



Inadvertent exposure to organochlorine pesticides DDT and derivatives in people from the Canary Islands (Spain)

Manuel Zumbado^{a,b}, Muriel Goethals^{a,b}, Eva E. Álvarez-León^{b,c}, Octavio P. Luzardo^{a,b}, Félix Cabrera^{a,b}, Lluís Serra-Majem^{b,c,d}, Luis Domínguez-Boada^{a,b,*}

^aToxicology Unit, Department of Clinical Sciences, University of Las Palmas de Gran Canaria, P.O. Box 550, 35080, Las Palmas de Gran Canaria, Spain

^bInstituto Canario de Investigación del Cáncer (ICIC), Spain

^cPreventive Medicine Service, Complejo Hospitalario Materno Insular de Gran Canaria, Canary Health Service, Avda. Marítima s/n, 35016, Las Palmas de Gran Canaria, Spain

^dPreventive Medicine and Public Health Unit, Department of Clinical Sciences, University of Las Palmas de Gran Canaria, P.O. Box 550, 35080, Las Palmas de Gran Canaria, Spain

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Abstract

In 1998, one of the largest determinations of organochlorine pesticides in a representative sample of a Spanish population (682 serum samples from the Canary Islands) was made in the context of the “Canary Islands Nutrition Survey” (ENCA). In the Canary Islands, extensive farming areas have been developed in these last decades, with greenhouses dedicated to intensive cultivation using DDT in huge amounts. In Spain, similarly to other European countries, DDT was banned in the late 1970s. The pesticide residues in human serum are indicative of past and present exposure to them. Our objective is to point out the differences of pesticide contamination between islands; and together with this, if a connection could be established with gender, age, or habitat of subjects. Concentration of selected persistent organochlorine pollutants (*p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD, and *o,p'*-DDD) was measured with gas chromatography-electron capture detector. Almost all of the samples (99.3%) presented detectable levels of some DDT-derivatives, being *p,p'*-DDE the most frequently detected organochlorine. The median concentration of total DDT body burden, expressed in ng/g fat, present in the Canary Islands (370 ng/g fat) was similar to that found in other European countries, although it was noteworthy that a fourth of the population showed a total DDT body burden higher than 715 ng/g. Interestingly, statistical significant differences were found in serum levels of organochlorine pesticides between islands, being these levels higher in people from Tenerife and Gran Canaria (415 and 612 ng/g fat, respectively), the islands that present both highest population and highest surface devoted to intensive agriculture. As expected, serum levels of both total DDT body burden and *p,p'*-DDE increased with age. Statistically significant differences were also found in relation to gender, women showing higher levels of these organochlorine pesticides

* Corresponding author. Toxicology Unit, Department of Clinical Sciences, University of Las Palmas de Gran Canaria, P.O. Box 550, 35080, Las Palmas de Gran Canaria, Spain. Tel.: +34 928 453 472; fax: 34 928 451 416.

E-mail address: ldominguez@dcc.ulpgc.es (L. Domínguez-Boada).

than men. One of the most relevant findings of this work is the presence of a very high DDT/DDE ratio in people from the Canary Islands, thus indicating a chronic exposure to DDT that persists nowadays. DDT and its derivatives are considered as xenoestrogens, and they have been linked to breast cancer. Having into account that the Canary Islands present high incidence and mortality for breast cancer, our results point to the possibility that these environmental contaminants may be playing a determining role that must be investigated in detail. The high levels of DDT and its derivatives in serum from people living in this Archipelago should be considered a matter of public health concern.

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1. Introduction

In 1998, a population study was set to assess nutritional factors in humans, between 6 and 75 years old, living in the Canary Islands. The study was called the “Canary Islands Nutrition Survey” (ENCA, in its Spanish initials) (Serra-Majem et al., 2000a,b; Díaz Romero et al., 2001). Serum from a representative sample of the total population of the Archipelago was obtained. As part of the study, serum concentration of the organochlorine pesticide DDT and its derivatives (OC-DDTs) was determined in 682 serum samples from ENCA.

It is well established that residues of pesticides and their metabolites in human fluids collected from a general population spectrum are indicative of the total body burden of these pesticides and of past and present exposure to them (Jaga and Dharmani, 2003). OC-DDTs are ubiquitous, they persist in the environment and have been measured in air, water, aquatic and marine sediments, and in fish and wildlife (Muckle et al., 2001; Covaci et al., 2002). Technical DDT (*p,p'*-DDT—the main component—and *o,p'*-DDT) was used extensively all over the world as a domestic and agriculture pesticide (Ecobichon, 1995). In most countries, it was banned in the 1970s due to its long residual life and its accumulation in food chains (Bolt and Degen, 2002). Environmental exposure to *p,p'*-DDT and its main metabolite *p,p'*-DDE or dietary sources exposure (especially, food of animal origin, but also through water, outdoor and indoor air, dust and soil) result in the bioaccumulation of these chemicals in the human body (especially, adipose tissue, serum, and breast milk) (DeVoto et al., 1998; Snedeker, 2001; Covaci et al., 2002; Cruz et al., 2003).

DDT has a very long average-life. In addition, it is metabolized to DDE, which tends to persist much longer in the body, and this metabolite is of greater concern as regards bioaccumulation, since it is a marker of chronic exposure (Jaga and Dharmani, 2003). These chlorinated pesticides present estrogenic properties, both in in vivo and in vitro studies, (Gellert et al., 1972; Soto et al., 1995). In fact, DDT and its metabolites are considered as endocrine disrupters (Colborn et al., 1993). Parental compounds (*p,p'*-DDT and *o,p'*-DDT) present in technical formulations of DDT are the most estrogenic among all DDT derivatives (Robinson et al., 1985). Environmental contaminants with estrogenic properties may exert a number of deleterious effects in human beings. It is remarkable that, although current available data are not considered to be consistent enough yet, these contaminants may be linked to environment-induced breast cancer (Wolff et al., 2000; Snedeker, 2001; Jaga and Dharmani, 2003; López-Cervantes et al., 2004). Although, as previously mentioned, most human exposure to OC-DDTs can probably be linked to dietary contamination, other factors, such as age, gender, and type of habitat, must also be taken into account (Glynn et al., 2003).

Certain number of studies that analyze the presence of organochlorine residues (pesticides and also other substances as PCBs, PCDD/PCDFs) in the Spanish population have been published, but most of them concentrate on biological samples other than serum (Camps et al., 1989; Ferrer et al., 1992; Gómez-Catalán et al., 1993; Falcon et al., 2004). Nevertheless, we can consider that 80–100% of the Spanish population present detectable levels of *p,p'*-DDE or any other organochlorine residues (Porta et al., 2002). Similarly, it has drawn our

attention that no analyses of the presence of OC-DDTs in the Canarian population have been done up to this day.

Although in Spain, as in most European countries, DDT was banned in the late 1970s (actually, in Spain it was banned in 1977) (Ferrer et al., 1992; Gómez-Catalán et al., 1993), it is currently used in African countries, such as Morocco. Having into account that the Canary Islands are located very close to Africa yet enjoy a European lifestyle, the comparison between the serum levels of OC-DDTs in Canarian people with the levels previously reported from both people from Europe (developed countries) and people from Africa (underdeveloped countries) could be of interest.

The purpose of this study was to determine the levels of OC-DDTs in serum from people of the Canary Islands and to look into possible associations between these levels and sociodemographic and geographical factors. It is remarkable the fact that this is an interesting group of study due to the condition of archipelago that makes this group a relatively isolated one, even more so in the case of the smaller islands. This is an important fact in order to establish the potential sources of these environmental contaminants. An additional source of interest could be to compare the results for the total DDT body burden with the results obtained from the DDT isomers and DDT metabolites (mainly DDE) separately, because geographical variations in body burden may vary depending on the local use of pesticides, mainly in agricultural practices, and subsequent environmental pollution. Furthermore, such differences may indicate the possibility that geographical

regions located near underdeveloped countries (which continue using DDT) could be exposed nowadays to this environmental pollutant.

2. Materials and methods

2.1. Study area

The Canary Islands are located 1600 km away from southwest Spain, in the Atlantic Ocean, and hardly 100 km from the nearest point of the North African coast (south west of Morocco). Geographically, the Islands are part of the African continent, yet from a historical, economic, political and socio-cultural point of view the Canaries are completely European. As shown in Fig. 1, the Archipelago consists of seven major islands, as well as a number of smaller, mainly uninhabited ones. The seven major islands are the ones considered in this study. The population of the Canary Islands between 6 and 75 years old, was, at the moment of the study, of approximately 1.3 million people, equally divided between the two Provinces of the Archipelago. Around half the people are concentrated in the metropolitan areas of the capital islands (the city of Las Palmas de Gran Canaria, in the island of Gran Canaria, and the city of Santa Cruz de Tenerife, in the island of Tenerife). Only in these islands real urban population exists (in the sense of metropolitan areas of more than 100,000 inhabitants), while the population of the rest of the islands should be considered as rural (<10,000 inhabitants) or semi-urban population (10,000–100,000 inhabitants). The economy of the

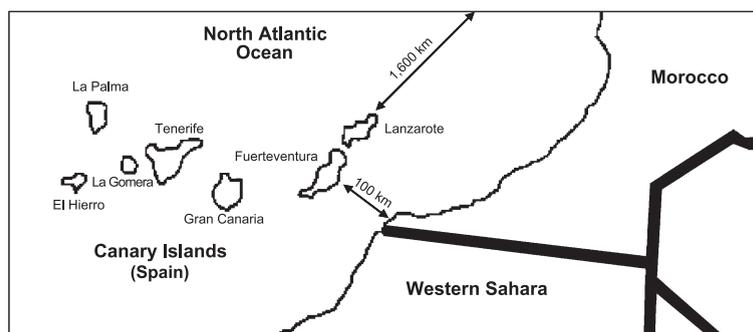


Fig. 1. Canary Islands Archipelago in northwestern African coast (arrows: minimal distance from Spanish and Moroccan coasts).

Canary Islands is based fundamentally on a few economic sectors: tourism and, to a much lesser extent, farming and fishing. Other economic sectors, such as traditional polluting industries, have a limited presence in the Islands.

In these last decades, farming in these Islands has turned to an intensive kind of agriculture (plastic greenhouses). It is well known that intensive agriculture uses pesticides in large amounts (Olea et al., 1999). In order to have a clearer idea of the current state of things, suffice it to say that approximately 15% (around 7000 ha) of the entire area devoted to farming in the Canary Islands belongs to intensive agriculture. The surface covered with plastic greenhouses is very important in the two capital islands (Gran Canaria and Tenerife); in fact, in the rest of the islands agriculture is mainly of a traditional type. Half of all surface devoted to intensive agriculture in the Archipelago is located in Gran Canaria (around 3500 ha). In 2001, it was calculated that the Canary Islands consumed 12 times as much pesticides by hectare than the rest of the country (Díaz-Díaz et al., 1999).

2.2. Study group and sample collection

The ENCA nutritional survey was conducted in 1997–1998 in the seven major Islands of the Canary Archipelago. The sample was stratified in two stages, and it was representative of the population between 6 and 75 years of age for both genders. A total of 1747 subjects participated in the first part of the study. This part consisted in two individual interviews with questions about dietary variables, life habits, and health condition. The participants had blood samples extracted after 12-h fasting in order to determine biochemical parameters and the presence of organochlorine pesticide residues. A total of 783 subjects participated in the biochemical part (participation rate, 44.8%). The OC-DDTs were measured in 682 subjects. The distribution of their sociodemographic characteristics is shown in Table 1.

Approximately 40 ml of blood was collected from each individual into a vacuum system tube and centrifuged at 4 °C (15 min×3000 rpm) in order to obtain serum. Serum was distributed in aliquots of 2–3 ml and kept frozen at –80 °C until analyzed. All

Table 1
Sociodemographic characteristics of the population studied

	N (%)
Total samples	682 (100)
Gender	
Men	302 (44.3)
Women	380 (55.7)
Age (years)	
<18	149 (21.8)
18–34	139 (20.4)
35–49	167 (24.5)
50–64	147 (21.6)
65–75	80 (11.7)
Island	
Gran Canaria	249 (36.5)
Lanzarote	37 (5.4)
Fuerteventura	44 (6.5)
Tenerife	193 (28.3)
La Palma	79 (11.6)
La Gomera	49 (7.2)
El Hierro	31 (4.5)
Habitat	
Rural (<10,000 inhabitants)	309 (45.3)
Semirural (10–100,000 inhabitants)	187 (27.4)
Urban (>100,000 inhabitants)	186 (27.3)

collection and handling equipment in contact with serum specimens was tested as possible sources of OC-DDTs contamination. No contaminating materials were identified.

The local ethics committee approved the design of this study, and informed consent was obtained from the study participants.

2.3. Materials and analytical methods

The OC-DDTs residues we measured in serum were DDT isomers, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT), 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane (*o,p'*-DDT), and DDT metabolites, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE), 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene (*o,p'*-DDE), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDD), and 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane (*o,p'*-DDD). The standard analytes under investigation were purchased from Pestanal (Riedel-de Haën, Sigma-Aldrich Laborchemikalien, Germany). Anhydrous sodium sulphate was provided from Scharlau Chemie (Barcelona, Spain). The chlorinated biphenyl (204) used as surrogate was

purchased from AccuStandard (CT, USA), at a concentration of 35 µg/mL in iso-octane. The internal standard (pentachloronitrobenzene) was obtained from Supelco (Sigma-Aldrich, PA, USA), at a concentration of 5000 µg/mL in methanol. Sulphuric acid was analytical grade (Scharlau Chemie).

Analytical methods to identify organochlorine pesticides by gas chromatography with electron capture detection and capillary columns have been reported. A simple, sensitive, and efficient liquid–liquid extraction method prior to analytical detection has been used in this study (Veierov and Aharonson, 1980; To-Figueras et al., 1995). In brief, sample extraction and cleaning up was as follows: PCB 204 (CAS number 74472529; 2,2',3,4,4',5,6,6'-octachlorobiphenyl) was added, as surrogate, to 500 µL of serum (Najam et al., 1999). The serum was extracted twice with 2 mL of hexane. The combined extracts were treated with concentrated sulphuric acid (200 µL). Then, the organic layer was removed, and 100 mg of anhydrous sodium sulphate was added. Finally, the transferred organic layer was concentrated to 500 µL under a stream of nitrogen at room temperature. The internal standard, pentachlorobenzene, was added to the final concentrate, prior to GC analysis. A volume of 1 µL of the sample was injected.

Quantitative analyses of the OC-DDTs were undertaken on a gas-chromatography (GC)-electron capture detector (^{63}Ni) (HP6890 with HP2397A µECD, Agilent Technologies, CA, USA). A 5% phenyl methyl siloxane capillary column of 30 m×0.22 mm i.d.×0.25 µm (HP5 Supelco, Sigma-Aldrich, PA, USA) was used throughout the analyses. The GC operating conditions were as follows: carrier gas helium and argon–methane (95+5) as make-up gas at a flow rate of 1.5 ml/min; pulsed splitless injection; an initial temperature of 50 °C was held for 2 min, then the temperature was increased to 200 °C at a rate of 30 °C/min, and finally to 280 °C at a rate of 5 °C/min. The analytical limit of detection was 1 ppb (ng/g fat) for all the analytes tested. The accuracy of the instrumental methods and analytical procedures were checked by using reference solutions of pure analytical grade. Recovery rates, conducted in serum samples at the fortification level 1–1000 ng/ml, were 90.9±1.18%, 96.8±1.62%, 89.9±0.98%,

93.2±1.24%, 89.60±1.09%, 91.89±1.28% for *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD and *p,p'*-DDD, respectively.

The serum OC-DDTs concentrations used in the analysis were lipid-adjusted. Total cholesterol and triglycerides were measured with an automatic Hitachi Analyser 717 (Boehringer Mannheim, IN, USA). Estimates of total serum lipids were calculated by the formula established previously by other experts (Phillips et al., 1989):

$$TL \text{ (total lipids)} = 2.27 \text{ TC (total cholesterol)} \\ + \text{ TG (triglycerides)} + 0.623$$

Lipid adjustment is recommended because temporary changes in serum lipid content can be noticed this way, and lipid-adjusted serum concentrations give a better estimate of the burden (Koppen et al., 2002). In this work we expressed the total DDT body burden as the sum of the six OC-DDTs measured (*p,p'*-DDT, *o,p'*-DDT isomers and their metabolites *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD). However, as in most of the articles cited in this text, the DDT/DDE ratio has been calculated only with the serum values of *p,p'*-DDT and *p,p'*-DDE (Jaga and Dharmani, 2003).

2.4. Statistical analysis

Database management and statistical analysis were performed with SPSS v 11.0 (SPSS, Chicago, IL, USA). The distribution of serum OC-DDTs levels are not normal, and they are expressed with the median and the percentiles 25–75. If median and both percentiles are 0 ng/g fat mean values and standard deviation of OC-DDTs are shown. The continuous variables that followed a normal distribution were presented as mean and standard deviation. Differences in the OC-DDTs levels between two groups or more were tested with the non-parametric Mann–Whitney *U*-test and Kruskal–Wallis test. The categorical variables were presented as percentages and were compared between variables with the χ^2 . The correlation of the organochlorines with the continuous variables was analyzed by the Spearman correlation test. *P* value of less than 0.05 (two-tail) was considered to be statistically significant.

3. Results

3.1. Occurrence of selected OC-DDTs residues in serum samples

As presented in Fig. 2, most of the serum samples showed the presence of some type of DDT-residue, being the most frequent one *p,p'*-DDE (the main metabolite of *p,p'*-DDT), which was present in 88% of the samples, while *o,p'*-DDE (the main metabolite of the other DDT-isomer, *o,p'*-DDT) showed detectable levels only in a very low number of samples (less than 2%). Oddly enough, DDT isomers used in the technical formulations of DDT nowadays prohibited, namely *o,p'*-DDT and *p,p'*-DDT, were present in nearly 40% of the samples. The other metabolites (*p,p'*-DDD and *o,p'*-DDD) were found in lower number of serum samples (20.6% and 6.43%, respectively). In any case, if we take the sum of the six DDT-derivatives measured as total DDT body burden, we could observe that almost all the samples showed detectable residues of OC-DDTs. In Table 2, we show the concentration of these pesticides in serum. The values of parental isomers of DDT (242 and 256 ng/g fat in percentile 75 for *p,p'*-DDT and *o,p'*-DDT, respectively) and the metabolite *p,p'*-DDE (216 ng/g fat in percentile 75) were the residues of OC-DDTs that we found in higher concentrations, while the levels of the rest of OC-DDTs measured were very low. The total DDT body burden, perhaps the most relevant value, was around

370 ng/g fat, although it was noteworthy that the values of total DDT body burden for percentile 75 was around 715 ng/g fat.

3.2. Sociodemographic and geographical factors affecting OC-DDTs residue levels

3.2.1. Age

The serum values of total DDT body burden and *p,p'*-DDE increased with age (*p* for trend <0.001) (Table 2). The highest levels of these OC-DDTs appeared in subjects that were born before the official prohibition to use DDT in Spain (age ≥ 20) (Table 3).

3.2.2. Gender

There were statistically significant differences between genders in serum levels of the main metabolite of DDT, *p,p'*-DDE; thus, women showed higher levels than men (Table 2). When the levels are analyzed by age and Islands, some interesting results are found. As it is shown in Fig. 3, women of 20 years or older living in Gran Canaria were the group of women with highest levels of *o,p'*-DDT, *p,p'*-DDE and total DDT (*p* < 0.001). It was remarkable that a quarter of these women showed around 1000 ng/g fat of total DDT body burden in serum.

3.2.3. Island

As shown in Table 2, the serum samples of the population of Gran Canaria showed the highest median levels of residues of total DDT (612 ng/g fat). It must

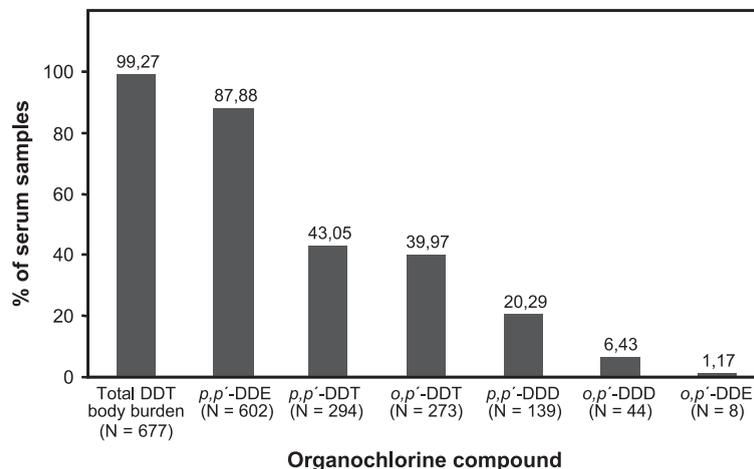


Fig. 2. Occurrence of organochlorines DDT and its derivatives in serum samples from studied population.

Table 2

Sociodemographic and geographical factors affecting the presence of DDT and its derivatives residues in the population studied (median values expressed in ng/g fat): gender, age, island, and habitat

Samples group	N (%)	<i>o,p'</i> -DDE	<i>p,p'</i> -DDE	<i>o,p'</i> -DDD	<i>p,p'</i> -DDD	<i>o,p'</i> -DDT	<i>p,p'</i> -DDT	Total DDT body burden	Ratio DDT/DDE
Total	682 (100)	1.75 (17)	118 (68.2–216)	36 (181)	31.2 (93.7)	0 (0–256)	0 (0–242)	370 (129–715)	0 (0–1.30)
Gender									
Male	302 (44.3)	2.76 (22.5)	96.1 (61.1–168)	33.4 (136)	30.1 (73.2)	0 (0–261)	0 (0–244)	344 (116–694)	0 (0–1.15)
Female	380 (55.7)	0.95 (10.8)	132 (78.3–237)**	38.1 (211)	32.2 (107)	0 (0–254)	0 (0–240)	390 (135–730)	0 (0–1.32)
Age									
<18	149 (21.8)	1.03 (12.5)	65.1 (0–93.3)	13.4 (66.4)	35.5 (97.2)	0 (0–287)	0 (0–273)	216 (74.3–599)	0 (0–0)
18–34	139 (20.4)	1 (11.9)	90.8 (60.3–134)	41.1 (182)	44 (144)	0 (0–267)	0 (0–262)	282 (84.8–694)	0 (0–1.94)
35–49	167 (24.5)	2.44 (18.5)	134 (83.5–225)	55.8 (274)	36.1 (87.3)	0 (0–248)	0 (0–217)	390 (162–730)	0 (0–1.54)
50–64	147 (21.6)	2.65 (24.3)	177 (120–335)	39.4 (156)	15.5 (36.4)	0 (0–267)	0 (0–238)	475 (211–827)	0 (0–1.29)
65–75	80 (11.7)	1.32 (11.9)	224 (128–417)** ^f	21.7 (111)	19.7 (44.5)	0 (0–229)	0 (0–215)	511 (228–887)** ^f	0 (0–0.73)
Island									
Gran Canaria	249 (36.5)	1.01 (11.5)	117.8 (44.6–188)	23 (130)	13.1 (94.4)	250 (0–343)**	233 (0–326)**	612 (305–836)**	0.98 (0–2.11)*
Lanzarote	37 (5.4)	0 (0–0)	104 (71.4–239)	0 (0–0)	42 (53.4)	N.A.	0 (0–0)	170 (74.6–301)	0 (0–0)
Fuerteventura	44 (6.5)	0 (0–0)	94.4 (68.5–159)	0 (0–0)	27.7 (51.6)	N.A.	N.A.	118 (69.1–215)	N.A.
Tenerife	193 (28.3)	4.86 (28.9)	118 (71.2–224)	94 (298)	40.1 (120)	0 (0–256)	106 (0–264)	415 (139–839)	0 (0–1.90)
La Palma	79 (11.6)	0 (0–0)	140 (93.5–348)**	1.84 (16.3)	81 (66)**	0 (0–0)	0 (0–0)	268 (190–465)	0 (0–0)
La Gomera	49 (7.2)	0 (0–0)	117 (74.6–292)	3.9 (19.1)	19.2 (42.6)	N.A.	0 (0–0)	136 (80.7–386)	0 (0–0)
El Hierro	31 (4.5)	0 (0–0)	94.5 (58.1–151)	9.67 (41.7)	6.43 (25.3)	0 (0–0)	0 (0–0)	113 (63.6–255)	N.A.
Habitat									
Rural	309 (45.3)	0.95 (11.8)	110 (63.7–195)	12.8 (102)	33.6 (108)	0 (0–194)	0 (0–194)	263 (94.4–664)	0 (0–0)
Semirural	187 (27.4)	0 (0–0)	126 (78.9–226)*	6.83 (68.3)	42 (99.8)*	0 (0–212)	0 (0–196)	345 (161–649)	0 (0–1.08)
Urban	186 (27.3)	4.82 (28.6)**	120 (64.7–192)	104 (304)**	16.6 (51.3)	216 (0–363)**	196 (0–342)**	542 (178–929)**	0.78 (0–2.19)** ^f

The values are expressed in median (percentil 25–percentil 75); or in mean (Standard Deviation) in case the median and percentiles were 0.00 ng/g.

N.A.: Not Applicable (no detection of OC-DDTs in any individual).

* $p < 0.05$.

** $p < 0.01$.

**^f p tendency > 0.01 .

Table 3
Difference in levels of organochlorines (ng/g fat) in people born before or after the official prohibition of DDT use

Compound	Age (years)	N	Median (p25–p75)	Mean (S.D.)	p
<i>o,p'</i> -DDE	<20	167	0 (0–0)	1.90 (19.7)	N.S.
	≥20	518	0 (0–0)	1.64 (14.6)	
<i>p,p'</i> -DDE	<20	167	80.2 (61.8–106)	85.7 (75.1)	<0.001
	≥20	518	151(98.6–269)	262 (305)	
<i>o,p'</i> -DDD	<20	167	0 (0–0)	28.2 (139)	N.S.
	≥20	518	0 (0–0)	42 (208)	
<i>p,p'</i> -DDD	<20	167	0 (0–99.4)	39 (121)	N.S.
	≥20	518	0 (0–0)	25.3 (65.2)	
<i>o,p'</i> -DDT	<20	167	0 (0–0)	160 (307)	N.S.
	≥20	517	0 (0–219)	161 (269)	
<i>p,p'</i> -DDT	<20	167	0 (0–0)	171 (325)	N.S.
	≥20	517	0 (0–205)	181 (363)	
Total DDT body burden	<20	167	110 (65.3–253)	480 (730)	<0.001
	≥20	516	388 (139–731)	673 (782)	
Ratio DDT/DDE	<20	67	0 (0–0)	0.58 (1.52)	<0.001
	≥20	16	0 (0–0)	1.08 (2.53)	

p=Kruskal-Wallis; N.S.:Not significant.

be highlighted that one fourth of the population of Gran Canaria and Tenerife showed a very high total DDT body burden (more than 835 ng/g fat). The

samples from the population of Gran Canaria showed (see Table 2) higher values of the more estrogenic compounds (*p,p'*-DDT and *o,p'*-DDT), *id est* the parental compounds, than those from the rest of the Islands. Nevertheless, serum samples from La Palma island showed the highest values of the main metabolites of the parental isomers most frequently used in technical formulations of DDT (*p,p'*-DDE and *p,p'*-DDD) (Table 2).

3.2.4. Habitat

The type of habitat did exert a clear influence on serum values of OC-DDTs. People from semiurban areas showed significantly higher levels of the metabolites *p,p'*-DDE and *p,p'*-DDD, whereas people from urban areas showed higher levels of the non-metabolized pesticides (*p,p'*-DDT and *o,p'*-DDT) and the Total DDT body burden, and also *o,p'*-DDD (Table 2).

3.3. DTT/DDE ratio

It has been established that the DDT/DDE ratio is an indication of environmental exposure to DDT and/or occupational exposures that persist nowadays (Jaga

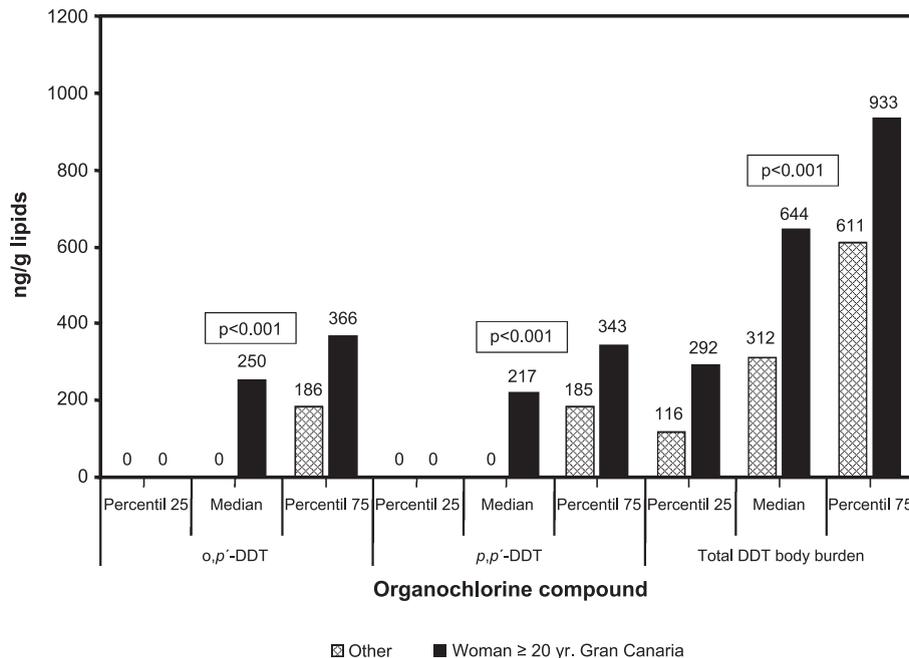


Fig. 3. Levels of *o,p'*-DDT, *p,p'*-DDT, and total DDT body burden in women ≥20 years old living in Gran Canaria, compared with the rest of Canary women.

and Dharmani, 2003). In this work, we found that the DDT/DDE ratio was very high (around 0.95). From the total of the population studied, more than one out of every four individuals, 28%, showed values of DDT/DDE ratio higher than 1 (Table 2). Furthermore, it was remarkable the high value of this ratio in the Gran Canarian population, where around 50% of the population showed values higher than 1. In the other capital island, Tenerife, the values for this ratio were, also, very high, with 38% of the population in levels of up to 1. When the DDT/DDE ratio is analyzed by age, the highest levels are found in the generation that was born between 1970 and 1980, in fact, around the time when the official prohibition to use DDT began to be enforced ($p < 0.001$). The lowest levels are found in the youngest generation (Tables 2 and 3).

Our study did not find differences in DDT/DDE ratio between genders, although if we analyzed only the women older than 20 living in Gran Canaria, we found that a fourth of these women have a DDT/DDE ratio of nearly 2 (Table 4). This excess of ratio is maintained in each group of age, especially in the younger generations, where the median ratio is 130 times higher in Gran Canaria women than in the rest of Canarian women (Table 4).

As regards the habitat, although in the global sample the urban inhabitants showed higher levels of DDT/DDE ratio ($p < 0.01$) (Table 2), if we look only at women, we can see that the women living in the

semirural habitat of Gran Canaria had the highest ratio than the rest of Canarian women (Table 4).

It must be highlighted that there were very marked differences in DDT/DDE ratio between islands. Thus, La Palma and Lanzarote showed very low ratio values, thus indicating geographical variations in relation to exposure to these environmental contaminants (Table 2).

4. Discussion

Our data confirm that almost all the people from the Canary Islands, just as the rest of the world population, present some type of OC-DDTs residues in serum (Snedeker, 2001). As expected, it is p,p' -DDE, the main metabolite of technical DDT, the most frequently detected residue (Snedeker, 2001; Glynn et al., 2003), while other DDT metabolites such as DDD isomers, are detectable only in a very low proportion of the population. These results are consistent with the data published previously in the sense that p,p' -DDT breaks down at a relatively rapid pace in the environment to become the most persistent form of this pesticide (p,p' -DDE) (Safe, 1994; Ecobichon, 1995). The levels of this metabolite are frequently used to evaluate chronic exposure to DDT.

Most studies show an association between p,p' -DDE levels and age (Glynn et al., 2003). In our study, we confirm such a circumstance. Among the elderly people from Canary Islands, lipid-adjusted serum concentration of p,p' -DDE and total DDT body burden increase with age. When we adjusted the population by age, we could observe, as expected, that people born before the official prohibition to use DDT in Spain (around 20 years old) show higher levels of residues than the people born afterwards.

As previously reported (Charlier and Plomteux, 2002), it is noticeable the important influence that gender exerts in total DDT body burden and p,p' -DDE levels. Women from Canary Islands present higher levels of these organochlorines than men. Older women present higher levels of metabolites of DDT (p,p' -DDE), while younger women present higher levels of the parental compound (p,p' -DDT).

In respect with the habitat, it is remarkable the fact that in this study, urban inhabitants showed the

Table 4
Differences in DDT/DDE Ratio between women from Gran Canaria and women from the rest of the Archipelago

	Ratio DDT/DDE				<i>p</i>
	Women ≥ 20 years in Gran Canaria		Rest of women		
	<i>N</i>	Median (p25–p75)	<i>N</i>	Median (p25–p75)	
Total	127	0.94 (0–2)	305	0 (0–0.42)	<0.001
Age					
20–34	36	1.3 (0–2.88)	58	0 (0–1.05)	<0.05
35–49	44	1.39 (0.56–2.7)	73	0 (0–0.39)	<0.001
50–64	33	0.84 (0.04–1.63)	60	0 (0–0.37)	<0.001
65–75	14	0.7 (0.19–1.35)	29	0 (0–0.35)	<0.01
Habitat					
Rural	53	0.73 (0–2.9)	141	0 (0–0)	<0.001
Semirural	28	1.2 (0.51–2.05)	87	0 (0–0)	<0.001
Urban	46	0.86 (0.11–1.58)	77	0.81 (0–2.99)	N.S.

p =Kruskal-Wallis; N.S.:Not significant.

highest levels of parental isomers of DDT and total DDT body burden. Attending to the nutritional data of this group from the ENCA survey (Serra-Majem et al., 2000a,b), this situation could be related with the high intake of animal and dairy products and also to the high consumption of animal- and vegetable-origin foods imported from Asia and Latin American countries, where DDT is still in use (Ahlborg et al., 1995; Cruz et al., 2003). However, people from semiurban areas presented highest levels of the *p,p'*-DDE (the main metabolite of the isomer most frequently used in technical formulations of DDT: *p,p'*-DDT). If we take into account that the largest farming areas with plastic greenhouses are located in semiurban populations of Gran Canaria and Tenerife, these results may well to indicate that agricultural practices in intensive cultivation could be related with the high serum levels of this DDT-metabolite.

Taking into account that the main source of OC-DDTs is the environment and food, we have studied the populations of the seven islands looking for differences between them. We find that people in the

two main islands (with many more inhabitants, with the largest urban areas and where the largest surface covered by plastic greenhouses are located) present higher levels of total DDT body burden than the people in the rest of the islands. It is noteworthy that people from Gran Canaria show the highest levels of the nowadays forbidden parental compounds *p,p'*-DDT and *o,p'*-DDT, in fact the more estrogenic forms of DDT. The population from Gran Canaria shows the highest intake of dairy products of the Canary Islands, and shows also one of the higher intakes of lipids and saturated fatty acids (Serra-Majem et al., 2000a,b).

As mentioned above, the median lipid-adjusted serum concentration of total DDT body burden measured in people from the Canary Islands (370 ng/g fat) is similar to that found in other European or American developed countries, if we compare our results with those published referring to Swedish, Belgian, North American, or Canadian people (Glynn et al., 2003; Koppen et al., 2002; Jaga and Dharmani, 2003). However, the situation is very different if we study the ratio DDT/DDE (Table 5). It is well known

Table 5
Status of DDT/DDE ratio in human serum from different countries in the world. Comparison with values obtained in the Canary Islands

Country	Year	N	<i>p,p'</i> -DDT (ng/g)	<i>p,p'</i> -DDE (ng/g)	DDT+DDE (ng/g)	Ratio DDT/DDE ¹
Mexico	1990–1995	246	190	3125	3315	0.06
Tanzania ²	1992	9	3034	2547	5581	1.19
Canada	1994–1997	532	12.6	486	498	0.02
Canada (population control)	1994–1997	305	11	480	491	0.02
Mexico	1994–1996	359	356	533	889	0.67
Brazil	1995–1996	493	–	692	–	–
Egypt	1996	48	3.9	38	41.9	0.10
Sweden	1996–1997	359	20.4	663	684	3
USA	1996–1997	190	–	1813	–	–
Canary Islands	1998	683	177	186	362	0.95
Gran Canaria		249	280	146	426	1.92
Tenerife		194	248	182	493	1.36
La Palma		79	8.9	276	285	0.03
La Gomera		49	32.4	250	283	0.13
Fuerteventura		44	0	131	131	0
Lanzarote		37	5.7	274	280	0.02
El Hierro		31	7.8	169	177	0.05
Australia ³	1999	68	–	3.9	–	–
Brazil	1999	33	–	357	–	–
Nicaragua	2000	52	–	7.1	–	–

¹ Ratio calculated with global data (mean *p,p'*-DDT/mean *p,p'*-DDE).

² Measured in adipose tissue.

³ Median values of *p,p'*-DDT.

that humans show a very limited capacity to metabolize DDT. We can consider that the p,p' -DDE found in human serum could have its origin in p,p' -DDE from dietary sources (Snedeker, 2001). In any case, the association of dietary patterns with levels of OC-DDTs in serum needs to be studied in depth.

Although p,p' -DDE is used as surrogate of past exposure to all sources of DDT and DDE, there is no way to distinguish how much of the p,p' -DDE present in human serum is a result of direct exposure to sprayed technical DDT, and hence to the more estrogenic forms of DDT (p,p' -DDT and o,p' -DDT), versus how much exposure is due to ingestion of dietary preformed p,p' -DDE. In an attempt to differentiate between historical exposure to DDT and current exposure, different authors have used the ratio DDT/DDE (Ahlborg et al., 1995; Jaga and Dharmani, 2003). A high DDT/DDE ratio is an indication of both chronic and also ongoing exposure to DDT. Conversely, a low DDT/DDE ratio implies high environmental persistence and ongoing bioaccumulation (Jaga and Dharmani, 2003).

In the population studied by us this ratio is very high, with one fourth of the population up to 1, thus indicating that, at present, there could be an active source of technical DDT in our islands. This result is more impressive in certain islands, especially Gran Canaria, where the people that present ratio levels of up to 1 constitute around half the entire population. These values are extremely high and, interestingly, they only have been reported previously in people from countries that currently use DDT in vector control and/or agriculture (Table 5), such as Tanzania and Mexico (countries whose populations are subjected to high exposure of technical DDT), although the results published previously about these countries were obtained in fat tissue, not in serum samples (van der Ven et al., 1992; Rivero-Rodríguez et al., 1997). All these results taken together point to the possibility that people from the Canary Islands could be currently exposed to a source of technical DDT. Such result is in accordance with those published previously (Villa et al., 2003).

It is well known that organochlorine pollutants, such as DDT-derivatives, are persistent, lipophilic chemicals and have low vapour pressures, favouring their widespread dispersal and accumulation (Olea et al., 1999). As cited previously, DDT was widely used as an insecticide (Ecobichon, 1995). Although its use

was banned nearly 30 years ago in North America and Europe, its production is allowed for export, and, in fact, it is not well documented when its use was definitely ended in the Canary Islands. After DDT was banned in the late 1970s, the only source of exposure to DDT in developed countries is, theoretically, via the ingestion of contaminated food (Hanaoka et al., 2002; Andersson and Skakkebaek, 1999). However, other sources must be taken in account.

The fact that in underdeveloped countries, a number of organochlorine pesticides (among them, DDT) are still running making it possible for the people living there and in the neighbouring areas to be under inadvertent exposure. This could be the case of people living in Canary Islands. In this Archipelago, as in the rest of the Spanish territory, DDT was banned in the late 1970s. Nevertheless, this organochlorine pesticide is currently used as an insecticide in the neighbouring State of Morocco. An explanation of the high levels of parental DDT in people from the Canary Islands is the possibility that there exists some deposition of DDT onto this Archipelago soils via volatilization and atmospheric transport from Morocco (Rapaport et al., 1985; Fries, 1995). However, the transport magnitude through this route is generally considered to be small, and we do not find a correlation between the proximity of every Island to the African coast and the serum levels of DDT.

As mentioned above, another interesting possibility could be the existence, in the Canary Islands market, of foods with high levels of residues of parental DDT isomers. Just as it happens in many other countries of Occidental Europe, Spain imports animal- and vegetable-origin foods from countries where DDT is still permitted (Cruz et al., 2003). Even more so, it is remarkable that due to its geographical, economic, and cultural circumstances, these imports are higher in the Canary Archipelago than in the mainland (Serra-Majem et al., 2000a,b).

In any case, whatever the considerations born in mind, there also could be a huge contamination of soil and water by DDT because of its uncontrolled use in previous decades. Island ecosystems, such as the Canary Islands, present geological features and chemical use patterns that contribute to potential soil and groundwater contamination (Allen et al., 1997; Díaz-Díaz and Loague, 2000). Further studies are

necessary to evaluate this possibility. Drastic geographical variations in DDT/DDE ratio between islands could indicate a very different use of DDT in the last decades, and, in fact, possibly, the existence of dramatic differences in soil and water contamination. In our opinion, although the possibility exists that the huge contamination of soil and water could be a current source of DDT, we cannot discard the existence of other sources of DDT. As cited previously, other authors have evaluated the presence of organochlorine pesticides in pine needles from Tenerife Island (Villa et al., 2003). Their results are in accordance with those described in this work, and allow us to hypothesize that DDT has been used recently in these islands. The differences found between islands reinforce such a possibility. Geographical differences seem to be related to agricultural practices. Thus, the Islands with lower DDT/DDE ratios (La Palma and La Gomera) have both a small surface devoted to intensive agriculture and a recent introduction (late 1980s) of this kind of activity (Gobierno de Canarias, 2001).

Despite the fact that the overall objective of this work is not to study the influence of OC-DDTs serum levels in the health status of people from the Canary Islands, we can but consider the possible influence of the high values of OC-DDTs residues in people living in the Canary Islands. *p,p'*-DDT is the most estrogenic among all OC-DDTs and is classified by the IARC as group 2B, that is “possibly carcinogenic to humans” (IARC, 1991). Although epidemiological research has not definitely proved that DDT causes cancer or other harmful effects in humans, it is well known that DDT and DDE are carcinogenic in animals (Snedeker, 2001; Turusov et al., 2002). As previously said, DDT and DDE bind to estrogen receptors and mimic the action of natural estrogens, so they may be involved in endocrine pathologies related with estrogenic effects (Welch et al., 1969; Gellert et al., 1972).

The province of Las Palmas (whose main inhabited area is Gran Canaria) presents one of the highest mortality rates of Spain for breast cancer (Rojas-Martín et al., 2003; López-Abente et al., 2003), and Gran Canaria is the island whose people show higher DDT/DDE ratios and higher values of parental DDT isomers. Even more so, from all the women living in the Canary Islands, the women

older than 20 living in Gran Canaria are the population strata with highest serum levels of parental DDT isomers, total DDT body burden and DDT/DDE ratio. The possibility that the estrogenic *p,p'*-DDT could be involved in the incidence and/or mortality for breast cancer in Gran Canaria island must be considered and requires further research. In general, detailed geographically based studies should be undertaken in the Canary Islands to elucidate the potential role of environmental factors in the development of breast cancer and other diseases.

To sum up, in the present study, concentrations of DDT-derivatives were measured in serum of 6- to 75-year-old Canarian people. This is one of the few published studies that analyze OC-DDTs residues in a representative sample of around 700 individuals, and the only one for the Spanish Archipelago of the Canary Islands. The high DDT/DDE ratio found here indicates high chronic exposure to DDT that could be persisting nowadays, mainly in the most populated and urbanized island, Gran Canaria. All these findings taken together may well talk about a matter of public health concern because, as reported previously, the problem of organochlorine pesticides' residues goes beyond occupational exposure and affects the general population living in areas devoted to intensive agriculture. These results must be considered by local authorities, who should take it into account, if necessary, for immediate intervention in agricultural policies.

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