SIX YEARS OF CONSECUTIVE HUMAN BIOMONITORING ADDRESSED TO DIOXIN EXPOSURE AS DETERMINED BY PCDD/PCDF LEVELS IN HUMAN MILK

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Introduction

Dioxins and dioxin-like compounds are persistent organic pollutants that may bioaccumulate in human bodies, mainly in the lipids, increasing the potential for human health hazards. These toxic compounds are produced in a wide range of industrial and thermal processes, being emissions from municipal solid waste (MSW) incineration one of the most relevant sources of the pollutants[1]. As part of an Environmental Health Surveillance Program relative to a modern MSW incinerator operating in Lisbon[2], a human biomonitoring project focused on regular determinations of dioxins and dioxin-like compounds levels in milk samples has been implemented on the population residing in the vicinity of this facility. Main objectives of this project are: 1) to determine whether living in the potentially exposed area (near to the VALORSUL incinerator) increases maternal body burden and accordingly perinatal exposure to the pollutants; 2) to monitor spatial and temporal trends of human exposure to this kind of compounds.
The present paper shows detailed results of dioxins in human milk collected during the evaluation of the third potential specific impact of the MSW incinerator under study relatively to the established baseline. Temporal trend based on present and previous results – namely baseline levels (reference period, 1999), as well as first (2001) and second (2003) evaluations published elsewhere[3] – is also investigated and conclusions are drawn in relation to health hazard for the residents in the vicinity of the incinerator.

Materials and methods

Study group: Pregnant women, apparently healthy, non-occupationally exposed to dioxins, primiparous and/or breastfeeding first child or, at least, 3 years after breastfeeding the last child, living at residing area for more than 1 year, volunteered to participate in the biomonitoring study after giving written informed consent. Participants were classified as “exposed” if they lived and/or worked within a 5 km distance of the MSW incinerator and as “controls” if they lived at a greater distance from the facility. Volunteers from both study groups were as much as possible similar in relevant socio-demographic characteristics in order to avoid between-group bias.

Sample and data collection: From the study group, milk samples from 30 women (15 from each study area) who were still breastfeeding four weeks after delivery have been collected under adequate protocol during a visit to the women residence. For gathering relevant information on study participants (for example age, residence, parity, smoking habits, use of medicines, occupational exposure), and also on their newborns, a questionnaire was applied. Complementary information was obtained from mother and newborn records at Maternity Dr. Alfredo da Costa, in Lisbon, also after informed consent given by the women. Both the Ethics Committees of the Faculty of Medicine, Lisbon University, and of the Maternity Dr. Alfredo da Costa have approved the study protocol.

Analytical procedures: As for previous observations, to determine dioxin levels and congener profiles, collected milk samples have been analyzed for the determination of PCDDs and PCDFs via high-resolution gas chromatography and mass spectrometry (HRGC/HRMS) at ERGO Laboratory, Germany, and PCDD/F levels were converted into TEQ-levels using WHO-TEFs[4]. Details of the procedures are described elsewhere[5].

Statistical analyses: 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, Fisher Exact and Kruskal-Wallis) were used to compare means, medians and proportions across the two areas of residence, between age and other relevant related groups, as well as along time. Single and 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 specific living area, the exposed and control groups seem to be relatively homogeneous since differences in studied variables such as age, occupational exposure, and relevant dietary and smoking habits were not statistically significant. These findings led to the conclusion that results are not likely to be confounded by a selection bias. For the global study population of the present observation, mean and median age values were 32 years, within a range of 23 to 41.

Levels of PCDD/Fs in human milk: In present investigation and similarly to what has been seen in previous monitoring periods, no statistically significant differences were observed between both study groups for the levels of dioxins in human milk (Table 1). Confirming conclusions from previous studies[3, 6, 7], these results suggest that dioxin emissions from the incinerator under study seem to be controlled, at least in what it is possible to verify from these six consecutive years of observation.
For the whole population, PCDD/F levels for the most recent observation ranged from 2.6 to 16.6 pg TEQ-WHO/g fat with a mean value of 9.0 ± 3.2 pg TEQ-WHO/g fat. Although PCDD/F levels did not change significantly over time (p=0.095), it seems that these levels are decreasing along the whole monitoring period, suggesting that, in particular for the population living near to the incinerator under study, there is a consistent trend for reduction of the environmental exposure to dioxins as estimated by PCDD/F concentrations on human milk (Table 1).

Congener's profile of PCDD/Fs in human milk: The profile of the single congeners for PCDD/Fs was quite similar to those observed during the baseline and subsequent observational periods, as well as in other industrialized countries[2, 3, 8]. From the 17 PCDD/Fs analyzed, the most significant contributors to TEQs in human milk were 2378-TCDD, 12378-PCDD, 123678-HCDD and 23478-PCDF, accounting for approximately 83% of the total toxicity (Figure 1).

Other determinants of breast milk dioxin-like compounds: For all the participants enrolled in the study in any of the four observations, several environmental and personal variables have been studied using single and multiple regression analysis to investigate possible influencing factors on dioxins in human milk of study population. Criteria for variables selection in single and multiple regressions were relevance for study objectives and missing information lesser than 10% for each variable. According to single regression analysis (Table 2), the positive association of PCDD/F levels in human milk with age of the subjects is the only statistically significant. When variables were included in multiple model, a significant age-dependent trend towards higher body burden of dioxins in aged subjects (increase of 0.26 WHO-TEQs per year) was again evident. Except for study period in T3, associations with the remaining tested variables were not significant confirming the relative homogeneity between groups. Age proved to be one of the strongest factors determining dioxin body burden of the studied population and a trend for decreasing levels of PCDD/Fs with time is evident, being significantly lower in T3. Altogether investigated variables could not explain more than 20.2% of the dioxin body burden variation. In summary, results of six years of investigation of the population living in the area of influence of the VALORSUL incinerator have shown that: i) living area was not a statistically important factor influencing PCDD/F levels in human milk, suggesting similar dioxin body burden for exposed and controls; ii) there is a good agreement of the congener profile with those reported for background exposure of similar populations and conditions, meaning that PCDD/F levels in human milk of the study population can be considered as environmental not specific exposure to dioxins; iii) there is a trend for lower levels of PCDD/Fs along time of observation, more significant in recent years, which points to a decreasing environmental exposure to dioxins of the population under study. Therefore, conclusion can be drawn that no additional health hazard for the residents near to the incinerator may be derived from these results.

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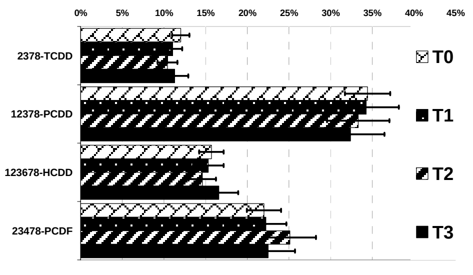

Figure 1 – Pattern for PCDD/Fs congeners in human milk

Table 1 – PCDD/F levels in human milk in relation to study period, living area, and age of mothers

Factor	Group	TEQ-WHO (pg/g fat)	T0[a]	T1[b]	T2[c]	T3[d]	
Living area	Exposed	N	9	27	15	15	
		Mean	10.4	10.9	10.9	8.8	
		Median	9.4	10.4	11.0	8.7	
		Standard deviation	3.4	4.1	2.9	2.7	
		Min.-Max.	5.7-16.2	4.4-20.9	5.9-15.6	4.5-13.6	
	Control	N	43	14	15	15	
		Mean	10.9	9.8	10.2	9.2	
		Median	10.8	10.5	10.4	9.2	
		Standard deviation	2.9	2.5	3.0	3.5	
		Min.-Max	4.8-19.9	5.4-12.7	5.5-16.9	2.6-16.6	
		p [e]	0.536	0.541	0.512	0.744	
	Global population	N	52	41	30	30	**p**[f] = 0.095
		Mean	10.8	10.5	10.5	9.0	
		Median	10.8	10.5	10.7	9.1	
		Standard deviation	2.9	3.6	2.9	3.1	
		Min.-Max	4.8-19.9	4.4-20.9	5.5-16.9	2.6-16.6	
Age (years)		**R^g (p)**	0.45 (**0.001**)	0.43 (**0.005**)	0.56 (**0.001**)	0.38 (**0.036**)	

a, b, c, d – 1st (T0), 2nd (T1), 3rd (T2) and 4th (T3) study periods, respectively; e – Mann-Whitney test; f – Kruskal-Wallis test; g – Spearman coefficient correlation

Table 2 – Single/Multiple linear regression for PCDD/F levels in human milk

Co-variables	n[a]	Single linear regression			Multiple linear regression		
		B[b]	R^2 (%)[c]	p-value[d]	B[b]	R^2 Partial (%)[c]	p-value[d]
Area (Exposed)	153	0.031	0.0	0.953	0.678	1.1	0.211
Age -Years	153	0.196	11.6	**<0.001**	0.256	15.0	**<0.001**
Parity (Primiparous)	149	-0.407	0.4	0.444	0.616	0.8	0.282
Study period (T0)							
T1	153				-0.935	1.3	0.174
T2	153				-0.959	1.2	0.184
T3	153				-2.631	8.4	**<0.001**
					R^2_{model} [e]= 20.2% (p < 0.001)		

a – Number of research units; b – Coefficient of regression: variation of PCDD/Fs in breast milk per unit of co-variable or in relation to reference; c – Explained variation; d – Probability that association is explained by chance; e – Percentage of variance explained by multiple regression model.