First results from dioxins and dioxin-like compounds in the population from Madeira Island, Portugal. Part 2 - Biomonitoring in breast milk of women living near to a solid waste incinerator

M. Fatima Reis[1], Carla Sampaio[1], J. Mauricio Melim[2], J. Pereira Miguel[1]

[1]Institute of Preventive Medicine, Lisbon
[2]Public Health Regional Department, Funchal

Introduction

Measuring internal dose of chemicals rather than exposed dose, biomonitoring accounts for exposure from all sources, pathways and routes of absorption. Therefore, it has several advantages over environmental monitoring for large-scale survey programs, when objective is to monitor exposure to relevant chemicals and to determine spatial and temporal trends of human exposure to those chemicals.

Breast milk, as well as adipose tissue and blood or its components, has been widely used in biomonitoring programs[1, 2] to assess human exposure to dioxins and dioxin-like compounds. The WHO European Centre for Environment and Health has conducted several studies[3] in breast milk in countries worldwide, designed to assess levels and changes in levels of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and certain polychlorinated biphenyls (PCBs) known to have "dioxin-like" properties.

Although breast-feeding women cannot be representative of the general population, for biomonitoring programs carried out to ultimately assist in policy design to improve public health and safety, it is important to monitor dioxin exposure of this demographic segment through breast milk due to several reasons: 1) breast milk reflects the maternal body burden of lipophilic chemicals and thus it is a measure of prenatal exposure to those compounds; 2) being a human food and the first and main foodstuff for most newborn babies during first lifetime-period, breast milk can be a very significant pathway for infant exposure to dioxins; 3) because large volumes can be collected non-invasively, breast milk is also a convenient sampling specimen for biomonitoring purposes if it is collected taking into consideration all the relevant factors influencing fat content and thus levels of lipophilic compounds, namely the time of sampling during lactation, breast-feeding patterns and maternal characteristics.

As part of an Environmental Health Survey Program[4], relative to an updated incinerator at Meia Serra, Madeira Island, Portugal, dioxin breast milk levels have been determined to provide indicative data on the extent and pattern of exposure of the general population to dioxins and dioxin-like compounds and to investigate potential determinants of dioxin exposure in age reproductive women for prevention priorities. The ultimate objective was to determine whether living in the vicinity of the incinerator increases the maternal dioxin

exposure and accordingly their breast-fed infants. Data will also be collected longitudinally in order to provide information on temporal trends in breast milk dioxin levels, which will indicate whether controls on sources of these pollutants are effective.

Materials and methods

Study group: **122 apparently healthy pregnant women, not known occupationally exposed to dioxins, primiparous and/or breast-feeding first child or, at least, 3 years after breast-feeding the last child, volunteered to participate in the study giving written informed consent. Study group included 42 individuals living in the small town of Camacha or its surroundings, at a distance less than 3 km from the incineration facility. As controls, another group of 80 volunteers has been recruited among the residents at Estreito-Jardim da Serra, living far from the plant for more than 20 km, but as much as possible similar to those from Camacha in relevant socio-demographic characteristics, in order to avoid between-group bias.**

Sample and data collection: **From the study group, all women 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 Maternidade Dr. Alfredo da Costa in Lisbon, also after informed consent given by the women. Both the Ethics Committees of the Faculty of Medicine, University of Lisbon, and of the Maternidade Dr. Alfredo da Costa have approved the study protocol.**

Analytical procedures: **In total, 58 breast milk samples, 22 from exposed women and 36 from controls, were already analysed, via high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS, ERGO, Hamburg, Germany), for the determination of levels and congener profiles of PCDD/PCDFs and mono-ortho and non-ortho PCBs. For every congener, in addition to concentration value in pg/g, lipid based, calculation of the toxicity equivalent (TEQ) according to the WHO-system was carried out and reported by the ERGO Laboratory. Concentrations below the detection limit were set to one detection limit for calculation of WHO-TEQs.**

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 α=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 58 participants, differences in the studied variables such as age, parity, relevant dietary habits and main professional activity were not statistically significant. Mean age was about 30±6 years within a range of variation from 20 to 52 years and number of primiparous is similar in exposed and control areas. 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.**

Dioxins and PCBs in human milk: **No statistically significant differences were observed for the levels of breast milk dioxin and dioxin-like PCBs in relation to the specific living area of the 58 participants. For the whole group, dioxin levels, calculated as WHO toxicity equivalents and measured by PCDD/Fs in human milk, showed distribution close to normal, with a mean of 6,5±2,4 pg/g WHO-TEQ/g fat, within a range from 2,8 to 13,7 pg/g WHO-TEQ/g fat.**

Relatively to mono-ortho and non-ortho PCBs, a mean value of 11,8±7,2 pg/g WHO-TEQ/g fat was found, spread over the range 3,1 to 32,4 pg/g WHO-TEQ/g fat. Therefore, in general terms, exposure to dioxin and dioxin-like compounds determined in breast milk for this population in the first phase of the monitoring process was characterized by a slightly asymmetric distribution (C_{as}=1,2) with a mean value of 18,3±8,9 pg/g WHO-TEQ/g fat, within a range from 6,1 to 43,9 pg/g WHO-TEQ/g fat.

Age-dependence of breast milk dioxin and dioxin-like PCB levels: **The results for total TEQs in breast milk were positively associated with age of women, showing very significant correlations for the total population (r = 0,460; p<0,001) and regional-specific groups, with r=0,698 and p<0,001 for exposed participants and r=0,406 and p=0,014, for controls. When the women were stratified by parity, positive associations with age were also observed (r=0,396; p=0,041 and r=0,368; p=0,041) for primiparous and multiparous, respectively. From these associations it could be seen that including multiparous in the study, even those with last breast-fed child for more than three years, can modify the relationship between dioxin body burden and age.**

Other determinants of breast milk dioxin and PCB levels: **To investigate possible influencing factors on dioxin body burden as determined in breast milk, besides age, parity and living area of the subjects, several other personal and environmental variables (namely professional activity and hobbies considered risky for higher exposure, present or past smoking habits, intake frequencies of fruit and vegetables, and preferential consumption of meat or fish) have been studied using single and multiple regression analysis. 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%.**

From the regression analysis no additional determinants have been identified, having age and living area been confirmed as the only significant factors influencing dioxin body burden. The breast milk levels of PCDD/Fs and PCBs were about 5 WHO-TEQs higher for controls compared with residents living in the vicinity of the plant under study. Living in Estreito-Jardim da Serra proved to be one of the strongest factors influencing dioxin body burden of the studied population.

A significant age-dependent trend towards higher body burden of dioxins in aged subjects (increase of 0,6 WHO-TEQs per year) was also evident from the multiple regression model.

Congener profile of PCDD/Fs and PCBs in human milk: **The profile of the single congeners for PCDD/Fs and PCBs (Fig. 1) was similar to those generally observed in industrialized countries[2]. Most contributors to the toxicity were, by descending order, non-ortho PCB 126, mono-ortho PCB 156, 12378-PCDD, mono-ortho PCB-118, 23478-PCDF, 2378-TCDD and 123678-HCDD. Altogether, these individual congeners accounted for 86% of the total identified dioxin and dioxin-like body burden in the studied group. The non-ortho PCB 126 alone was responsible for more than 30% of this total toxicity.**

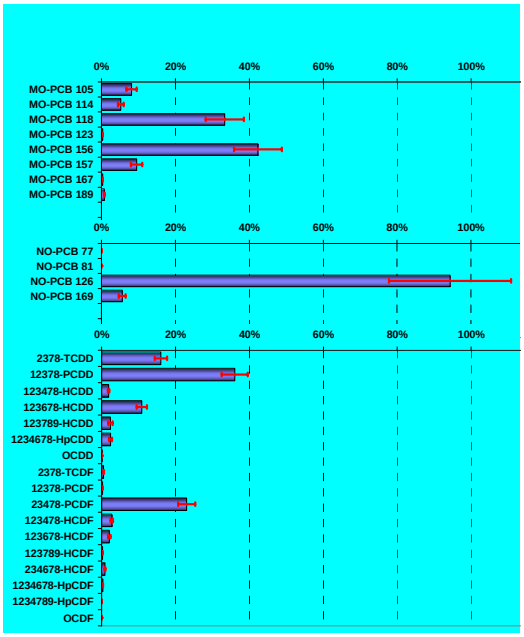

Fig. 1 – Congener profile of PCDD/Fs and PCBs in human milk

Infant exposure to PCDD/Fs and PCBs through daily intake of human milk: **Based on the assumptions of mean infant body weight (BW) of 5 kg, mean milk daily intake of 800 ml and a breast milk fat content of 3%, mean daily intake of PCDD/Fs through breast milk can be estimated as 28 pg/kg BW/day for the breast-feeding babies of the population under study, which is almost half of the mean value recently found for a similar Portuguese population[5] (Fig. 2 [2, 6-10]).**

Conclusions

Due to the reduced dioxin body burden found, as well as the good agreement of the congener profile with those reported for breast milk in similar populations and conditions, one might conclude that no additional health hazard for the residents in the vicinity of the Meia Serra incinerator may be derived from the present results. Observed trend for higher levels in regions farther from Meia Serra should be investigated.

Being representative of background exposure, as suggested by the analysis of congener profile for the global area under study, results from the present work were compared with results from relatively recent studies carried out in similar conditions and populations, either in the country or abroad. From Fig. 3[9-15], it could be concluded that observed levels for PCDD/Fs in this population were the lowest in the range defined by those studies.

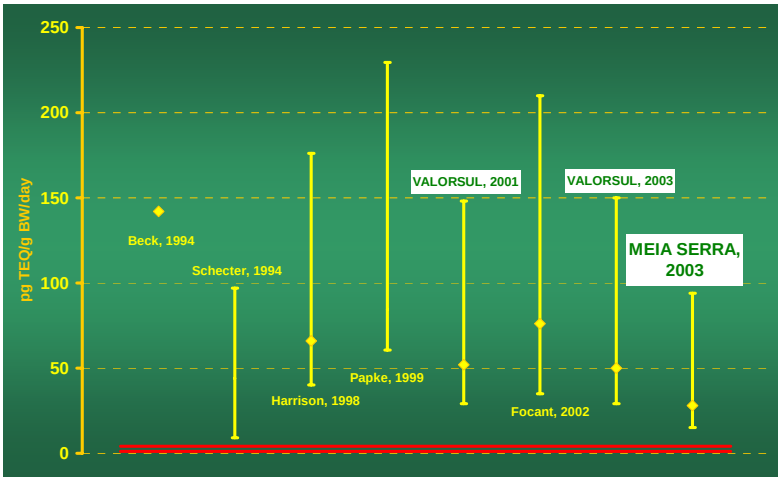

Fig. 2 – Infant exposure to PCDD/Fs and PCBs through daily intake of human milk

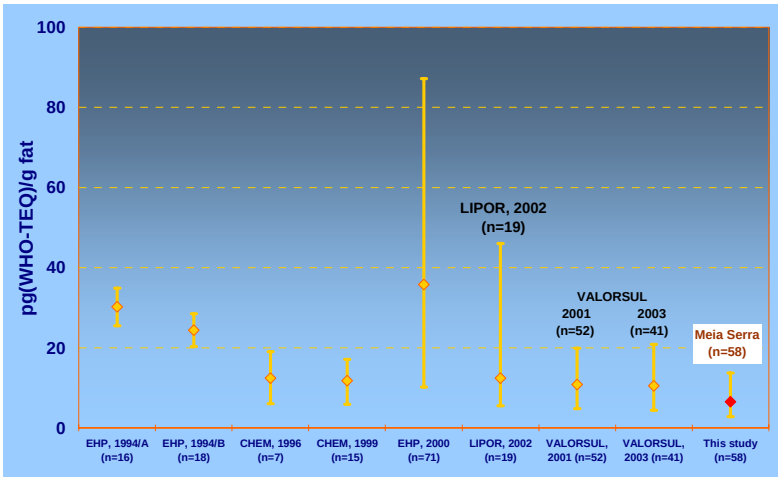

Fig. 3 – PCDD/Fs levels in Portuguese breast milk samples compared with published background levels

Although the estimated daily intake of PCDD/Fs is much higher than the WHO recommended threshold value, breast-feeding should continue to be encouraged in this population considering duration of lactation in comparison with lifespan and actual breast-feeding patterns for the one-month-old babies studied under the present survey.

From a public health perspective, continuous surveillance of dioxin body burden through monitoring of breast milk should be conducted in order to ensure, by a suitable and not very expensive way, at least maintenance of the present patterns.

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