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Exposure of women to organochlorine pesticides in Southern Spain☆

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Abstract

Organochlorine pesticides are lipophilic compounds that persist in the environment. Because of their lipid solubility and resistance to metabolism, some of these chemicals accumulate in human tissues. The largest area of intensive greenhouse agriculture in Europe is near the Mediterranean coast of Southern Spain, where this type of farming has greatly expanded since the 1960s. We determined and compared the levels of 15 organochlorine pesticides in the adipose tissue and blood of 200 women living in Southern Spain. Aldrin, dieldrin, endrin, lindane, methoxychlor, endosulfans, and DDT and its metabolites were identified. Detectable concentrations of *p,p′*-DDE were found in 100% of adipose tissue and serum samples. Among the remaining DDTs, *p,p′*-DDT was the most common, being detected in 39% of adipose tissue and 76.5% of serum samples, followed by endosulfans I and II, which also were found in both adipose tissue and serum samples but at lower concentrations. Endosulfans were followed in frequency by lindane, aldrin, and dieldrin. Endrin and methoxychlor were present at a much lower frequency compared to those of the other organochlorines. Serum concentrations of *p,p′*-DDE *o,p′*-DDD, and endosulfan -I, -sulfate, -lactone, and -diol were significantly correlated with their adipose tissue concentrations. No significant relationships were found between the serum and adipose tissue concentrations of the remaining nine compounds determined, raising doubts about the equivalent use of fat/serum samples for the exposure assessment of some pesticides in epidemiological studies. The results suggested that women of reproductive age in Southern Spain have been and are currently exposed to organochlorine pesticides. Because many of these chemicals can mobilize during pregnancy and lactation, further research is warranted to interpret the health consequences for the children of such exposure.

Keywords: Pesticides; Serum; Adipose tissue; Human; Exposure

1. Introduction

Organochlorine pesticides are a wide group of chemicals, many of which persist in the environment. They are of interest because of reports, after their widespread use, of their ubiquitous persistence in different environmental media, their ability to bioaccumulate and biomagnify in food chains, and their capacity for long-range atmospheric transport (Negoita et al., 2003). Although industrialized nations have restricted or banned many organochlorine pesticides, they continue to be manufactured for export to other countries. Moreover, some of these chemicals (e.g., endosulfans) are still used in industrialized countries, with the assumption that they are safer (Olea et al., 1999a, b).

Humans can be exposed to pesticides through their occupational activity (Cocco, 2002), their environment, or their food (Herrera et al., 1996; Jandacek and Tso, 2001), among other routes. Some of these chemicals accumulate and persist in human tissues due to their lipid solubility and resistance to metabolism (Jandacek and Tso, 2001). The presence of organochlorine pesticides in human serum and adipose tissue has been reported throughout the world (Archibeque-Engle et al., 1997; Longnecker et al., 1997; Stellman et al., 1998; López-Carrillo et al., 1999; Rivas et al., 2001; Pauwels et al., 2000; Waliszewski et al., 2001; Covaci et al., 2001). Although adipose tissue levels have been preferentially used as an indicator of historical human exposure to

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organochlorine pesticides, serum levels have been adopted in epidemiological studies as a less invasive and more practical alternative (Snedcker, 2001).

In Spain, the use of some organochlorine pesticides was restricted in the mid-1980s. However, recent reports have shown a high prevalence of pesticide residues in food (Ariño et al., 1995; Porta et al., 1999), and detectable concentrations of organochlorines have been observed in 80–100% of the population (Van't Veer et al., 1997; Olea et al., 1999a, b; Porta et al., 1999, 2002; Sala et al., 1999, 2001; Campoy et al., 2001; Rivas et al., 2001).

Human health effects after exposure to organochlorine pesticides are not adequately understood. In this regard, endocrine-disrupting activity has been reported for some organochlorine pesticides in biological test systems (Soto et al., 1995; Andersen et al., 1999). Some of these have been implicated in the etiology of various diseases and endocrine-related disorders, such as pancreatic cancer (Porta et al., 1999, 2002), breast cancer (Wolff et al., 1993; Hoyer et al., 1998; Güttes et al., 1998), non-Hodgkin's lymphoma (Woods et al., 1987; Flodin et al., 1988; Blair et al., 1998), leukemia (Brown et al., 1990), uterine cancer (Saxena et al., 1987), liver cancer (Cocco et al., 2000), sexual precocity (Krstevska-Konstantinova et al., 2001), cryptorchidism, (Garcia-Rodriguez, 1996; Hosie et al., 2000), and low sperm concentration (Abell et al., 2000).

The largest area of intensive greenhouse agriculture in Europe is Southern Spain, near the Mediterranean coast, where it has been practiced since the 1960s. This type of farming requires the use of large amounts of pesticides (Olea et al., 1999a, b). There have been sparse reports on human exposure to pesticides in Southern Spain. The present study determined the levels of 15 organochlorine pesticide residues in the adipose tissue and blood of 200 women living in this area and compared the relationship between pesticides levels in each compartment.

2. Materials and methods

2.1. Samples

Two hundred postmenopausal women undergoing surgery for malignancies ($n = 86$), bile vesicle disease ($n = 71$), abdominal or inguinal hernia ($n = 26$), varices ($n = 6$), or other diseases ($n = 11$) were enrolled in this study. The mean age was 53 years. Adipose tissue and blood samples were collected during surgery in the three largest public hospitals serving the Granada and Almeria provinces in Southern Spain. Adipose tissue was placed in a glass vial on ice, coded, and frozen to $-70°C$, always within 30 min of being excised, the temperature at which it was then stored in our laboratory until processing. In fasting women, approximately 10 mL whole blood was collected during surgery in glass vials (no anticoagulant or serum separator). Serum samples were stored at $-70°C$ until laboratory analysis.

2.2. Reagents

All solvents used were of high purity for high-performance liquid chromatography (HPLC): methanol, 2-isopropanol, hexane, ethanol, chloroform, and hydrochloric acid (Panreac, Barcelona, Spain). The chemicals used (and suppliers) were: aldrin, dieldrin, endrin, lindane, methoxychlor, endosulfans I and II, *p,p′*-DDT, and *o,p′*-DDT (Sulpeco, Bellefonte, PA, USA); *o,p′*-DDD, and *p,p′*-dichlorobenzophenone (Dr. Ehrenstorfer Laboratory, Ausburg, Germany); *p,p′*-DDE (Chem Service, Westchester, PA, USA); and endosulfan-diol, -sulfate, -lactone, and -ether (Hoechst Schering AgrEvo, Frankfurt, Germany).

2.3. Apparatus

2.3.1. High-performance liquid chromatography

Waters Model 501 Millipore apparatus (Marlborough, MA, USA) equipped with two pumps and a U6 K injector with a 500-μL load capacity were used. The ultraviolet/visible detector was a Waters Model 490 Millipore device, and Millennium Chromatography Manager software was applied. A Lichrocart column was used (Merck, Darmstadt, Germany) and packed with Lichrospher Si-60 of 5-μm particle size.

2.3.2. Gas chromatography and mass spectrometry

Saturn 2000 Varian equipment (Walnut Creek, CA, USA) with Varian Injector 1177 and a CP5860 WCOT fused silica column ($30\,m \times 0.25\,mm$) were used.

2.3.3. Gas chromatography and electron capture detector

A Varian-3350 with an electron-capture detector (^{63}Ni) was used, with Millennium Chromatography Manager software. A CP SIL8 CB column ($30\,m \times 0.25\,mm$) was used.

2.4. Sample preparation

2.4.1. Adipose tissue

The adipose tissue sample preparation has been described in detail elsewhere (Rivas et al., 2001). Briefly, an aliquot of 200 mg of adipose tissue was extracted in hexane, and pooled fractions were separated by HPLC. The fraction in which the pesticides were eluted underwent chemical analysis. The lipid content was quantified gravimetrically (Rivas et al., 2001).

2.4.2. Serum

The serum samples were analyzed according to a previously described method (Moreno Frías et al., 2001). The analytical methodology combines serum extraction with organic solvents, clean-up of the organic extract using acid treatment with H_2SO_4, elution of the cleaned-up extract through a liquid column chromatography system, and gas chromatography analysis of the fraction in which the organochlorine pesticides are eluted.

2.5. Gas chromatography

The presence of aldrin, dieldrin, endrin, lindane, *o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDD, *p,p′*-DDE, methoxychlor, endosulfans I and II, and endosulfan-diol, -sulfate, -lactone, and -ether was determined by gas chromatography with electron-capture detection, using *p,p′*-dichlorobenzophenone as an internal standard. The method has been previously described (Rivas et al., 2001). The identity of all chemicals was confirmed by gas chromatography and mass spectrometry, as described elsewhere (Martinez Vidal et al., 2000; Hernandez et al., 2002). The operational quality control and limits of detection and quantification have been reported previously (Rivas et al., 2001).

2.6. Data analysis

Arithmetic means were calculated for adipose tissue and serum pesticide levels using the corresponding concentration in lipids and on a wet basis, respectively. The association between organochlorine pesticides in adipose tissue and serum was estimated by calculating the Spearman correlation coefficient. A P value of <0.05 was regarded as significant. Individual organochlorine pesticide adipose tissue/serum ratios were calculated as the average of the adipose tissue:serum ratios of all samples.

3. Results

The mean values of the organochlorine concentrations measured in adipose tissue and serum are shown in Tables 1 and 2, respectively. All samples studied were positive for one or more chemicals. All pesticides, with the exception of endosulfan-ether and endosulfan-diol, were detected at a higher level in adipose tissue than in serum samples. Three of the pesticides were detected in at least half of the adipose tissue (*p,p′*-DDE, endosulfan-ether, lindane) and serum (*p,p′*-DDE, endosulfan-ether, *p,p′*-DDT) specimens. Detectable concentrations of *p,p′*-DDE were found in 100% of the adipose tissue and serum samples, with means of 508.83 ng/g and 8.11 ng/mL, respectively. Among the remaining DDTs, *p,p′*-DDT was the most frequently detected and was present in 39% of adipose tissue and 76.5% of serum samples. The *p,p′*-DDT/DDE ratio, which may indicate how long ago DDT contamination occurred, was 0.179.

Endosulfans I and II and their metabolites followed DDTs in the frequency of their presence in adipose tissue and serum samples but were found at lower concentrations. Among endosulfan metabolites, endosulfan-ether was the most predominant compound in both adipose tissue (68%) and serum (86%) samples. Endosulfans were followed in frequency by lindane, which was detected in at least 55% of adipose samples

Table 1
Residues of organochlorine pesticides in adipose tissue

	Mean ng/g lipid	SD ng/g lipid	Maximum ng/g lipid	% frequency
Aldrin	25.56	24.66	137.20	40
Endrin	47.43	36.74	148.13	7
Dieldrin	17.01	16.75	84.05	28.5
Endosulfan-ether	1.04	0.78	3.98	68
Endosulfan-lactone	2.02	1.14	4.23	10.5
Endosulfan-diol	9.23	12.31	64.99	26
Endosulfan-sulfate	12.17	13.04	48.50	13.5
Endosulfan I	6.02	6.69	23.07	17
Endosulfan II	73.36	103.73	414.15	14
Total endosulfans	21.37	54.63	417.59	78
Lindane	17.44	17.84	113.31	55
o,p′-DDT	13.46	12.93	57.07	12
p,p′-DDT	61.01	51.20	246.51	39
p,p′-DDD	95.66	75.18	297.43	10.5
p,p′-DDE	508.83	410.54	2637.67	100
Total DDTs	543.25	432.51	2806.22	100
Methoxychlor	29.86	43.71	155.58	5.5

SD, standard deviation.

Table 2
Residues of organochlorine pesticides in serum

	Mean ng/mL	SD	Maximum ng/mL	% frequency
Aldrin	2.17	2.40	14.16	56
Endrin	2.25	1.34	6.24	9
Dieldrin	1.21	1.22	6.35	47
Endosulfan-ether	1.66	2.01	12.77	86
Endosulfan-lactone	0.76	0.83	3.03	18.5
Endosulfan-diol	12.81	25.74	180.37	33
Endosulfan-sulfate	2.01	1.86	8.47	25.5
Endosulfan-I	1.72	1.55	7.27	37.5
Endosulfan-II	7.66	10.12	35.92	9.5
Total endosulfans	8.85	19.08	210.99	96
Lindane	1.53	2.26	12.77	54.5
o,p′-DDT	1.35	1.58	10.12	24.5
p,p′-DDT	3.15	2.56	16.50	76.5
p,p′-DDD	5.68	2.03	14.56	28.5
p,p′-DDE	8.11	12.76	80.27	100
Total DDTs	12.10	39.43	87.48	100
Methoxychlor	0.38	0.002	0.39	1

SD, standard deviation.

Table 3
Spearman correlation coefficients (ρ) between means of organochlorine pesticides in serum and adipose tissue samples

Pesticide	Correlation
o,p′-DDE	$\rho = 0.337$
o,p′-DDD	$\rho = 0.207$
Endosulfan-sulfate	$\rho = -0.148$
Endosulfan I	$\rho = 0.146$
Endosulfan-lactone	$\rho = 0.190$
Endosulfan-diol	$\rho = 0.254$

Table 4
Individual organochlorine pesticide adipose tissue/serum ratios

	Mean	SD	Maximum
Aldrin	24.81	40.79	244.00
Dieldrin	16.38	11.98	37.91
Endosulfan-ether	2.85	4.86	28.64
Endosulfan-lactone	6.50	4.64	14.10
Endosulfan-diol	3.71	5.77	19.35
Endosulfan-sulfate	62.68	79.91	183.43
Endosulfan I	4.21	4.70	15.13
Endosulfan II	159.47	235.64	430.53
Total endosulfans	8.29	21.14	134.84
Lindane	24.81	40.79	219.11
o,p′-DDT	17.22	25.82	55.28
p,p′-DDT	25.53	23.57	122.23
p,p′-DDD	25.46	15.58	59.49
p,p′-DDE	150.68	212.28	1675.60
Total DDTs	84.80	123.98	1464.77

SD, standard deviation.

and over 54.5% of serum samples. Aldrin, dieldrin, endrin, and methoxychlor were present in less than 40% of the study population and at lower levels.

Serum concentrations of *o,p′*-DDE, *o,p′*-DDD, endosulfan I, and endosulfan-sulfate, -lactone, and -diol were significantly correlated with their concentrations in adipose tissue (Table 3). No significant relationships were found between the serum concentrations and the adipose tissue residues of 9 of the 15 compounds determined. Adipose tissue/serum concentration ratios for all pesticides were in favor of the former, with coefficients ranging from 2.85 for endosulfan-ether to 159.47 and 150.68 for endosulfan II and *p,p′*-DDE, respectively (Table 4).

4. Discussion

The present study reports the presence of organochlorine pesticide residues in women living in agricultural areas of Southern Spain. Both adipose tissue and serum were analyzed, and comparisons between the residues in the two substrates confirmed the fat solubility of these organochlorine chemicals. Adipose tissue may act as a reservoir for the continuous release of chemicals that are therefore present in blood and may reflect historical exposure to pesticides that are not now in use.

In this regard, residue levels of DDT and metabolites were included in this study, despite their prohibition, because their presence in the body reflects either a relatively recent exposure or cumulative past exposure. DDT was extensively used in Spain between the 1950s and the 1970s (Porta et al., 2002). Although its use in agriculture was banned in 1977 (Porta et al., 2002), it can still be employed for vector control in some neighboring countries and it is allowed in the manufacture of pesticides currently produced and in use in Spain, such as dicophol (Di Muccio et al., 1988; Porta et al., 2002). In fact, *p,p′*-DDE, the major metabolite of DDT, was present in 100% of the human samples studied and at the highest concentrations. These findings are consistent with worldwide reports that around 90–100% of the population have detectable concentrations of DDE (Gómez-Catalán et al., 1993, 1995; López-Carrillo et al., 1997; Waliszewski et al., 2001; Sala et al., 1999; Millikan et al., 2000; Aronson et al., 2000; Rivas et al., 2001; Campoy et al., 2001). Regarding the concentrations, DDE mean values in adipose tissue (508.83 ng/g) and serum (8.11 ng/mL) were similar to those recently reported in the USA (Hunter et al., 1997; Wolff et al., 2000; Aronson et al., 2000) and Europe (Dello Lacovo et al., 1999; Guttes et al., 1998; Strucinski et al., 2002). Interestingly, recent studies (Smeds and Saukko, 2001) described mean values of DDE in adipose tissue (range 567 ng/g) that are very similar to the levels found in our samples and very much lower than those reported some years ago in the same geographical areas. This decrease may explain the lower DDE levels in the present samples compared with those in older studies in both male and female populations in other parts of Spain (Gómez-Catalán et al., 1993, 1995; Sala et al., 1999; Porta et al., 2002), although the mean ages of the studied populations, sex effects, and regional differences in pesticide use cannot be ruled out. Differences in the prevalence and duration of breast-feeding may also influence results, because breast feeding is the main mechanism whereby *p,p′*-DDT and its metabolites are eliminated (Lopez-Carrillo et al., 2001). On the other hand, this mechanism is less important for the estimation of the *p,p′*-DDE fat/serum ratio in postmenopausal women. Interestingly, the *p,p′*-DDT to *p,p′*-DDE ratio, which may indicate whether the exposure was in the distant (low ratio) or recent (high ratio) past, was estimated at 0.17 in the present study, which is similar to the ratios recently described in Spanish populations (Porta et al., 2002). These results suggest the influence of the restriction and prohibition of DDT and the decrease in exposure to these compounds over the past few decades.

Mean values of *o,p′*-DDT, *p,p′*-DDT, and the metabolite *p,p′*-DDD in our series were similar to those reported in other populations (Aronson et al., 2000) but were again much lower than those reported in a series from other regions of Spain (Gómez-Catalán et al., 1993, 1995). The frequency of DDT in serum samples (77%) suggests a current exposure to this compound in Southern Spain, although the source of this exposure is unknown. However, findings of DDT in the Guadalquivir River and its tributaries (Espigares et al., 1997) in the southwest of the Iberian Peninsula (CSIC), and in Southern Madrid (Fernandez et al., 2000) suggest the possibility of current exposure. In fact, a similar suggestion has been made for a population in North Italy (Galelli et al., 1995).

Lindane was detected in more than half of the adipose tissue and serum samples. These results are consistent with the past heavy use of lindane-based pesticides in Spain (Gómez-Catalán et al., 1995; Porta et al., 2002), and lindane is still permitted in the formulation of pharmaceuticals and indoor applications (United Nations Economic Commission for Europe, 2003). The mean level of lindane in adipose tissue was similar to that reported in other Spanish populations (Gómez-Catalán et al., 1995). Aldrin, endrin, and dieldrin were found at a frequency lower than but at concentrations similar to those in other Spanish populations (Gómez-Catalán et al., 1995). Even though the environmental and agricultural use of these pesticides is prohibited, residues are frequently found, as is the case of aldrin in waters from a section of the Guadalquivir River and its effluents in Southern Spain (Espigares et al., 1997) and in surface waters in Northern Greece (Golfinopoulos et al., 2003). Regional differences in the use of aldrin/dieldrin around the world (Jorgenson, 2001) may explain the distinct frequencies of exposure to these two chemicals.

Some types of organochlorine pesticides are still being used in Spain (Olea et al., 1999a, b, 1996) on the assumption that they are safer, despite their prohibition in some industrialized countries. This is the case with endosulfans, which were also included in this study because of their widespread use in Europe (Rivas et al., 2001; Rufingier et al., 1999; United Nations Economic Commission for Europe, 2003) and the frequency of their residue in European fruits and vegetables (Fernández et al., 2001; Arrebola et al., 2001). Among endosulfans, endosulfan-ether was the most predominant compound found in both adipose tissue (68%) and serum (86%) samples. Recently, there have been several reports of endosulfan residues in human samples from Southern Spain (Hernández et al., 2002; Martinez Vidal et al., 2002) and worldwide (Younglai et al., 2002; Ramesh and Ravi, 2003), although data on exposure to these compounds are scarce. More attention should be paid to endosulfans because of the frequency of their residues in human tissues and their reported estrogenic activity (Soto et al., 1995; Rivas et al., 2001). Finally, methoxychlor showed a very low frequency in our samples (<1–5%), indicating either a low exposure to this pesticide or its high metabolism.

Serum levels of organochlorines are frequently used as biomarkers of exposure, on the assumption that they represent fat reservoir content and are reliable indicators of total body burden (Snedeker, 2001; Hunter et al., 1997). However, a perfect relationship between serum levels and adipose tissue content is not always evident, and several authors have recommended the direct measurement of adipose tissue content (Woodruff et al., 1994; Kohlmeier and Kohkmeier, 1995; Aronson et al., 2000). The significant correlation coefficient found in this study between DDE or endosulfan residues in serum and adipose tissue is consistent with previous studies and indicates that adipose tissue and serum are equivalent for those chemicals (Stellman et al., 1998; López-Carrillo et al., 1999). However, we found no correlation between serum concentration and adipose tissue residues for 9 of the 15 compounds determined. This lack of a correlation between organochlorine pesticide residues in adipose tissue and serum, reported in several studies (Archibeque-Engle et al., 1997; Waliszewski et al., 2003), indicates that the serum concentrations of some organochlorines do not represent their adipose tissue concentrations and are, therefore, a poor predictor in epidemiological studies.

Our results suggest that women are currently exposed to organochlorine pesticides in Southern Spain. The frequency and level of these compounds were similar to those reported in humans in other areas of the world where many of these organochlorines have been banned. Because organochlorines can be stored in the mother's body and transferred prenatally to the developing fetus or postnatally from breast milk to the nursing infant, the exposure of women is of the greatest concern. The endocrine-disrupting activity reported for many of these chemicals means that a better understanding of infant exposure to these chemicals is essential. Further research is needed to interpret the human health consequences of the residue levels found in tissues.

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