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Association between organochlorine pesticide exposure and thyroid hormones in floriculture workers

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ABSTRACT

Several studies have suggested that exposure to DDT may be related to changes in thyroid hormone levels in animals and humans, even though results across studies are inconsistent. The aim of this study was to assess the association between exposure to *p,p'*-DDE (a stable metabolite of DDT) and serum levels of thyroid hormones in floriculture workers. A longitudinal study was conducted on 136 male subjects from the States of Mexico and Morelos, Mexico, who were occupationally exposed to pesticides, during agricultural periods of high (rainy season) and low (dry season) levels of pesticide application. Using a structured questionnaire, a survey was carried out on socio-demographic characteristics, anthropometry, clinical history, alcohol and tobacco consumption, residential chemical exposure, and occupational history. Blood and urine samples were collected to determine serum levels of TSH, total T_3, total T_4, and *p,p'*-DDE, and metabolites of organophosphate pesticides (OP), respectively. The analysis of the associations between *p,p'*-DDE levels and thyroid hormone profile adjusting by potential confounding variables including urinary OP metabolites was carried out using multivariate generalized estimating equation (GEE) models.

Our results showed that the geometric means of *p,p'*-DDE levels were 6.17 ng/ml and 4.71 ng/ml in the rainy and dry seasons, respectively. We observed positive associations between the serum levels of *p,p'*-DDE and those of total T_3 (β=0.01, 95% CI: −0.009, 0.03), and total T_4 (β=0.08, 95% CI:0.03, 0.14) and negative but no significant changes in TSH in male floricultural workers, supporting the hypothesis that acts as thyroid disruptor in humans.

1. Introduction

DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) is a broad spectrum synthetic insecticide, used since the 1940s for pest control in agriculture and at the residential level. Its high persistence in the environment and the known evidence of toxicity for some animal and vegetable species (Carson, 1962) led to its use being discontinued in most of the world in the 1970s and 1980s. However, its use has continued for malaria control in countries where this problem is endemic. In Mexico, DDT was used for agricultural pest control until 1991 and for malaria control until 1999, as its use for this purpose was restricted in the year 2000 (Yáñez et al., 2002). Technical grade DDT is a mixture of two isomeres, mainly *p,p'*-DDT(85%) and *o,p'*-DDT (15%), and traces of *o,o'*-DDT. Technical grade DDT may also contain *p,p'*-DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) and *p,p'*-DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane) as contaminants (ATSDR, 2002). DDT is almost completely metabolized and the greatest part is transformed to *p,p'*-DDE, with a small percentage remaining as *o,p'*-DDT (Torres-Sánchez and López-Carrillo, 2007a). DDT and its metabolites are highly persistent and lipophilic compounds that

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bioaccumulate and magnify through the food chain so that, even today, it is possible to detect residues in most human populations; *p,p'*-DDE is even more persistent than DDT in both the environment and live organisms (Beard, 2006). Several studies have documented the presence of *p,p'*-DDE in biological samples (serum, maternal milk, cord blood and adipose tissue) in Mexican populations (Galván-Portillo et al., 2002; Torres-Sánchez et al., 2007b; Barraza-Vázquez et al., 2008; Pérez-Maldonado et al., 2014). Several organochlorine (OC) compounds were shown to be associated with changes in human serum thyroid hormone levels (Langer, 2010). Among many different pesticides, the thyroid-disrupting effect of DDT has been one of the most studied (Boas et al., 2006).

Thyroid hormones have a fundamental role in the metabolism, and are virtually active in all organs and systems (Galofré et al., 2006; Hall and Guyton 2011), and it is estimated that approximately 10% of the population suffers from some degree of thyroid dysfunction, representing a significant public health problem (Galofré et al., 2006). Although the health consequences of subclinical changes in the circulating levels of these hormones are unknown, there is growing interest in ascertaining the effect of environmental exposures on such changes.

Regarding the effect of DDT and its metabolites, several studies performed on animal models showed, in general terms, a hypothyroid like effect (Goldman, 1981; Grässle and Biessmann, 1982; Capen, 1992). Recently, studies in Sprague-Dawley rats have shown that *p,p'*-DDE exposure significantly reduced total thyroxine (TT4), free thyroxine (FT4) and transthyretin serum levels, accompanied by elevated mRNA expression in α1 and β1 thyroid hormone receptors in the hypothalamus (Liu et al., 2011). Furthermore exposure to PCB153 and *p,p'*-DDE significantly increase the expressions of TRα1, TRβ1 and TRHr (Liu et al., 2015). However, few studies have been carried out on humans and the results found to date have been inconsistent. In adult men, several authors did not find associations between thyroid profile and *p,p'*-DDE exposure (Hagmar et al., 2001; Persky et al., 2001, Abdelouahab et al., 2008), whereas other have shown associations between *p,p'*-DDE and one or more thyroid hormones, but results have not been consistent and have reported TSH increases (Rylander et al., 2006), non-significant decreases in total T4 (Turyk et al., 2007), or decreased TSH and significant increases in total T3 and free T4 (Meeker et al., 2007).

The aim of this analysis was to assess the association between serum levels of *p,p'*-DDE and serum levels of thyroid hormones, in Mexican male floriculture workers who have been exposed to DDT, adjusting by potential confounding variables including urinary dialkylphosphates (DAPs), since organophosphate pesticides have been considered as thyroid endocrine disruptors (Meeker et al., 2006; Fortenberry et al., 2012) and were largely used by these workers (Lacasaña et al., 2010).

2. Methods

2.1. Study population

A longitudinal study was conducted on male floriculture workers, in the States of Mexico and Morelos (Mexico) during the periods July-October 2004 (rainy season) and December 2004 to May 2005 (dry season). These seasons correspond to two main agricultural periods, the first in which large quantities of pesticides were sprayed (rainy season) and the second, when pesticides were used to a lesser extent (dry season). The objective of the main study was to explore the potential associations of past (OC) and current (OP) pesticide exposure with male hormonal profile and thyroid hormones. OP used widely were: diazinon, metamidophos, omethoate, glyphosate and methyl parathion. Chemicals were usually applied biweekly for preventive purposes, but during the rainy season pesticide applications increased.

Details of population and design of the study have been reported elsewhere (Lacasaña et al., 2010). Workers were identified using the employee records of 57 businesses belonging to two associations of floriculture growers. First, an appointment was made with each of the managers of the companies, who provided access to their working population. Later, workers were invited to an informative session about the study, where their participation was requested. Using a selection questionnaire, which include information on age, seniority and medical history, workers who met the eligibility criteria were identified: age between 18 and 52 years old and having been at the job for at least 6 months, Men with a prior diagnosis of infertility and endocrine or chronic diseases (thyroid disease, diabetes mellitus, liver disease, renal insufficiency or cancer) were excluded from the study. There were no workers taking hormone supplements or medication affecting thyroid hormone levels. A total of 143 workers were eligible, and 136 (95%). agreed to participate and signed informed consent forms; later they completed the questionnaire and gave urine and blood samples. Eighty four workers (62%) again provided biological samples during the dry season.

The study was approved by the Ethics Committee of the National Institute of Public Health of Mexico.

2.2. Information collection

A structured questionnaire was completed by the floriculture workers included in the study, containing questions on socio-demographic characteristics (age, marital/cohabitant status, education and family income), anthropometric measures (weight and height), alcohol and smoking habits, pathological history, type of activity in the floriculture industry and its duration (occupational exposure), as well as exposure to specific pesticides or other chemicals at home (residential exposure). The questionnaires were administered by previously trained nursing personnel, who had no knowledge of the study hypothesis.

2.3. Samples collection

For the two periods of sampling, blood samples (10 ml) were collected in fasting conditions between 8 and 9:30 AM by nursing personnel, using non-heparinized Vacutainer® tubes. Samples were centrifuged for 10 min at 2500 rpm and serum was kept at −70 °C in Eppendorf vials and glass vials prewashed with hexane grade pesticide and covered with a Teflon cap (for thyroid hormones and *p,p'*-DDE quantification, respectively) until analysis. The urine samples (first morning samples) were self-collected by workers at home in a supplied container and kept at −20 °C until analysis.

Samples were taken from each participant the day after non persistent pesticides were applied in the work place.

2.4. TSH and thyroid hormones measurement

Thyrotropin (TSH) and total thyroxine (T_4), and total triiodothyronine (T_3) were determined in serum by enzyme immunoassay using commercial tests from Diagnostic Automation, Inc., and from Diagnostic Systems Laboratories, Inc., respectively. The assay sensitivities for TSH, T_4 and T_3 were 0.2 μIU/ml, 0.18 μg/dl and 0.4 ng/dl, respectively. The inter-assay CV for these hormones was less than 8% in all three cases.

The reference values for hormone levels were provided by the laboratory according to commercial kits information.