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High serum organochlorine pesticide concentrations in diabetics of a cotton producing area of the Benin Republic (West Africa)

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ABSTRACT

The Borgou region of northern Benin is a major cotton producing area and consistently uses higher amounts of pesticides than other areas of the country. Organochlorine pesticides (OCPs), poorly handled, have been widely used and are still illegally present. We therefore hypothesized that serum OCP levels would be high in Borgou. As part of a case–control study on diabetes status and pesticide exposure, we measured the distribution of serum concentrations of 14 OCPs by gas chromatography with mass spectrometry. A sample of 118 diabetic subjects was selected using a four-stage cluster sampling with 54.2% of men and 45.8% of women; 43% lived in urban areas, 14.4% were obese and 39.8% had high economic status. The four detected OCPs were *p,p′*-DDT, *p,p′*-DDE, β-HCH and *trans*-nonachlor with respective geometric means (geometric standard deviation) of 497.1 (4.5), 20.6 (7.9), 2.9 (3.4), and 2.0 (2.3) ng/g of total serum lipids. OCP levels were significantly higher in obese, wealthier and more educated subjects and in those living in urban areas as compared to the other groups, particularly for *p,p′*-DDE, *p,p′*-DDT and β-HCH. Levels of DDT and DDE were higher than reported in other countries where DDT is no longer permitted. The low DDT/DDE ratio of 0.05 suggests past human exposure through food contamination. There is thus a need to reinforce governmental regulations for a more responsible use of pesticides in the country, in order to reduce health risks associated with persistent organic pollutants.

1. Introduction

The use of pesticides in developing countries is continuously increasing where urbanization and intensive agriculture are growing (United Nations, 2012). Although most organochlorine pesticides (OCPs) have been banned by the Stockholm Convention (United Nations Environment Programme, 2001), some are still used in developing countries for various reasons including disease control, malaria in particular (World Health Organization, 2011).

In the Republic of Benin, OCPs have been used for disease control in public health and for crop protection in agriculture. Dichlorodiphenyltrichloroethane (DDT) has been used since 1960. Dieldrin, endrin, aldrin, heptachlor and other OCPs were used around 1980 until they were banned in the country in 2004. Endosulfan was used until 2010 in cotton production (Watts, 2008). Although prohibited, OCPs have been illegally sold or stored in adverse conditions for the environment and for population health (Williamson, 2003). Additionally, as in several other African countries, inadequate management of pesticides and their wastes, low use of individual and collective protective equipments and inappropriate uses are common (Ahouangninou, 2011; Dalvie et al., 2009; Ngowi et al., 2007). Consequently, pesticides accumulate in the environment and along the trophic chain in the country and there have been reports of environmental concentrations above tolerable limits (Adam et al., 2010; Assogba-Komlan et al., 2007; Okoumassoun et al., 2002; Pazou et al., 2006a, 2006b; Rosendahl et al., 2009).

Abbreviations: OCPs, Organochlorine pesticides; μg/L, micrograms per liter; ng/L, nanograms per liter.

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There is increasing evidence supporting that exposure to persistent organic pollutants such as OCPs could be involved in the occurrence of several non-communicable diseases including diabetes (Ha et al., 2007, 2009; Hong et al., 2012; Howard and Lee, 2012; Howard et al., 2011; Lee, 2010, 2012; Lee et al., 2006a, 2006b, 2010; Porta, 2006a, 2006b; Porta and Lee, 2009; Son et al., 2010; Taylor et al., 2013; Thayer et al., 2012). In this context, measuring serum concentrations of some OCPs in the general population of the Borgou area appeared relevant in order to assess the level of environmental risk incurred by the population. The Borgou, one of the 12 departments of Benin, is the second highest pesticide user in the country for cotton production. It is also the department with the highest diabetes prevalence of 4.6% compared with the national average of 2.6% (Houinato et al., 2007).

This paper reports on the distribution of serum concentrations of OCPs in diabetics of this population, as assessed in an epidemiological case–control study on pesticide exposure and type 2 diabetes. Levels of OCPs in this study were compared with reported levels in other African and non-African countries. The associations between socio-economic factors (education, occupation, and wealth index, Body Mass Index (BMI), demographics (gender and age) and residence area (urban, rural or semi-rural) and exposure biomarker levels were documented.

2. Materials and methods

2.1. Site of the study

The study took place in the Borgou area, one of the 12 departments of Benin Republic. Located in the North-East of the country, Borgou is divided in eight municipalities (Parakou, N'dali, Tchaourou, Nikki, Kalale, Perere, Sinende, Bembereke). In these municipalities, we count 43 districts and 310 villages. Borgou covers an area of 25,856 km^2 (23% of the country) including 13,962 km^2 of arable land, 54% of the total area of the department. Borgou has 969,896 inhabitants with 20% living in the main town of Parakou. From the economic point of view, this area is characterized by agriculture along with cattle breeding and trade. The Borgou Department is regarded as the breadbasket of the country. It is also the second largest producer of cotton. Large amounts of pesticides are used for cotton production and also for food crops such as legumes and cereals. OCPs were used in the past for pest control in agriculture and for malaria control. Currently prohibited, there are still stocked in worst conditions in certain parts of the department.

2.2. Population and study design

Subjects were identified from the database of a diabetes prevalence survey in the Borgou Department, in the northern part of Benin. The survey included 4740 adults selected using a four-stage cluster sampling. In the first stage, 22 districts of the eight municipalities of Borgou were randomly selected without replacement. The second step consisted of randomly selecting half of the villages in each district. The third stage involved the random selection of compounds to visit in each village. From the center of the village, direction was chosen randomly. Each third compound was selected in the given direction after randomly selecting the first compound. Lastly, half of households living in the same compounds were randomly selected and all eligible adults in the selected households were interviewed. This study was conducted in 80 villages of 24 districts located in eight municipalities. Data collection for the prevalence study was conducted on October 3–18, 2011 and the present survey was carried out from October 5 to December 30 of the same year. The current study focused on adults aged 18–65 years who had been living in the Borgou area for at least ten years. Sample size was calculated by taking the standard deviation (SD) of DDT mean serum concentration (SD = 1.20 ng/g total lipids) in cotton farmers of Ghana, a neighboring country. For an expected mean difference of 0.43 ng/g of total lipids between subjects with and without diabetes, estimated sample size was 125 subjects per group at significance level of 0.05 and power of 80%. In the prevalence survey, capillary blood glucose was tested after a 12-hour fast. For our study, we measured venous blood glucose among subjects screened for hyperglycemia in the survey in order to identify those with diabetes (fasting glycemia ≥ 7 mmol/L). Venous glucose test was done 48–72 h after the capillary glucose test. A total of 65 subjects with diabetes were thereby identified out of the 125 required subjects. The additional subjects with diabetes were recruited in health centers of the eight municipalities where the 65 initial subjects were previously detected using a ratio of 1:1. Using health center records, 64 persons with diabetes were randomly selected using SPSS software for random digits. Of the 129 subjects enrolled in the study, 118 subjects had enough sampled serum for analysis of OCP concentrations and were therefore included in the present report. OCP levels were also measured in the 129 control subjects paired with the diabetics by age, gender, ethnicity and residence location. Data were also available for 116 control-subjects but these are not presented in this paper since this sub-population was not representative of the whole population of non-diabetic subjects in the area.

This study was approved by the Ethics Committee of the Faculty of Medicine of the University of Montreal (Canada) and by the Ministry of Health of Benin. The informed consent of the subjects was obtained for the conduct of the study and anonymous publication of results.

2.3. Laboratory methods

2.3.1. Glucose determination

Capillary blood glucose was measured using “One Touch Ultra” glucometers with a drop of blood from a finger-tip. Plasma was collected for glucose analysis 24 to 72 h later, after a 12-hour overnight fast. The blood samples were collected in tubes containing fluoride oxalate and immediately stored in a cooler with frozen icepacks for 3–5 h before being brought to the nearest laboratory for centrifugation and separation of plasma. Plasma was frozen at −20 °C before being brought to the Biochemistry Laboratory of the Institute of Applied Biomedical Sciences (ISBA) in Cotonou, Benin, for the determination of glucose using the glucose oxidase enzymatic method.

2.3.2. Determination of OC pesticide concentrations

For the analysis of OCPs, serum samples were collected in EDTA tubes according to the protocol provided by the Toxicology Laboratory of the National Public Health Institute of Quebec (INSPQ) in Canada. After centrifugation, serum samples were stored at −20 °C in Benin before being shipped on dry ice by air cargo to the INSPQ laboratory (Quebec City).

The E-458 method was used to analyze the following compounds: aldrin, dieldrin, endrin, endosulfan I (alpha), endosulfan II (beta), α-chlordane, γ-chlordane, α-hexachlorocyclohexane (α-HCH), β-HCH, γ-HCH, *cis*-nonachlor, *trans*-nonachlor, *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) and *p,p′*-DDT, (all from Ultra Scientific, RI, USA).

Serum samples (2 mL) were spiked with labeled internal standards (hexachlorobenzene-$^{13}C_6$, α-HCH-$^{13}C_6$, oxychlordane-$^{13}C_6$, *trans*-nonachlor-$^{13}C_{10}$, *p,p′*-DDE-$^{13}C_{12}$, dieldrin-$^{13}C_{12}$, endrin-$^{13}C_{12}$, endosulfan I-$^{13}C_9$; all from Cambridge Isotope Laboratories (CIL), MA, USA) and proteins were denatured with 2 mL of reagent alcohol. Organohalogenated compounds were extracted from the aqueous matrix by liquid–liquid extraction with 8 mL of hexane. The extracts were evaporated to dryness before they were dissolved in 0.5 mL of hexane. These extracts were cleaned up on deactivated 0.5% Florisil columns. The elution was performed in two steps: the first fraction was eluted with a mixture of dichloromethane:hexane (9 mL; 25:75) and contained all compounds except endrin, dieldrin, endosulfan I and endosulfan II, which were eluted in the second fraction. The second fraction was eluted with a mixture of acetone:dichloromethane (4 mL; 2:98). The solvents of these 2