First results from dioxins and dioxin-like compounds in the population from Madeira Island, Portugal. Part 1 - Biomonitoring in blood of the general population living near to a solid waste incinerator

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Introduction

Dioxins and dioxin-like compounds are among the most toxic environmental pollutants that can be produced through incineration of municipal or hazardous waste. Studies of human exposure to this kind of compounds have shown dioxin blood and breast milk levels as significant biomarkers of exposure [1-5]. There is limited information on the dioxin body burden of the general population or specific groups in Portugal. Reis *et al*[6] and Calheiros *et al*[7] reported levels of PCDD/Fs in whole blood and breast milk from residents near to municipal solid waste incinerators in the region of Lisbon and Oporto, respectively. In relation to suchlike facilities, similar data are of utmost relevance in environmental health.

In Madeira Island, an old incinerator at Meia Serra has been updated and, through modern technology, recommenced operating (June, 2002) to incinerate solid waste, either municipal, or from hospital or slaughterhouse. The Regional Ministry of Environment and Natural Resources of the Autonomous Region of Madeira invited the Institute of Preventive Medicine to develop an Environmental Health Survey Program relative to the population living in the vicinity of the plant. The main goal of the Program is to monitor magnitude as well as spatial and temporal trends of specific indicators of both exposure to the environmental agents of concern and potential adverse health effects in subjects living in the area under influence of the facility.

Within the scope of this Environmental Health Survey Program, several human biomonitoring projects have therefore been started, mainly focussed in heavy metals and dioxins and dioxin-like compounds. In a first phase, baseline levels for human exposure to those pollutants will be established through their determination in blood from subjects living in the area under potential influence of the facility and, for comparison, from individuals with farther place of residence and work. Measurements will be repeated periodically in order to monitor space and time trends. Analysis of results will be made not only in terms of proximity of plant, but also of sex, age and other relevant socio-demographic, life style and general health factors. The protocol of the survey was approved by the Ethics Committee of the Lisbon Faculty of Medicine and has been published in detail elsewhere[8].

The present study is one of a series of papers describing selected results of the ongoing projects, designed to ultimately evaluate the potential impact on public health of the updated solid waste incinerator. Addressing dioxins and dioxin-like compounds, specific aims of this study were: i) to determine whether living in the vicinity of the Meia Serra incinerator increases the dioxin body burden of the general population; ii) to investigate other potential determinants of dioxin exposure in this population for prevention priorities; iii) to provide data on the extent and pattern of exposure of the general population to dioxins and dioxin-like compounds by determining respective toxicity levels and congeners profile in blood samples.

Materials and methods

Study group: In total, 200 apparently healthy adults, not-known occupationally exposed to dioxins, volunteered to participate in the study, after written informed consent was given. Study group included 100 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 100 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 each fasting participant, nurses from both local Health Centres (Camacha and Estreito-Jardim da Serra) collected a venous blood sample (about 35 ml), which was divided into different tubes, “dry” or prepared to prevent coagulation, in order to obtain whole blood and serum samples for heavy metal and dioxin determinations, as well as for tests such as platelet counts, and haematological and biochemistry studies. Immediately after sample delivery to the Regional Public Health Laboratory, part of the whole blood tubes was frozen to further analysis of heavy metals. From the remaining, serum has been separated and kept frozen until analysis of the heavy metals requiring this type of matrix and also for the determination of exposure to dioxin-like compounds. The 110 (55 from each group of sex and age paired participants) out of 200 blood samples collected during this first phase of the Program were subsequently analysed for the determination of heavy metal and dioxin levels. Because of the repeated cross-sectional type of the survey, the remaining samples are kept frozen, in order to habilitate to adequate substitution of “follow-up” losses, in a cost-effective way. By the end of the present survey, all the still existing coherent sets of samples will be analysed, if necessary.

Due to the large volume blood needed for all the measurements and due to ethical reasons, only twenty participants (10%) were recruited, three weeks after the first collection period, to give an additional sample of venous blood (around 70 ml) to measure the concentration of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and certain polychlorinated biphenyls (PCBs) known to have “dioxin-like” properties.

Simultaneously with the main blood collection, same nurses administered questionnaires to gather data on individual characteristics, smoking and drinking habits, specific dietary habits (mainly consumption of meat, fish, dairy and local food), professional activity and specific hobbies, residential and work conditions, physical activity, past history of diseases and treatments and, recently and only for women, parity, number of breastfed children and breastfeeding duration.

Analytical procedures: The 110 serum samples (55 from each group of participants) have been analyzed for the determination of exposure to dioxin-like compounds via the “Dioxin Responsive – Chemically Activated LUciferase gene eXpression assay” (DR CALUX bioassay, BioDetection Systems B.V., Amsterdam, The Netherlands). DR CALUX assay reported toxic equivalents (pg 2,3,7,8 TCDD TEQ/g fat) as DR CALUX TEQs.

The 20 whole blood samples with higher volume were analysed, via high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS, ERGO, Hamburg, Germany), for the determination of the congener profiles of PCDD/PCDFs and mono-ortho and non-ortho PCBs in the blood of the population under study. 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 calculations of either DR CALUX-TEQs or 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 sex, age or 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 participants, differences in the studied personal attributes such as age, sex, consumption pattern of specific food-items, smoking and drinking habits and main professional activity were not statistically significant. Women were predominant (> 60%) in both areas and mean age of the total group (n=110) was 38 $\pm$ 9 years, with a range from 19 to 64 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.

Concerning dietary habits and life styles of the population under study, more than 80% of them produce vegetables for their own consumption and about 95% consume local food-items, mainly fruit and vegetables. In spite of living in an island with very common fishing activities, food consumption preferences go to meat (> 80%). The prevalence of regular smokers, mainly among men (> 60%), was not very high (< 20%), although heavier tobacco use (> 10 cigarettes/day) was dominant (> 60%) among active smokers.

Most correlations between total, HDL and VLDL cholesterol and triglycerides in serum of the participants were statistically significant and not weak. Therefore, influence by collinearity has to be considered on the statistical parameters to be estimated by multiple linear regression analysis.

Regional and sex-related differences of CALUX-TEQs in serum: Distribution of CALUX-TEQ values in serum of the studied population were slightly asymmetrically distributed and significantly (p<0,001) higher in the control area of Estreito-Jardim da Serra – namely 76 (38-173) pg CALUX-TEQ/g fat – compared to exposed area of Camacha, with a median (mean) concentration of 55 (56) pg CALUX-TEQ/g fat spread over the range 9 to 111 pg TEQ/g fat. Thus, no indication of an enhanced exposure related with the updated Meia Serra incineration facility could be derived from the obtained results.

Sex-related differences of dioxin body burden as evaluated by CALUX-TEQs in serum were not statistically significant (p=0,30). The CALUX-TEQ values found for sex groups were almost normally distributed and showed rather similar means and ranges (69,6±27,0 (19-173) and 64,5±22,6 (9-116) pg TEQ/g fat in 40 men and 69 women, respectively).

Determinants of serum CALUX-TEQs: No significant correlation between age and serum CALUX-TEQ values could be observed, either for total population or for sex- or regional-specific groups, possibly due to large interindividual variations. Besides sex, age and living area of the subjects, several other personal and environmental characteristics have been investigated as possible influencing factors on dioxin body burden as determined by serum CALUX-TEQ values. Potential determinants of the serum concentration have been identified by single and multiple linear regression analysis being the p-value for variable selection set at 0,10 and considering missing information lesser than 5% to decide on variables to enter and stay in the multiple model.

Dioxin body burden was significantly higher for professional activities classified as risky, hobbies with chemical products and farming activities. Differences were however not statistically significant between mean values of specific groups related to general health status, drinking pattern, smoking habits (either present or past), intake frequencies of fruit and vegetables, and preferential consumption of meat or fish. According to simple regression analysis, serum CALUX-TEQ values (Table 1) were positively associated with residence, own hobby, or from somebody else at home, with chemical products, gender and risky professional activity, although association was not significant for the last two factors.

Table 1 – Single and multiple linear regression analysis for serum CALUX-TEQ values

Co-variables	n	Single linear regression			Multiple linear regression		
		B	R^2 (%)	p-value	B	$R^2_{Partial}$ (%)	p-value
Living area (Ref: near to plant)	109	19,9	16,9	0,001	14,3	4,9	0,045
Profession of risk of exposure	96	10,5	2,2	0,148	3,9	0,3	0,596
Hobby with chemicals	109	13,1	6,0	0,010	1,1	0,0	0,869
Somebody's hobby with chemicals	105	14,8	6,6	0,008	4,1	0,4	0,573
VLDL cholesterol in blood (mg/dl)	108	-0,3	3,8	0,042	-3,6	2,0	0,203
Triglycerides in blood (mg/dl)	109	-0,1	3,6	0,047	0,7	1,7	0,243
Age (years)	109	-0,2	0,3	0,547	-0,1	0,2	0,726
Sex (Ref: women)	109	5,1	1,1	0,289	9,1	3,0	0,115
Consumption of local vegetables	108	-15,1	2,0	0,140	21,3	3,6	0,088
					R^2_{model} = 23,7% (p = 0,007)		

n – Number of research units; B – Coefficient of regression: variation of serum CALUX-TEQs per unit of co-variable or in relation to reference; R^2 – Explained variation; p-value – Probability that association is explained by chance. Set at 0,10 to decide on variables to enter and stay in the model; R^2_{model} – Percentage of variance explained by multiple regression model.

Significant negative weak associations were observed with serum VLDL cholesterol and serum triglycerides, and not significantly with age and consumption of vegetables from local production.

From the analysis of results it could be seen that, in multiple linear regression, the dioxin body burden was about 14 and 9 CALUX-TEQs higher for controls compared with residents living in the vicinity of the plant under study and for men, respectively. Living in Estreito-Jardim da Serra proved to be one of the strongest factors influencing dioxin body burden of the studied population. A certain not significant age-dependent trend towards lower body burden of dioxins in aged subjects (decrease of 0,1 CALUX-TEQs per year) was also apparent from the multiple regression model. Associations with the remaining tested variables were no longer significant. Altogether investigated variables could not explain more than 16% of the dioxin body burden variation. Therefore, two conclusions could be drawn: i) in single regression model, the only relevant contribution (17%) to explain dioxin body burden variation is that from residence. Although several variables were statistically significant, they could not explain independently more than 7%. ii) When in multiple regression analysis, even the partial importance of each variable (with p≤ 0,10 or interesting for research) was "diluted", since living area was the only statistically important factor, showing higher dioxin body burden for controls.

PCDD/Fs and PCBs in blood fat: Based on PCDDs and PCDFs and calculated as WHO toxicity equivalents, the median concentrations and ranges of dioxins and dioxin-like compounds in the individual whole blood samples after stratifying for living area were 9,6 (5,8-28,8) and 9,8 (5,2-23,0) WHO-TEQ pg/g fat for exposed and control groups, respectively. These values increased to 23,7 (10,9-39,2) and 28,6 (9,6-100,8) WHO-TEQ pg/g fat when toxicities were added from non- and mono-ortho PCBs.

Similarly to CALUX-TEQ results, a trend for higher levels in controls was evident, although the difference between the WHO-TEQ measured concentrations was not significant (p=0,218). It should be stated, however that these regional subgroups were rather small. Thus, only large differences would have been detected. The overall mean (median) concentration of the total group amounted to 29,7 (25,6) pg/g blood fat with a range from 9,6 to 100,8 pg WHO-TEQ / g fat.

Compared with results from relatively recent studies carried out in similar conditions and populations[2, 4, 9, 10], the results observed for PCDD/Fs were among the lowest in the range defined by those studies.

The pattern of the single congeners for PCDD/Fs and PCBs (Table 2) was similar to those described in comparable studies on non-occupationally exposed individuals [2-4,11,12]. The highest contributions to the PCDD/Fs toxicity came from 12378-PCDD, 23478-PCDF, Hexa-CDD, 2378-TCDD and Hexa-CDF. Mono-ortho PCBs 156, 118 and 157 and non-ortho PCB 126 made the principal contribution to the toxicity of dioxin-like PCBs. Altogether, these nine individual congeners accounted for 91,6% of the total identified dioxin and dioxin-like body burden in the studied group. The non-ortho PCB 126 alone was responsible for almost 30% of this total toxicity.

Due to both the reduced number of whole blood samples analysed by HRGC/HRMS and the inherent limitations of the conclusions to be drawn from the corresponding set of results, they are considered in the present paper mainly for informative purposes. However, because of the reduced dioxin body burden found, as well as the good agreement of the congener profile with those reported for whole blood 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 these results (similarly of what has been concluded from the CALUX-TEQ results). Observed trend for higher levels in regions farther from Meia Serra should be investigated.

Table 2 – Concentration of PCDD/F and dioxin-like PCB congeners in whole blood samples

PCDD/Fs	*WHO-TEQ* (pg/g fat)	**%**	**PCBs**	**WHO-TEQ** (pg/g fat)	**%**
2378-TeD	1,73	**5,7%**	**NOPCB-77**	0,01	0,0%
12378-PeD	4,29	**14,1%**	**NOPCB-81**	0,00	0,0%
Σ Hexa-CDD	1,88	**6,2%**	**NOPCB-126**	8,40	**27,6%**
1234678HpD	0,32	1,0%	**NOPCB-169**	0,65	2,1%
OCDD	0,02	0,1%			
			MOPCB-105	0,69	2,2%
2378-TeF	0,11	0,3%	**MOPCB-114**	0,48	1,6%
12378-PeF	0,05	0,2%	**MOPCB-118**	3,05	**10,0%**
23478-PeF	2,27	**7,4%**	**MOPCB-123**	0,03	0,1%
Σ Hexa-CDF	1,03	**3,4%**	**MOPCB-156**	4,30	**14,1%**
Σ Hepta-CDF	0,06	0,2%	**MOPCB-157**	0,96	**3,1%**
OCDF	0,00	0,0%	**MOPCB-167**	0,04	0,1%
			MOPCB-189	0,09	0,3%
PCDD/Fs WHO-TEQ	11,76	**38,6%**	**PCBs WHO-TEQ**	18,70	**61,4%**

Acknowledgements

In the ambit of the Environmental Health Survey Program, the Autonomous Region of Madeira, Regional Ministry of Environment and Natural Resources financed the present research.
Authors of this study wish to express their gratitude to Dr.ª Conceição Estudante, Head of the Regional Ministry of Social Affairs, for her excellent continuous support. Special thanks are also due to the Regional Public Health Department for all the assistance in the logistics of field activities and University of Madeira also for practical arrangements mainly for sample preparation and storage. The authors are very grateful for the excellent assistance in fieldwork, sample collection and analytical procedures of the technical staff from the local Health Centres (Camacha and Estreito-Jardim da Serra) and Regional Public Health Laboratory. Very special thanks are also due to the volunteers for their unselfish participation.

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