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Organochlorine pesticides and polychlorinated biphenyls in human adipose tissue from northern Tunisia: Current extent of contamination and contributions of socio-demographic characteristics and dietary habits

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ABSTRACT

The aims of the present study were to investigate the current exposure levels of persistent organochlorine compounds (OCs) in adipose tissues intraoperatively collected from 40 patients over 20 years undergoing non-cancer-related surgery residing in Northern region of Tunisia (Bizerte), which constitutes an exemplary case, and examined association between levels of contamination and both socio-demographic characteristics and dietary habits. Concentration of hexachlorobenzene (HCB), hexachlorocyclohexane isomers (α-HCH, β-HCH, γ-HCH and δ-HCH), dichlorodiphenyltrichloroethane isomers (p,p′-DDT and o,p′-DDT) and metabolites (p,p′-DDE, o,p′-DDE, p,p′-DDD and o,p′-DDD) and 12 polychlorinated biphenyls (PCBs) congeners were measured using capillary gas chromatography with electron capture detector. Overall, residue levels of OCs followed the decreasing order of DDTs > PCBs > HCB > HCHs. DDTs levels ranged from 74.49 to 1834.76 ng g^{-1} lipid and contributing to more than 90% to the sum of organochlorine pesticides (OCPs). p,p′-DDE was the most abundant in all samples and the p,p′-DDT/p,p′-DDE ratio (range between 1.85% and 58.45%) suggesting recent and ongoing exposure to banned commercial DDT products. PCB concentrations varied from 29.27 to 322.58 ng g^{-1} lipid and PCB-180, PCB-153 and PCB-138 were the dominant congeners accounting for 70% of total PCBs. We did not find significant correlations between OC exposure levels and sex, parity, habitat areas and smoking habits. In females, the adipose tissue concentrations of DDTs, HCB and PCB-118 were positively correlated with age. There was statistically significant relationship between body mass index (BMI) changes and the adipose tissue levels of HCB and HCHs. No association was found between OCPs levels and dietary factors. However, our study suggests that fish consumption may be an important contributor of PCBs adipose tissue content of PCBs in Tunisian people. The presented work is highly significant, being the first study pointing out the chronic exposure to OCs in Bizerte.

1. Introduction

Synthetic organochlorine compounds (OCs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) have long been produced and used over the world and recognized as persistent organic pollutants (POPs). Due to the high lipophilicity and resistance to biological, chemical and photolytic degradation, POPs tend to persist in the environment for many years and will therefore bioconcentrate and biomagnify in the food chains (Muir et al., 1988; Olsson et al., 2000). Because humans occupy the top position in the trophic levels, lipid rich tissues accumulate the highest concentrations of these pollutants and can become more vulnerable to their toxic effects (Djien Liem et al., 2000; Schecter and Gasiewicz, 2003). Human samples, such as serum/plasma, milk, and adipose tissue have been used in monitoring the extent of human exposure to lipophilic contaminants (Covaci et al., 2008; Hardell et al., 2010; Jin et al., 2009). OC concentrations are known to be higher in adipose tissues and provide more representative of the cumulative internal exposure

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(Kohlmeier and Kohlmeier, 1995; Pearce et al., 1995). The measurements in blood or milk of these compounds will fluctuate during lipid mobilization through body weight loss (Chevrier et al., 2000; Quinsey et al., 1995), pregnancy and breast-feeding period (Ostrea et al., 2009; Torres et al., 2006; Wang et al., 2009). Thus, adipose tissue samples are preferable bioindicators of body burden when available (Allam and Lucena, 2001; Hardell et al., 1996; Quintana et al., 2004).

Chronic exposure of humans to OCPs and PCBs occurs through different pathways; consumption of contaminated foods, specifically with high fat content (fish, meat, chicken etc.), local use and environmental contamination (Schildkraut et al., 1999). The variability of the stored amount depends on dietary exposure (food habits and the quality and quantity of POPs in available food) and on individual disposition to store these substances (genetic traits and size of adipose tissue and its dynamic changes) (Müllerová and Kopecký, 2007).

Bizerte Governorate is located in the extreme north coast of Tunisia with a wide opening onto the Mediterranean Sea and it represents more than 5% of its population and 2.3% of its area. Because of its natural resources and climate, it's one of the most important poles of Tunisia and constitute an economic hub based essentially on agriculture, industry and services. These anthropogenic activities may be attributable to the contamination by chemical pollutants such as organochlorine compounds. Despite the use OCPs and PCBs, has been banned or restricted in Tunisia according to the Stockholm Convention, these compounds are still found in various environmental matrices collected in Bizerte region such as sediment (Barhoumi et al., 2014b; Derouiche et al., 2004), fish (Ameur et al., 2013a, 2013b) and mussels (Barhoumi et al., 2014a).

Until now, no data is available in Tunisia on OCPs and PCBs levels in human adipose tissue and while few studies have reported concentrations of these contaminants in serum (Ennaceur and Driss, 2010; Hassine et al., 2014) and breast milk (Ennaceur et al., 2007, 2008; Hassine et al., 2012). In this background, monitoring OC residue levels in Bizerte living areas will provide better indications of contamination and also for assessing human health risks of Tunisian population. The relationships between dietary habits and socio-demographic characteristics of the volunteers and the accumulation of OC residues were also investigated.

2. Materials and methods

2.1. Subjects and sample collection

Forty volunteers were chosen among patients undergoing non-cancer-related surgery (appendectomy, inguinal hernia surgery, gall bladder removal…) between January and June 2014 in regional hospitals of Bizerte Governorate (Fig. 1. for location). An informed consent was obtained from all participants to the study. A structured questionnaire included questions on their age, weight, height, occupation, residence, clinical history, dietary habits, smoking history and number of children (women) were filled for all participants.

The age of the contributors varied from 20 to 85 years, with a median age of 54.5 years. Body mass index (BMI) was calculated as the weight/height squared (kg m^{-2}) and ranged between 16.53 and 40.40 (mean 27.68). Among the 40 donors, 22 were males. 8 of the female volunteers did not have a child and the rest had one or more children (range 1–6, median =2 children). 50% of the participants reported having resided in the rural area of studied region and 25% were tobacco consumers. Dietary information was obtained and only white and/or blue fish and red meat consumption were categorized.

The human adipose tissue samples were collected from the abdominal fat region and stored in precleaned glass bottles at −20 °C until analysis.

2.2. Chemical analysis

A standard reference materials SRM 2273 (chlorinated pesticides (DDTs) and metabolites in isooctane) were provided by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). The other organochlorine pesticides under investigation were α, β, γ, δ-HCH and HCB were obtained from Supelco (Bellefonte, PA, USA) and were about 99% pure. Individual standard solution of each pesticide was prepared in hexane at 1000 mg mL^{-1} except β-HCH which is dissolved in acetone. A standard mixture of twelve PCB congeners (PCB-18, −28, −31, −52, −44, −101, −149, −118, −153, −138, −180 and −194) at 10 μg mL^{-1} in heptane and PCB-170 analytical standard were purchased, as target PCB, from Supelco (CIL, USA). PCB congeners −15, −155 and −198, for use as surrogate standards, as well as an internal standard of PCB-209 were obtained by Cluzeau info labo (Sainte Foy La Grande, France, purity 99%) and diluted in isooctane to suitable concentrations. The working standard solutions were prepared by dilution of the above standard solutions in n-hexane to appropriate concentration levels. All of the standard solutions were stored at 4 °C and left for 1 h at ambient temperature prior to use.

All organic solvents used in this study (n-hexane, isooctane, dichloromethane, chloroform and methanol) were high purity grade and were obtained from Panreac (Barcelona, Spain). Hydrochloric acid and sulfuric acid was obtained from Biotechnica. The water used was from a MilliQ system (Milford, MA, USA). Silica gel (63–230 mesh), basic alumina (70–230 mesh) and anhydrous sodium sulfate were supplied by Fluka. Acidified silica was prepared by adding 35.5 mL concentrated sulfuric acid to 100 g silica gel and mixing thoroughly. Preparation of deactivated alumina was done by adding 5 mL water to 45 g of the alumina.

The studied PCBs and OCPs were analyzed following the method described elsewhere with some modifications (Naert et al., 2004). Approximately 5 g adipose tissue was put on a folded Whatman filter paper in a glass funnel together with anhydrous sodium sulfate (5 g). The fat was melted in a microwave oven (600 W for 2 min) and received in a glass recipient. Surrogate standards PCB-15, PCB-155 and PCB-198 together with 1 mL n-hexane were added to 1 g of melted fat. A glass column containing a wad of silane-treated glass wool at the bottom was used for the clean-up. This column was subsequently filled with n-hexane (25 mL), acidified silica (12 g), deactivated alumina (3 g) and anhydrous sodium sulfate (3 g). After removal of any excess n-hexane from the column, the fat solution was brought onto the column and elution was performed with 40 mL of a dichloromethane/n-hexane mixture (1/9; v/v). The eluate was concentrated to near dryness and after addition of internal standard PCB-209, the final volume of the extracts was adjusted to 100 μL in isooctane.

100% lipid content of melted fat using for determination procedure was verified using a previously reported method by Rivas et al. (2001). Briefly, 100 mg aliquot of this sample was homogenized with 2.5 mL of chloroform:methanol:hydrochloric acid (20:10:0.1) (v/v/v). After repeating the process, 5 mL of 0.1 N HCl were added and centrifuged at 3000 rpm for 10 min. The organic phase was then collected. The sample extract was evaporated to dryness and the lipid content of the sample was measured gravimetrically.

A Hewlett-Packard 6890 gas chromatograph equipped with ^{63}Ni electron capture detector (GC-ECD) operated by HP Chemstation software was used for PCB and OCP determinations. The column used for analysis was fused silica capillary SLB5-MS (30 m × 0.25 mm i.d. × 0.25 μm thickness). A second SPB-608 fused silica column 30 m × 0.32 mm ID, 0.25 μm film thickness was used as a confirmatory column. The operating conditions were as follows: injector temperature 250 °C; detector temperature 300 °C; oven temperature: initial 100 °C for 1 min, programed to 200 °C at 10 °C/min, followed at 1 °C/min to 220 °C, then ramped to 280 °C and held for 10 min; carrier gas: helium at a flow rate (constant flow) of 1 mL min^{-1}; detector make-up gas was nitrogen at a flow rate of 60 mL min^{-1}; sample injection volume 2 μL;