



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.

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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. Dichlorodiphenyl-trichloroethane (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; μ g/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 km² (23% of the country) including 13,962 km² 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 mean 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-¹³C₆, α -HCH-¹³C₆, oxychlordane-¹³C₆, *trans*-nonachlor-¹³C₁₀, *p,p'*-DDE-¹³C₁₂, dieldrin-¹³C₁₂, endrin-¹³C₁₂, endosulfan I-¹³C₉; 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

fractions were evaporated to dryness and taken up in 20 μL of hexane for the first fraction and 20 μL of acetonitrile for the second fraction. The samples were analyzed for OCPs in two injections: the first fraction was analyzed on an Agilent 6890 Network gas chromatograph (GC) coupled to an Agilent 5973 Network mass spectrometer (MS) (Agilent Technologies; Mississauga, Ontario, Canada), and the second fraction on an Agilent 7890A gas chromatograph (GC) coupled to an Agilent 5975C mass spectrometer (MS) (Agilent Technologies). Both GCs were fitted with an Agilent 60 m DB-XLB column (0.25 mm i.d., 0.25 μm film thickness) to the MS. The temperature gradient for the first fraction was as follows: 2 min at 100 °C followed by an increase to 200 °C at a rate of 20 °C/min, increase to 245 °C at a rate of 1.5 °C/min, hold 10 min, increase to 280 °C at a rate of 20 °C/min, hold 5 min and finally an increase to 330 °C at a rate of 30 °C/min, hold 15 min. The constant flow rate was 0.8 mL/min and the total run time was 70.42 min. The temperature gradient for the second fraction was as follows: 1 min at 100 °C followed by an increase to 255 °C at a rate of 25 °C/min, increase to 265 °C at a rate of 1.5 °C/min, hold 5.5 min and finally an increase to 330 °C at a rate of 50 °C/min, hold 12 min. The constant flow rate was 0.8 mL/min and the total run time was 32.67 min. The carrier gas was helium and the injections were 3 μL in pulsed splitless mode. The mass spectrometers were operated in selected ion monitoring (SIM), using negative chemical ionization (NCI) with methane (99.97%) as the reagent gas. Analyte concentrations were evaluated by considering the ratio of the analyte area on the internal standard area and by considering the % recovery of labeled internal standards.

Limits of detection (LODs) were determined by first estimating concentrations of analytes yielding a signal to noise ratio of 3. A serum sample spiked with analytes in concentrations ranging from 4 to 10 times the estimated LODs was analyzed in 10 replicates. The calculated LOD was the value equivalent to 3-fold the standard deviation of those 10 replicates. The intra-day precision (repeatability) ranged from 3.3 to 8.3% and the inter-day precision (reproducibility) was between 3.9 and 11% depending on the analyzed OCPs. The method recovery was between 57 and 84% for the different OCP compounds.

Reference materials from Arctic Monitoring and Assessment Program (AMAP) (W-12-05; AMAP Ring Test for Persistent Organic Pollutants; Toxicology Laboratory, INSPQ) and certified reference material in human serum (SRM-1958; National Institute of Standards & Technology [NIST], Gaithersburg, MD, USA) were used for internal quality control. The overall quality and accuracy of the analyses were monitored by regular participation in inter-laboratory programs: German External Quality Assessment Scheme (G-EQUAS) and AMAP Ring Test. INSPQ toxicology laboratory is accredited under ISO/CEI 17025.

Levels of total cholesterol, free cholesterol, triglycerides and phospholipids were measured in the serum samples by a colorimetric enzymatic method (in g/L) at the 'Centre Hospitalier de l'Université Laval' (CHUL), a subcontractor of INSPQ. INSPQ material was used for quality control according to between lab control protocols. Total lipid levels were calculated using the following formula (Akins et al., 1989; Patterson et al., 1991): total serum lipids = $1.677 * (\text{total cholesterol} - \text{free cholesterol}) + \text{free cholesterol} + \text{triglycerides} + \text{phospholipids}$. Lipid-adjusted OCP concentrations (ng/g lipids) were obtained by dividing each OCP serum concentration ($\mu\text{g/L}$) by total serum lipid contents ($\mu\text{g/g}$) multiplied by 1000. A value of half the LOD was assigned to undetectable OCP concentrations, prior to adjustment.

2.4. Demographic and socio-economic features

Subjects were categorized in four age-groups: <30, 30–39.9, 40–54.9 and 55 years or above. Gender was considered as male or female. Socio-economic status was determined based on a wealth index as the reflection of economic status, education level and occupation (Liberatos et al., 1988). Three levels were identified for education: no formal schooling,

functional literacy or primary school level, and high school or university. Subjects' main occupations were divided into farmers, manual workers and office workers. A wealth index was computed based on household assets, source of energy for light and cooking, size of the household and home employees. Assets included land plots, housing and its features, furniture and appliances, transportation vehicle, communication equipment, size of livestock or farm (Higgs, 2002; Kobiane, 2004; Shavers, 2007). Variables were weighted according to their relative importance as markers of affluence. For example, scores attributed to ownership of a bicycle, a motorcycle and a car were respectively 1, 2, 4, and otherwise the score was 0. The total wealth index was split into tertiles for data analyses.

We collapsed the eight districts into urban, semi-urban and rural areas according to their degree of urbanization as defined by government services based on demographic, administrative and economic characteristics (Thomas and Djaouga, 2008). The only urban area was the town of Parakou and represented 44.9% of the whole sample. The semi-urban area was comprised of N'Dali, Tchaourou, Bembereke and Nikki districts and included 48.3% of the study subjects. Districts of Kalalé, Perere and Sinende were considered rural and represented 6.8% of sample.

2.5. Anthropometric data

Weight was measured in fasting subjects with minimal clothing. Seca® scale for 150 kg was used in standing position with 0.5 kg of measurement error. Height was measured without shoes. Measurement was done with 0.5 cm of error. Body mass index was computed with the following formula: $\text{BMI} = \text{weight (kg)} / \text{height (m)}^2$.

Subjects' BMI was as underweight (<18.5), normal (18.5–24.9), overweight (25–29.9) and obese (≥ 30).

2.6. Comparison of OCP concentrations in Borgou with other countries

Pesticide levels in Borgou were compared with data from other African or non-African countries. Some data were available in sub-Saharan Africa for the general population or specific groups; however, there is no published data concerning serum levels of pesticides in diabetic individuals. Selected surveys for comparison were those reporting serum OCP concentrations adjusted for total lipids. All data available in African countries from year 2000 were taken into account (Ben Hassine et al., 2013b; Channa et al., 2012a; Linderholm et al., 2010; Manirakiza et al., 2002; Ntow, 2001). Non-adjusted data were not considered in tables. For non-African countries, few were chosen (France, Belgium, Finland, Sweden, Korea, Canada and USA) according to data availability (Airaksinen et al., 2011; Department of Health and Human Services, 2009; Dirinck et al., 2011; Fréry et al., 2011; Health Canada, 2010; Rignell-Hydbom et al., 2007; Son et al., 2010).

2.7. Statistical analyses

Kolmogorov test was used to check normality of the distribution of OCP concentrations in serum. Serum concentrations of aldrin and α -chlordane were normally distributed. Despite log transformation, data distribution was not normalized in the case of the other 12 OCPs, although approaching log-normality. Geometric means of log transformed data, medians, ranges and percentiles were used to describe serum OCPs. Bivariate analysis of the relationship between OCP concentrations and various factors (education, occupation, economic status [wealth index], gender, age, residence area and BMI) were conducted for the four detected pesticides using the Chi square test, Student-t test or ANOVA with Bonferroni post-test for normally or log-normally distributed variables. Nonparametric tests such as the Mann-Whitney or Kruskal-Wallis were used otherwise. Multivariate linear regression analyses were also conducted to access relation between OCP levels (log-transformed) and personal or socioeconomic factors: BMI, wealth

index, residence area and education level. Adjustments for suitable confounders were performed. Associations were considered significant at p-values less than 0.05. The relation between OCP concentrations and diabetes status is not reported in this paper.

3. Results

3.1. Characteristics of the subjects

The sample of diabetic subjects included 54.2% men and 45.8% women. The mean age was 50.3 ± 11.3 years and the majority of subjects were in the range of 55–65 years (44.9%). Almost half of the subjects (45.8%) had no schooling (Table 1). Office workers were less numerous (28.0%) while farmers and manual workers were in the same proportion. Out of the total sample, half of the subjects lived in semi-urban areas and 43.2% in urban areas. According to BMI, 5.9% of the subjects were underweight, 42.4% in the normal range, 37.3% were overweight and 14.4% were obese. In the upper tertile of the wealth index, 53.2% had high levels of education, 66% lived in urban areas, 48.9% worked in offices and only 10.6% were farmers. Among obese subjects, 64.7% were in the upper wealth index tertile.

3.2. Organochlorine serum concentrations

Out of the 14 analyzed OCPs, ten had concentration values mostly below the LOD. This was not the case for four OCPs: *p,p'*-DDT, *p,p'*-DDE, β -HCH and *trans*-nonachlor. Percent detection was 95.8%, 85.6%, 53.8% and 48.3%, respectively, for the latter OCPs. Serum OCP values adjusted for total serum lipids are presented in Table 2. Mean

concentrations were particularly high for *p,p'*-DDE and *p,p'*-DDT compared to the other OCPs.

3.3. Organochlorine pesticide concentrations according to demographic and socio-economic features

Table 2 also shows that, according to bivariate analyses, there were no significant differences in OCP concentrations according to age or gender. However, the area of residence had an impact on OCP levels and it was observed that levels of the four pesticides were higher in urban areas than in semi-urban and rural areas. In pairwise comparisons, a significant difference between urban and semi-urban areas was observed for *p,p'*-DDE ($p = 0.013$) and *p,p'*-DDT ($p = 0.003$), while a difference was detected between urban and rural areas for *trans*-nonachlor ($p = 0.014$) and β -HCH ($p = 0.021$). Table 2 also shows that OCP concentrations were higher in people with more education, in particular when comparing *p,p'*-DDE and *p,p'*-DDT levels in subjects with high-school or university education with those with no formal or primary level education ($p = 0.034$ and 0.014 , respectively). There was no difference in OCP levels according to the occupation of subjects. A significant and positive association was observed between *p,p'*-DDE, *p,p'*-DDT and β -HCH levels and wealth index, when comparing the upper tertile of wealth index with the first tertile ($p = 0.034$, 0.005 and 0.002 , respectively). Concerning BMI, obese people had higher levels of *p,p'*-DDT ($p = 0.013$) and β -HCH ($p = 0.029$) than underweight subjects.

We performed linear regression analysis to determine main predictors of each identified pesticide concentration. As shown in Table 3, the wealth index predicted β -HCH concentration independently of age and gender ($\beta = 0.27$; $p = 0.004$) as well as BMI ($\beta = 0.20$; $p = 0.030$).

Table 1
Characteristics of Borgou diabetic subjects.

Variables	N = 118	%	% of subjects according to BMI category				p-Value	% of subjects according to wealth index tertile			p-Value
			Underweight	Normal	Overweight	Obese		Low	Medium	High	
<i>Age (years)</i>											
18–29	9	7.6	0.0	14.0	2.3	5.9	0.189	5.9	2.7	12.8	0.141
30–39	11	9.3	14.3	4.0	15.9	5.9		2.9	16.2	8.5	
40–54	45	38.1	28.6	30.0	45.5	47.1		38.2	29.7	44.7	
55–65	53	44.9	57.1	52.0	36.4	41.2		52.9	51.4	34.0	
<i>Gender</i>											
Male	64	54.2	57.1	58.0	52.3	47.1	0.866	41.2	59.5	59.6	0.194
Female	54	45.8	42.9	42.0	47.7	52.9		58.8	40.5	40.4	
<i>Education</i>											
No schooling	54	45.8	57.1	52.0	40.9	35.3	0.207	73.5	45.9	25.5	<0.001*
Literacy and primary school	25	21.2	42.9	14.0	20.5	35.3		17.6	24.3	21.3	
High school and university	39	33.1	0.0	34.0	38.6	29.4		8.8	29.7	53.2	
<i>Occupation</i>											
Farmers	43	34.6	42.9	46.0	31.8	17.6	0.349	58.8	48.6	10.6	<0.001*
Manual workers	42	35.6	42.9	26.0	38.6	52.9		35.3	29.7	40.4	
Office workers	33	28.0	14.3	28.0	29.5	29.4		5.9	21.6	48.9	
<i>Wealth index</i>											
Low	34	28.8	71.4	24.0	31.8	17.6	0.037*				
Medium	37	31.4	28.6	32.0	36.4	17.6					
High	47	39.8	0.0	44.0	31.8	64.7					
<i>Residence location</i>											
Urban	51	43.2	42.9	38.0	38.6	70.6	0.154	17.6	37.8	66.0	<0.001*
Semi-urban	59	50.0	57.1	50.0	56.8	29.4		73.5	56.8	27.7	
Rural	8	6.8	0.0	12.0	4.5	0.0		8.8	5.4	6.4	
<i>BMI</i>											
Underweight	7	5.9						14.7	5.4	0.0	<0.001*
Normal	50	42.4						35.4	43.2	46.8	
Overweight	44	37.3						41.2	43.2	29.8	
Obese	17	14.4						8.8	8.1	23.4	

* $p < 0.05$ with Chi-square test.

Table 2
Adjusted serum concentrations of organochlorine pesticides in Borgou study subjects.

	Lipid-adjusted serum concentrations (ng/g total serum lipids)			
	<i>p,p'</i> -DDE	<i>p,p'</i> -DDT	β -HCH	<i>trans</i> -Nonachlor
<i>All participants</i>				
GM (CI 95%)	607.2 (453.0–813.9)	32.0 (22.8–45.0)	3.9 (3.0–5.0)	2.3 (2.0–2.8)
Median (25th–75th)	746.2 (269.8–1823.5)	43.1 (18.8–108.7)	3.3 (1.1–11.7)	1.8 (1.1–4.2)
Range	2.2–8360	0.3–1086	0.6–190.2	0.6–80.5
95th percentile	4598.3	495.3	42.9	12.4
<i>Age (years)</i>				
18–29	253.4 (26.7–2400.5)	23.0 (2.6–198.3)	2.3 (0.8–6.1)	1.7 (0.9–3.2)
30–39	244.4 (48.4–1233.0)	12.6 (2.2–69.5)	3.5 (1.1–11.0)	1.9 (1.0–3.6)
40–54	622.9 (407.5–952.2)	34.9 (21.1–57.7)	3.6 (2.5–5.2)	2.2 (1.7–2.8)
55–65	832.5 (609.5–1137.2)	38.2 (24.2–60.4)	4.6 (3.2–6.8)	2.7 (2.0–3.5)
<i>Gender</i>				
Male	797.7 (562.5–1131.1)	31.0 (19.3–49.9)	4.3 (3.0–6.2)	2.7 (2.1–3.5)
Female	439.5 (270.1–715.0)	33.2 (20.1–54.8)	3.4 (2.4–4.8)	1.9 (1.5–2.4)
<i>Residence area</i>				
Urban	1022.3 (738.4–1415.4) ^{*a}	55.9 (34.8–89.8) ^{*a}	4.8 (3.4–6.8) ^{*a}	2.8 (2.2–3.6) ^{*a}
Semi-urban	428.3 (270.9–677.1) ^b	22.1 (13.2–36.8) ^b	3.7 (2.6–5.4)	2.1 (1.6–2.7)
Rural	87.9 (50.2–1650.6)	14.3 (4.7–43.4) ^c	1.3 (0.6–2.8) ^b	1.3 (0.9–1.9) ^b
<i>Education</i>				
No schooling	450.1 (285.7–709.2) ^{*a}	24.4 (14.9–40.0) ^{*a}	3.0 (2.1–4.3)	1.9 (1.5–2.4)
Literacy and primary school	530.1 (280.8–1000–2)	22.5 (10.6–47.8)	5.2 (3.2–8.5)	2.8 (1.8–4.2)
High school and university	1002.8 (620.9–1619.7) ^b	58.4 (31.9–106.9) ^b	4.6 (2.8–7.5)	2.7 (2.0–3.7)
<i>Occupation</i>				
Farmers	528.5 (321.8–867.9)	23.1 (13.8–38.8)	3.5 (2.4–5.2)	2.1 (1.6–2.9)
Manual workers	532.9 (319.0–890.3)	31.0 (17.3–55.7)	4.1 (2.7–6.1)	2.6 (1.9–3.4)
Office workers	859.2 (497.0–1485.5)	50.9 (24.9–104.2)	4.1 (2.4–7.1)	2.3 (1.7–3.2)
<i>Wealth index</i>				
Low	463.3 (332.5–645.6) ^{*a}	19.7 (11.9–32.4) ^{*a}	2.1 (1.4–3.1) ^{*a}	2.0 (1.4–2.9)
Medium	619.0 (350.8–1092.4)	33.0 (17.3–63.0)	4.1 (2.6–6.4)	2.2 (1.6–3.0)
High	727.4 (418.0–1265.8) ^b	44.5 (24.5–80.8) ^b	5.8 (3.9–8.6) ^b	2.7 (2.1–3.5)
<i>BMI</i>				
Underweight	374.6 (231.6–605.8)	11.3 (2.6–48.2) ^{*a}	1.4 (0.8–2.6) ^{*a}	1.3 (0.9–1.8)
Normal range	675.9 (424.8–1075.2)	38.5 (23.6–62.8)	4.1 (2.8–6.1)	2.3 (1.8–3.1)
Overweight	539.1 (311.9–932.1)	24.8 (13.5–45.6)	3.4 (2.3–5.1)	2.5 (1.8–3.3)
Obese	735.8 (378.4–1430.6)	55.1 (20.9–145.2) ^b	6.8 (3.6–12.6) ^b	2.6 (1.7–3.9)

Values are expressed as geometric mean GM (95% CI).

For the other 10 OCPs, most concentration values were below the LOD in $\mu\text{g/L}$ and were therefore not reported as ng/g total serum lipids.

Geometric means with different superscript letters (a, b, c) were significantly different in Bonferroni multiple comparison test ($p < 0.05$) when upper groups were compared with the first, otherwise means were not statistically different.

* $p < 0.05$ for mean comparison with Kruskal–Wallis test.

However this relationship was not independent of the residence area. Similar results were found for BMI as predictor. Moreover, no significant relationship was found when considered BMI categories (results not shown). A univariate analysis, showed that residence area highly predicted each lipid-adjusted levels of *p,p'*-DDT ($\beta = -0.26$; $p = 0.004$), *p,p'*-DDE ($\beta = -0.28$; $p = 0.002$), β -HCH ($\beta = -0.20$; $p = 0.028$) and *trans*-nonachlor ($\beta = -0.22$; $p = 0.016$). Education level predicted lipid-adjusted levels of *p,p'*-DDT ($\beta = 0.19$; $p = 0.032$) and *p,p'*-DDE ($\beta = 0.21$; $p = 0.019$) but not β -HCH and *trans*-nonachlor. Although the estimates of variability were very low, BMI and wealth index are consistently associated with levels of β -HCH (Table 3).

3.4. Comparison of OCP levels in Borgou area and in other countries

Table 4 shows that mean lipid-adjusted concentrations of *p,p'*-DDT and *p,p'*-DDE in Borgou diabetics were higher than those reported for diabetics in case-control studies in Finland, Korea and Swedish. DDT compounds were higher in the current study than in some African countries including in Ghana and Tunisia, where DDT is no longer permitted. However, concentrations of *p,p'*-DDE and *p,p'*-DDT were much higher in South-Africa, Gambia and Guinea where these pesticides are still used for malaria control. Compared with developed countries, our study revealed higher *p,p'*-DDT and *p,p'*-DDE values than those reported in the

United States, Canada, France and Belgium populations. On the other hand, levels of β -HCH and *trans*-nonachlor were lower than those observed in all comparison countries.

4. Discussion

4.1. Levels of OCPs and sources of contamination

In our study, the four detected pesticides were present in high concentrations, in particular *p,p'*-DDT and its metabolite *p,p'*-DDE. The 10 other OCPs tested were not detected given their shorter half-life and their elimination from the environment since these are no longer used. DDT compound levels were higher than those observed in some African and non-African countries. However, comparison of current data with other studies requires cautious interpretation because study designs and methods vary with regard to sample size, types of populations and exposure, assay methods and data analysis. Some studies collected data in the general population while other assessed specific populations according to their particular degree of exposure to pesticides. Inadequate use including overdosage, uses for fishing or hunting, uses of cotton pesticides for food crop cultivations and uses of containers for household needs could contribute to these high levels in our study subjects.

Table 3
Linear regression of socio-economic or personal factors on organochlorine pesticide levels.

	Lipid-adjusted serum concentrations (ng/g total serum lipids)							
	<i>p,p'</i> -DDE		<i>p,p'</i> -DDT		β -HCH		<i>trans</i> -Nonachlor	
	β	p-Value	β	p-Value	β	p-Value	β	p-Value
Residence area ^a	−0.28	0.002*	−0.26	0.004*	−0.20	0.028*	−0.22	0.016*
Education ^a	0.21	0.019*	0.19	0.032*	0.14	0.123	0.17	0.06
Wealth index ^b	0.03	0.741	0.04	0.665	0.20	0.030*	0.12	0.211
Wealth index ^c	0.07	0.441	0.11	0.250	0.27	0.004*	0.14	0.130
Wealth index ^d	−0.04	0.705	0.01	0.910	0.19	0.060	0.05	0.585
BMI ^a	0.02	0.809	0.09	0.314	0.19	0.036*	0.08	0.38
BMI ^c	0.04	0.584	0.09	0.324	0.21	0.024*	0.10	0.238
BMI ^d	−0.001	0.994	0.050	0.582	0.18	0.052	0.07	0.428

Bold data are significant determinants of serum pesticide levels.

* $p < 0.05$ at multivariate linear regression.

^a Values are reported with no adjustment.

^b Adjusted model for BMI.

^c Adjusted model for age and gender.

^d Adjusted model for age and gender, BMI and residence area.

The *p,p'*-DDE metabolite of DDT was predominant among study subjects, suggesting a wide use of DDT and a long-term accumulation in Borgou people. A low ratio of *p,p'*-DDT/*p,p'*-DDE (0.05 in our study) is indicative of that past contamination, as observed in Ghana (Ntow et al., 2008). It is also possible that DDT was still being used after its prohibition in Benin in 2004 through fraudulent import of unlicensed pesticides. Unsafe storage of obsolete pesticides may be another contributing factor to OCP contamination. Pesticides, perhaps including prohibited ones, are available from retailers in the country and neighboring countries, which concurs to their use for fishing and food preservation (Hotton et al., 2011; Ntow et al., 2008). Acute pesticide intoxications and even deaths have been reported following consumption of staples stored with pesticides.

Owing to their persistence in human tissues and in the environment, contamination through the food chain likely continues to be the main source of exposure to these OCPs. Indeed, in Benin, DDT and other OCPs were detected recently in soils, river, fish, vegetables, cereals and beans at concentrations exceeding maximum acceptable limits as defined by the Food and Agriculture Organization and other organizations (Assogba-Komlan et al., 2007; Okoumassoun et al., 2002; Pazou et al., 2006a, 2006b).

Several other factors have to be considered in the interpretation of serum OCP concentrations at the individual level. Knowing that serum concentrations reflect adipose tissue levels, pesticide bioaccumulation in adipose tissues could be influenced by lifestyle, including physical activity and diet, weight loss and functional status of the colon (Imbeault

Table 4
Serum concentrations of organochlorine pesticides in Borgou diabetic subjects compared with findings in other countries.

Country (year)	Lipid-adjusted concentrations of organochlorine pesticides (ng/g total serum lipids)			
	<i>p,p'</i> -DDE	<i>p,p'</i> -DDT	β -HCH	<i>trans</i> -Nonachlor
Current study ^a (2011)				
AM \pm SD	1338.8 \pm 1536.8	99.6 \pm 170.3	10.0 \pm 20.4	4.1 \pm 8.2
GM (95% CI)	607.2 (453.0–813.9) ^c	32.0 (22.8–45.0)	3.9 (3.0–5.0)	2.3 (2.0–2.8)
Ghana (Ntow, 2001)	380 \pm 120			
Tunisia (Ben Hassine et al., 2013a)	168.8 \pm 158	24.3 \pm 18.8	26.3 \pm 34.7	
Guinea-Bissau ^b (Linderholm et al., 2010)	1400 ^f	210	38	
Gambia ^b (Manirakiza et al., 2002)	M: 4020 \pm 3960 F: 3250 \pm 2720	M: 1160 \pm 840 F: 900 \pm 920	M: 220 \pm 340 F: 240 \pm 440	
South Africa ^b (Channa et al., 2012a)				
Malaria area	3840 (3008–4902) ^c	2194 (1706–2823)		
Low risk	191 (116–315) ^c	38 (22–65)		
Non-malaria	29 (25–33) ^c	7 (6–7)		
Belgium (Dirinck et al., 2011)	205 (30.2–1073.2) ^d		19 (2–200)	
US, NHANES IV (CDC-Department of Health and Human Services, 2009)	238 (195–292) ^c	19.5 (15–27.5) ^e	56.5 (43.7–69.4) ^e	14.7 (13.1–16.5) ^c
France (Fréry et al., 2011)	120 (100–140) ^c	4 (3–5)	30 (28–38)	NA
Canada (Health Canada, 2010)	152 (127.0–182) ^c	15.9 (<LOD–25.8) ^e	6.39 (4.8–8.6) ^c	5.98 (5.3–6.8) ^c
Finland ^a (Airaksinen et al., 2011)	710 \pm 28			34 \pm 1.1
Sweden ^a (Rignell-Hydbom et al., 2007)	340 (93–970) ^e			
South Korea ^a (Son et al., 2010)	652.3 \pm 646.7	34.2 \pm 21.3	57.9 \pm 24.5	31.3 \pm 28.1

Note: Arithmetic means \pm standard deviations are presented as reported by authors unless indicated otherwise without any consideration about diabetes.

GM: geometric mean.

AM: arithmetic mean.

CI: confidence interval.

^a Data concern diabetic subjects only.

^b African country where DDT is still used for malaria control.

^c GM (95% CI).

^d Median (minimum–maximum).

^e 95th percentile (95% CI).

^f Mean.

et al., 2002; Lim et al., 2010). From a public health standpoint, the observed levels in the Borgou area are indicative of high exposure to DDT.

4.2. Pesticide levels and individual factors

We found no gender difference in OCP levels, which differs from results of the Ghana or Gambia studies where concentrations were higher in men than in women. Therefore, it cannot be argued that these pesticide levels are gender-dependent (Ntow et al., 2008). No significant difference was found for age although all the four OCP levels tended to be higher in the upper age-groups. This is consistent with a past contamination that is well expressed by the low DDE/DDT ratio.

4.3. Pesticide levels with socioeconomic status and obesity

In our study, wealthier people had higher serum concentrations of *p,p'*-DDE, *p,p'*-DDE and β -HCH. Moreover, β -HCH lipid-adjusted levels were significantly predicted by the wealth index independently of BMI but not residence area. Published research on socioeconomic status and pesticides is scarce and findings results are not consistent. Borrell et al. found no relationship between DDE levels and income among a population of pregnant women (Borrell et al., 2004) in the US while Porta et al. (2008) reported higher DDE levels among those classified on the lower occupational social classes (Porta et al., 2008). In addition, according to Porta et al. (2010) social and educational level did not show clear relationship with pesticides levels. In the context of Benin, our results could be explained by the fact that wealthier people often reside in the urban areas characterized by high environmental pollution through various sources such as: ambient air, industrial foods, building materials, furniture, plastics, cosmetics and perfumes. Indeed, we found that those in the urban areas demonstrated higher pesticides level than people living in rural areas. Moreover, residence area was a strong predictor of OCPs levels. Higher levels of serum OCPs in urban compared with rural areas have also been observed elsewhere (Channa et al., 2012a, 2012b).

We found that obese subjects presented higher levels of *p,p'*-DDT and β -HCH than other individuals. This was also observed in Finland and US studies (Dirinck et al., 2011; Lee, 2012). In low income countries such as Benin, the nutritional transition affecting those living in the urban areas has been associated with an increased obesogenic lifestyle, characterized by a physical inactivity and energy-dense diets (Aggarwal et al., 2011; Delisle et al., 2012; Sodjinou et al., 2009). The usually positive rural–urban gradient in fat intake could influence bioaccumulation of OCPs in adipose tissues. Additionally, exposure to multiple pollutants associated with city life, through air pollution, diverse food supply, industrial foods, building materials, furniture, plastics, cosmetics and perfumes must be taken into account since their effects may be synergistic on adipogenesis alteration and bioavailability of circulating pesticides in blood (Lubrano et al., 2013; Peters et al., 2011). The endocrine disruption induced by OCPs, with exacerbation of appetite, alteration of adipogenesis and energy balance (Ershow, 2009; Janesick and Blumberg, 2011; Pelletier et al., 2003), should also be taken into account when attempting to understand the association between higher socioeconomic status, lifestyle, obesity and diabetes.

Relation between diabetes, obesity and pesticide concentrations will be examined in more depth in other publications.

4.4. Study limitations

This study design does not allow extrapolating the findings to the entire population of Borgou but only to diabetics of this department. The study focused on diabetes and hence levels of OCPs found in this study can overestimate the real level in the whole population. Trophic chain is the main way by which humans can be contaminated with these pesticides especially in this study population since OCPs are no longer used; sources of exposure are being examined in the study

population. Further analysis will examine thoroughly the association between diabetes and those OCPs.

Area labeling according to extent of urbanization should be considered with caution. In a developing country context, the definition of an urban area is relative, as it takes into account the population, but also the availability of some facilities and services that are not available in other areas of the country. These facilities cannot always differentiate an urban city from a rural one (Thomas and Djaouga, 2008). Some areas described as 'urban', for instance, would not qualify as cities in other settings, as roads and other infrastructures are almost non-existent.

5. Conclusion

OCP contamination as revealed by high serum concentrations among subjects with diabetes should be regarded as a serious health concern in the study area and other similar areas of the country since many chronic diseases are currently associated with persistent pollutant exposure, even at low doses. Some OCPs are no longer used in Benin, but their levels in human serum remain high. It will be useful in the future, to measure serum levels of other pesticide groups such as pyrethroids, carbamates, organophosphates and newer categories as these are currently used in liberal amounts.

The set-up of a program for biomonitoring of exposure to environmental pollutants is compelling. Policies to minimize pesticide use should be seriously considered. Meanwhile, education focusing on better practices may help to reduce health risks associated with pesticide and other organic pollutant exposure.

Conflicts of interest statement

The authors declare there are no conflicts of interest.

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