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Serum levels of organochlorine pesticides in healthy adults from five regions of Spain

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ABSTRACT

The aim of this study was to measure of serum levels of *p,p′*-dichlorodiphenyl trichloroethane (*p,p′*-DDT), *p,p′*-dichlorodiphenyl dichlorethylene (*p,p′*-DDE), β-hexachlorocyclohexane (β-HCH), and hexachlorobenzene (HCB) in healthy adults in Spain. Furthermore, we also analyzed these levels according to dietary, other lifestyle factors and anthropometric characteristics. We measured the concentrations of such organochlorine pesticides (OCPs) in serum samples collected during 1992–1996 from 953 subjects aged 35–64 years, they were residents of five Spanish regions, they were randomly selected from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. OCPs were determined by means of gas chromatography with electron-capture detection (GC-ECD). The most frequent compound found in serum was *p,p′*-DDE, present in 98% of the samples, followed by HCB and β-HCH, found in 89% and 77% of samples, respectively, while *p,p′*-DDT could be measured only in 26% of subjects. The geometric means of serum concentrations (ng/g lipid) were 822 for *p,p′*-DDE, 167 for β-HCH, and 379 for HCB. The concentrations of all OCPs were positively associated with age and body mass index, and decreased along the period of blood collection. No association was found between OCPs levels and dietary factors. The concentrations of *p,p′*-DDE and β-HCB were higher in Murcia, one of southern regions, most likely associated with intensive past use of pesticides related to agricultural practices, while higher levels of HCB were found in Navarra, located in the north, maybe due to industrial use rather than agricultural application.

1. Introduction

Organochlorine pesticides (OCPs) are a group of synthetic chemicals effective against a variety of insects, many of which are highly persistent in the environment. Among them, *p,p′*-dichlorodiphenyl trichloroethane (*p,p′*-DDT) and its metabolite *p,p′*-dichlorodiphenyl dichlorethylene (*p,p′*-DDE), β-hexachlorocyclohexane (β-HCH, the isomeric form of HCH with longest half-life), and hexachlorobenzene (HCB) are some of the organochlorine compounds most commonly found (WHO, 2003).

Although OCPs are primarily environmental pollutants, they are very resistant to degradation and highly lipophilic, and consequently they become part of the food chain, where they tend to bioaccumulate, mainly in fatty foods. Thus, the main source of exposure in the general population is the diet (WHO, 2003). The current background body burden of OCPs is still of concern because

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of their estrogenic properties with potential adverse effects both to the environment and human (Safe, 2004). On the other hand, even though there is sufficient evidence of carcinogenicity for experimental animals, the epidemiological evidence in humans is inadequate for the three groups of compounds, and they are classified by the International Agency for Research on Cancer in the group 2B, as possibly carcinogenic to humans (IARC, 1987; IARC, 1991; IARC, 2001). However, the health effects of chronic exposure to OCPs on the general population at the current levels are still unknown.

Because of its persistence and bioaccumulation, as well as growing concern about adverse effects, a list of twelve organochlorine compounds were the objects of international restrictions, defined by the treaty called Stockholm Convention, aimed to eliminations of such compounds (UNEP, 2005). DDT and β-HCB are included in this list, but HCH is not covered by the Stockholm Convention, so the use of HCH in parts of the world is continuing. In Spain, although most OCPs were banned during the 1970s and the levels of such compounds seems to have decreased, most OCPs are often found in food, and detectable concentrations of some of these have been observed in a high proportion among the population (Porta et al., 2008).

Most OCPs can be measured in relatively small amounts of serum, and because of their long half-life, often of about 10 years, these levels may be considered good indicators of long-term exposure. However, comprehensive monitoring in the general population in Spain is scarce, and most studies focused on specific groups exposed because of occupation or residence (Porta et al., 2008). The aim of our study was to measure the serum levels of *p,p′*-DDT, *p,p′*-DDE, β-HCH and HCB in healthy adults belonging to the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Furthermore, we also aimed to analyze these levels according to dietary and other lifestyle factors and anthropometric measurements.

2. Methods

2.1. Study population

The EPIC-Spain cohort consisted of 15 632 men and 25 806 women aged 29–69 years, with different social and education levels. Detailed description of methods and population has been published elsewhere (González et al., 2004). Briefly, participants were recruited between 1992 and 1996 among healthy volunteers from five Spanish regions, three from the North (Asturias, Navarra, and Guipúzcoa) and two from the South (Murcia and Granada). At recruitment all the participants provided information on diet and other lifestyle factors, anthropometric measurements, and a sample of 30 mL of blood. All participants gave their informed consent and the study was approved by the Ethical Review Board. For this study, a random sample of 200 subjects from each centre was selected, stratified according to the age and sex structure of the Spanish population, aged 35–64 years. Biological material was not available for 14 individuals, or was of poor quality or insufficient amount to obtain valid measurement in another 33 individuals; thus, the final population of study included 953 subjects.

2.2. Diet and lifestyle questionnaires and anthropometry

The usual food intake during the preceding year, taking into account seasonal variations, was estimated by personal interview using a computerized dietary history questionnaire, developed and validated specifically for the EPIC study in Spain (EPIC Group of Spain, 1997). The questionnaire was structured according to occasions of food intake and included a list of more than 600 foods and beverages including local recipes. A questionnaire administered by an interviewer was used to collect information on sociodemographic characteristics, and work and leisure physical activity. The section dealing with current or past occupational exposures included an item asking whether the subject had ever worked in agriculture; those who answered yes to this question were also asked whether this job involved manipulation of pesticides. For women reproductive history was collected as well; this section gathered information about each live birth, including date of childbirth, whether the woman breastfed the newborn (either as exclusive or as complementary nourishment), and duration of breastfeeding. Thus, we estimated the cumulative duration of breastfeeding by adding up weeks or months of lactation periods from different newborns, as well as the time in years elapsed since cessation of the last period of breastfeeding. Weight and height were also taken at recruitment following standardized procedures, and were then used to compute the body mass index (BMI) as $kg\,m^{-2}$.

2.3. Analytical methods

Blood samples were divided into 0.5 mL aliquots of serum, plasma, concentrated red blood cells, and buffy coat, and stored in liquid nitrogen at −196 °C. An improved method for the determination of organochlorine pesticides was used to measure serum levels of *p,p′*-DDT, *p,p′*-DDE, β-HCH and HCB (Goñi et al., 2007). The method required that low volume of serum (500 μL) and 48–96 samples per day be prepared by one analyst without special automatic equipment. Initial extraction was performed using 96-well solid-phase extraction disk plates and was followed by a clean-up with silica gel/sulfuric acid. Quantification was carried out by gas chromatography with electron-capture detector (GC-ECD). A mass spectrometer detector (GC–MSD) was used for quantitative and qualitative confirmation. Intra-day relative standard deviation of *p,p′*-DDT, *p,p′*-DDE, β-HCH and HCB varied from 1% to 11%, depending on compound. Inter-day relative standard deviation (measured over a yearlong period) was <15% in all cases. Total cholesterol (TC) and triglycerides (TG) were determined enzymatically by developing colored compounds that were measured spectrophotometrically (Wahlefeld, 1974; Wiebe and Bernert, 1984). Total serum lipids (TL) were calculated from total cholesterol and triglycerides (all of them expressed as g/L) by applying the formula $TL = 2.27\ TC + TG + 0.623$ (Phillips et al., 1989). Then, we used this result to report the serum pesticides on lipid basis. The limit of quantification (LOQ) was 0.4 ng/mL on serum basis; for subjects whose values were below the detection level, a concentration of 0.2 ng/mL (half the LOQ) was assigned.

2.4. Statistical analysis

The serum levels of each pesticide were expressed as lipid-corrected concentrations in ng/g lipid. Since the distributions of concentrations were right skewed, the variables were transformed using natural logarithms. For descriptive purposes, apart from the geometric mean, the median and the 75th percentile, with their corresponding 95% confidence intervals (CI), were reported for the whole sample and separately for men and women. Given the sampling framework, all the means reported may be considered standardized according to the age–sex structure of the Spanish population 35–64 years old. Analysis of covariance was used to examine how the log-transformed serum concentration of each OCP varied by demographic, anthropometric, dietary and lifestyle variables (Armitage and Berry, 1994). Geometric means of lipid-corrected concentrations of each OCP were calculated from multiple linear regression models with log-transformed concentration (as ng/g lipid) as the dependent variable; these geometric means are adjusted by all the covariates included in the model. The linear