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Concentrations of polychlorinated dibenzodioxins, polychlorodibenzofurans, and polychlorobiphenyls in women of reproductive age in Italy: A human biomonitoring study

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ABSTRACT

Background: Polychlorinated dibenzodioxins (PCDDs), polychlorodibenzofurans (PCDFs), and polychlorobiphenyls (PCBs) are persistent organic pollutants that represent a major concern for women of reproductive age because of the neurodevelopmental effects associated to perinatal exposure.

Objectives: This study was aimed at characterizing exposure of women of reproductive age to PCDDs, PCDFs, and PCBs as a function of residence in different Italian Regions, in areas at presumable different environmental contamination and human exposure to these pollutants.

Methods: Study participants were enrolled in 2011–2012 in 6 Italian Regions representative of Northern, Central and Southern Italy; in each region, areas at presumed different exposure (rural, urban and industrial) were selected for enrolment. Each participant provided a serum sample for the analysis of PCDDs, PCDFs and PCBs.

Results: Median concentrations of PCDDs + PCDFs, DL-PCBs, NDL $_6$ -PCBs and NDL $_9$ -PCBs in serum samples were respectively 6.0 and 3.5 pgWHO-TE $_{05}$ /g fat, and 75 and 93 ng/g fat.

Age was the variable that most affected median serum concentrations.

Age adjusted concentrations were found significantly different between geographical zones: women from Northern Italy showed the highest values, followed by Central and Southern Italy.

PCDDs + PCDFs concentrations were significantly higher in the group of women residing in industrial areas compared to the group residing in rural areas.

A clear diminishing temporal trend was observed compared to levels reported in previous studies. *Conclusions*: This study produced the largest dataset on serum concentrations of PCDDs, PCDFs and PCBs in women of childbearing age in Italy.

Results: confirmed that environmental and lifestyle factors may influence exposure to these contaminants and thereby the body burden.

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A.M. Ingelido et al. / International Journal of Hygiene and Environmental Health xxx (2016) xxx-xxx

The observed marked temporal decline in body burden during three decades is in agreement with the general trend observed worldwide.

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1. Introduction

In spite of the declining temporal trend observed in most countries in the last couple of decades (WHO (World Health Organization), 2009), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs, divided into the two groups of dioxin-like congeners (DL-PCBs), and non dioxin-like congeners (NDL-PCBs)) still are the toxic persistent organic pollutants (POPs) associated with high concern, because of their toxicological profile (Wikoff et al., 2012). Among the main toxic effects exhibited by PCDDs and PCDFs (altogether referred to as "dioxins") and PCBs, neurodevelopmental effects associated to perinatal exposure are considered the most sensitive health end-points (FAO (Food and Agriculture Organization of the United Nations)/WHO (World Health Organization), 2006; Koopman-Esseboom et al., 1996; Nakajima et al., 2006; WHO (World Health Organization), 2002), and represent a major concern for women of reproductive age, especially those living in contaminated areas.

PCDDs, PCDFs, and PCBs were among the POPs included in the human biomonitoring project "Womenbiopop – Linking Environment and Health – A Country-based Study on Women of Reproductive Age" (April 2010–May 2013). This project was funded by the financial instrument "Life Plus" (EC, DG Environment) and co-funded by the Istituto Superiore di Sanità (Italian National Institute of Health) and by the Italian Ministry of Environment, Land and Territory. The aim of the study was to characterize exposure of women of reproductive age to a group of priority pollutants of environmental origin, namely PCDDs, PCDFs, PCBs and other POPs, and to explore a possible correlation between exposure and women's reproductive health (De Felip, 2013).

Five hundred forty nine (549) nulliparous women were enrolled in the Project. These women resided in six different regions that were representative of Northern, Central, and Southern Italy, and lived in areas characterized by different levels of anthropogenic activity.

The present paper reports the results of the determinations of PCDDs, PCDFs, DL- and NDL-PCBs carried out on women's serum samples.

2. Methods

2.1. Study design

Study participants were enrolled in 2011–2012 in 6 Italian regions (Fig. 1). Regions included in the study were Trentino-Alto Adige and Piemonte (Northern Italy); Umbria and Lazio (Central Italy); and Puglia and Sicilia (Southern Italy).

Areas at different (presumed) exposure to POPs were included in all regions, such as areas at background exposure (rural or mountainous areas with no major industrial settlements), and areas at possible incremental exposure (urban areas, and/or areas with industrial settlements). About 50 subjects per area were contacted for recruitment. Five hundred forty nine (549) subjects agreed to participate, signed an informed consent form, and compiled a questionnaire aimed at collecting information on the participant's socio-demographic profile, dietary habits, and lifestyle. Enrollment and sampling were performed by the local health units of each

region. Inclusion criteria were: age between 20 and 40 years, having never breastfed, having resided in the area for at least 10 years.

Women were enrolled in the following areas:

In Trentino Alto-Adige, the town of Trento, and two mountainous areas in the Province of Trento, i.e. Val di Non (background area) and Valsugana, where a steel plant has been operating for the last thirty years;

In Piemonte, the town of Torino, and two mountainous areas in the Province of Torino, i.e. Val Chisone (background area) and Val di Susa, where a steel plant has been operating for decades;

In Umbria, the town of Terni (where a large steel plant has been operating since the end of the 19th century), and country areas in the Province of Terni;

In Lazio, the town of Rome, the town of Latina and country areas in the Province of Latina;

In Puglia, the town of Taranto (where one of the largest steel plants in Europe has been operating since 1960) and a country area (Laterza) in the Province of Taranto;

In Sicilia, the town of Palermo, and country areas in the Province of Palermo.

The Project was approved by the local Ethics Committees.

2.2. Blood withdrawal and serum analysis

Prior to blood withdrawal, a signed informed consent was acquired from each woman, and a questionnaire administered by medical doctors or trained nurses.

About 50 mL of blood were withdrawn from each woman and centrifuged to obtain serum. Serum samples (approximately 25 mL each) were added with ¹³C-labelled PCDD, PCDF, and PCB internal standards (Wellington Laboratories Inc., Guelph, Ontario, Canada), stirred, and allowed to rest overnight at 4°C. Before extraction, the spiked samples were allowed to warm up at room temperature, added with a mixture of formic acid and iso-propanol, and sonicated. Extraction was performed with *n*-hexane, followed by centrifugation. Extraction was repeated three times. Supernatant *n*-hexane layers were collected in a tube, and concentrated under a gentle nitrogen stream. Lipid removal was performed by elution on a glass column containing ExtrelutTM (Merk Millipore, Milan, Italy) impregnated with concentrated sulfuric acid. The prepurified extract was reduced to a small volume and cleaned up with an automatic Power-PrepTM system (FMS – Fluid Management Systems, Inc. Watertown, MA, USA) (Abballe et al., 2013). Each purified extract was reduced to a small volume and transferred to a vial to undergo instrumental determination. Total cholesterol and triglycerides were determined by enzymatic methods by the Laboratory of Clinical Pathology, Università di Roma "Sapienza" and total lipid content was calculated by the Rylander's formula (Rylander et al., 2006).

PCDD, PCDF, and non-ortho DL-PCB quantification was performed by HRGC-HRMS (Thermo-DFS, Thermo Fisher Scientific Inc., Waltham, MA, USA) applying the selected ion monitoring mode (SIM). Mono-ortho PCB and NDL-PCB quantification was performed by a HRGC-triple quadrupole system (TSQ Quantum GC, Thermo Fisher Scientific Inc., Waltham, MA, USA) operated in the electron impact SRM mode. The isotope dilution technique was applied throughout.

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2

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