fat). Two identified outliers, defined as three times the standard deviation of the global set of results, have not been included in the present analysis.

**Age-dependence of breast milk CALUX-TEQs:** Results for CALUX-TEQs in breast milk have shown a weak positive, but not statistically significant association with maternal age ( $r = 0,133$ ;  $p = 0,373$ ).

**Other determinants of breast milk dioxin-like compounds:** Several other personal and environmental variables (namely professional activity and hobbies considered risky for higher dioxin exposure, time of residence in the area – being, on average, of 13 and 10 years for control and exposed, respectively –, as well as present or past smoking habits, dietary pattern relative to frequency of fruit and vegetables, and preferential consumption of meat or fish) have been analysed for contribution to the concentration of CALUX-TEQs in breast milk. When adequate, single and multiple regression analysis have been used. To decide on variables to enter and stay in the multiple model, p-value was set at 0,10 and missing values lesser than 1%.

In single regression, living in exposed versus control area was the only factor with p-value lower than 0,10. However, due to its intrinsic interest, age was also included in the multiple model.

From the regression analysis it has been confirmed that living area and age were significant factors influencing CALUX-TEQ levels. Breast milk levels of dioxin-like compounds were about 12 pg CALUX-TEQs/g lipid higher for exposed women than for controls and an increase of 0,8 pg CALUX-TEQs/g lipid per year of aging was also evident.

**PCDD/PCDFs and dioxin-like PCBs in human milk:** In contrast to results obtained from DR-CALUX, no statistically significant differences were found for levels of PCDD/Fs and dioxin-like PCBs in breast milk collected from both study areas. Therefore, concerning PCDD/Fs, the whole group could be characterized by a mean level of  $10,5 \pm 3,6$  pg/g WHO-TEQ/g fat, spread over a range from 4,4 to 20,9 pg/g WHO-TEQ/g fat. For non-ortho and mono-ortho PCBs, mean values of  $4,6 \pm 2,3$  (1,7-12,8) and  $2,8 \pm 1,7$  (0,8-6,7) pg/g WHO-TEQ/g fat, respectively were found.

**Congener profile of PCDD/Fs and PCBs in human milk:** The profile of the single congeners for PCDD/Fs and PCBs was quite similar to those generally observed in industrialized countries<sup>17</sup>. Most contributors to the TEQs were, by descending order, non-ortho PCB 126, 12378-PCDD, 23478-PCDF, 123678-HCDD, mono-ortho PCB 156, 2378-TCDD, and mono-ortho PCB-118. Altogether, these individual congeners accounted for 85% of the total identified dioxin and dioxin-like body burden in the studied group. The non-ortho PCB 126 alone was responsible for almost 25% of this total TEQ.

**Comparison of CALUX-based and GC/MS-derived TEQ levels:** Results from CALUX-TEQ levels in human milk samples and chemically measured WHO-TEQs were slightly positively associated, as shown in Figure 1. However, correlations were statistically significant only for PCDD/Fs ( $r = 0,436$ ;  $p = 0,005$ ) and "PCDD/Fs + Non-ortho PCBs" ( $r = 0,412$ ;  $p = 0,008$ ), being Spearman correlation coefficient for "PCDD/Fs + Non-ortho PCBs + Mono-ortho PCBs" equal to 0,343, with p-value of 0,276. The gradual decrease of the regression coefficient for increasing number of dioxin-like compounds considered in the model seems to confirm that DR-CALUX bioassay is able to detect other eventual compounds present in the milk samples with dioxin-like activity rather than PCDD/Fs and a few dioxin-like PCBs. However, the results from the present analysis are not able to show the usual better correlation between GC-MS TEQs and CALUX-TEQs documented by other authors<sup>14, 18</sup> when a more elaborated number of PCBs is analysed.

Although different methods for lipid-extraction were applied, fat extracted from samples used for determination of CALUX-TEQ levels in human milk (mean level  $3,6 \pm 1,2$  g fat/100 g) was strongly correlated ( $r = 0,942$ ;  $p < 0,001$ ) with fat content of the same samples ( $3,2 \pm 1,2$  g fat/100 g sample) prepared for chemical analysis.

**Dioxin-like compounds in samples from animal origin:** 4 sets of 2 eggs each and 4 individual eggs, collected from both study areas, have shown a mean (median) value of  $13 \pm 7$  (12) pg TEQ/g fat, within a range from 8 to 28 pg TEQs/g fat. Mixed milk samples collected from animals grazing at the area of control have shown very different mean value when compared with samples collected in exposed area. However, due to the limited number of samples, a global average (median) value of  $7 \pm 8$  (5) pg TEQ/g fat, spread over a range of 2 to 22 pg TEQ/g fat, has been considered to characterize the collected animal milk samples.

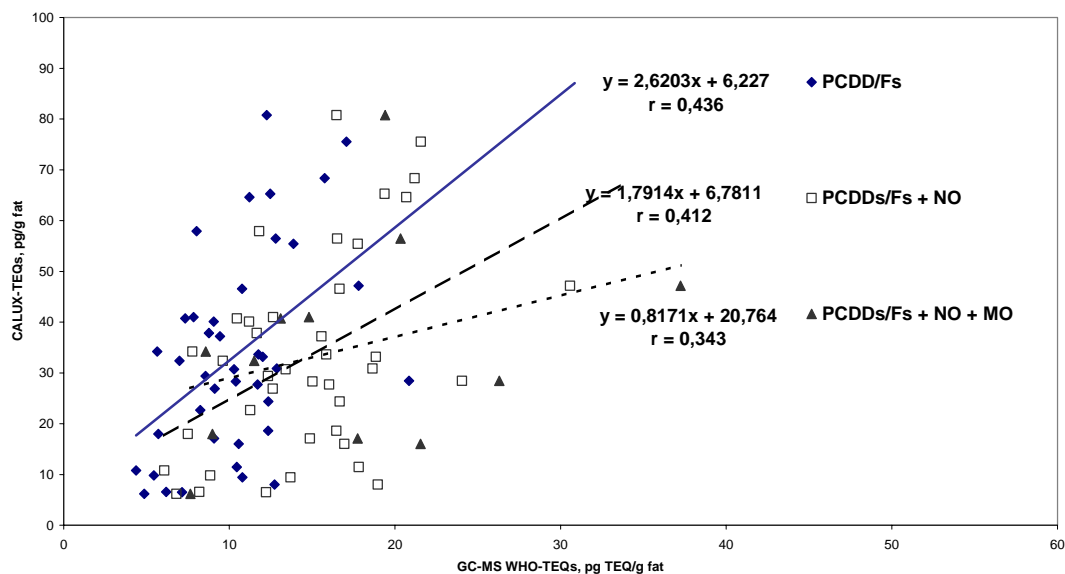


Figure 1 – Comparison of CALUX based and GC-MS derived TEQ levels

These results suggest that CALUX-TEQs in locally produced food from animal origin, mainly in eggs from free-range chickens, do reflect relatively high environmental contamination, very similar in both areas and, not attributable to any specific source. Having into consideration an expected contribution of no more than 50% from PCDDs and PCDFs for the total sample TEQ<sup>19</sup>, results found on average still exceed the maximum of 3 pg WHO-PCDD/F-TEQ/g proposed by the EU<sup>20</sup> for eggs, milk and their products. In conclusion, it might be expected that habitual consumption of these food items may exceed the acceptable daily intake of TEQs depending on pattern of consumption.

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