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Pesticide exposure and thyroid function in an agricultural population in Brazil

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ABSTRACT

Although numerous pesticides may interfere with thyroid function, however, epidemiological evidence supporting this relationship is limited, particularly regarding modern non-persistent pesticides. We sought to evaluate the association of agricultural work practices, use of contemporary-use pesticides, and OC pesticides residue levels in serum with circulating thyroid hormone levels in an agricultural population. A cross-sectional study was conducted with a random sample of 275 male and female farm residents in Farroupilha, South of Brazil. Information on sociodemographics, lifestyle and agricultural work was obtained through questionnaire. Blood samples were collected on all participants and analyzed for cholinesterase activity, serum residues of OC pesticides, and levels of free T4 (FT4), total T3 (TT3) and TSH. Non-persistent pesticides exposure assessment was based on questionnaire information on current use of pesticides, and frequency and duration of use, among others. Associations were explored using multivariate linear regression models. Total lifetime years of use of fungicides, herbicides and dithiocarbamates in men was associated with increased TSH accompanied by decrease in FT4, with evidence of a linear trend. In addition, there was an association between being sampled in the high pesticide-use season and increased TSH levels. Conversely, farm work and lifetime use of all pesticides were related with slight decrease in TSH and increased TT3 and FT4, respectively. In general, pesticide use was not associated with thyroid hormones in women. Subjects with detected serum concentrations of β-hexachlorocyclohexane, endrin, dieldrin, heptachlor epoxide B, γ-chlordane, transnonachlor, heptachlor, *p,p*′-dichlorodiphenylethane and endosulfan II experienced slight changes in TT3; however, associations were weak and inconsistent. These findings suggest that both cumulative and recent occupational exposure to agricultural pesticides may affect the thyroid function causing hypothyroid-like effects, particularly in men.

1. Introduction

Pesticides are extensively used today in agricultural settings to prevent and control pests. Numerous pesticides, including banned organochlorines (OCs) and modern non-persistent pesticides, have demonstrated thyroid-disrupting activity, affecting the homeostasis of the thyroid system (Boas et al., 2012; Diamanti-Kandarakis et al., 2009).

Animal studies have shown that OC pesticides may interfere with the thyroid system through multiple mechanisms of action, as they can affect deiodination of thyroid hormones (*i.e.*, thyroxine –T4– and triiodothyronine –T3–) by inducing or increasing type III deiodinase expression, bind to thyroid hormone-binding proteins, interfere with thyroid hormone binding to hormonal receptors, interfere with TSH receptor function and bind to T3 and T4 receptors, altering thyroid hormone-mediated gene expression (Crofton, 2008; Boas et al., 2012). In humans, several epidemiological studies conducted in the last decade have investigated the association between exposure to OC pesticides and serum levels of

Abbreviations: AChE, Acetylcholinesterase; BChE, Butyrylcholinesterase; DDT, Dichlorodiphenyltrichloroethane; DDE, Dichlorodiphenylethane; DDD, Dichlorodiphenyldichloroethane; EBDC, Ethylene-bis-dithiocarbamate; FT4, Free thyroxine; HCH, Hexachlorocyclohexane; HCB, Hexachlorobenzene; OC, Organochlorine; OP, Organophosphate; TSH, Thyroid stimulating hormone; TT3, Total triiodothyronine

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T4, T3 and thyroid stimulating hormones (TSH) in adult population, reporting negative, positive and null associations with conflicting results (Bloom et al., 2003; Freire et al., 2013; Meeker et al., 2007; Persky et al., 2011; Rathore et al., 2002; Rylander et al., 2006; Sala, 2001; Schell et al., 2009; Turyk et al., 2006, 2007).

Experimental studies also suggest that a variety of non-persistent pesticides, including organophosphates (OPs), carbamates, synthetic pyrethroids and dithiocarbamates, may act as thyroid disrupters, affecting the hypothalamic-pituitary-thyroid axis at different levels (Crofton, 2008; Diamanti-Kandarakis et al., 2009). However, current knowledge regarding the impact of modern pesticides on human thyroid function is still limited (Goldner et al., 2010, 2013), although a growing number of human studies has examined levels of circulating T4, T3 and TSH in relation to non-persistent pesticides (Campos and Freire, 2016). A majority of such studies focused on occupational exposure and used urinary pesticide biomarkers. Overall, while existing evidence is insufficient, results from a few studies are consistent with experimental data supporting that exposure to non-persistent pesticides exerts hormonal changes consistent with hypothyroidism (Fortenberry et al., 2012; Lacasaña et al., 2010; Meeker et al., 2006; Steenland et al., 1997; Toft et al., 2006).

Brazilian agriculture has grown exponentially in the last few years, and today Brazil is the world's top consumer of pesticides (ANVISA, 2012). Serra Gaúcha is a mountainous region in the South of Brazil settled by German and Italian immigrants characterized by family farms dedicated to fruit growing, especially grapes for wine production. In the present study it was hypothesized that current and/or cumulative lifetime exposure of agricultural workers may be related to thyroid disturbances. This was evaluated by exploring the association of agricultural work practices, current and lifetime use of non-persistent pesticides, and OC pesticides residue levels in serum with circulating levels of thyroid hormones in agricultural population of this region.

2. Materials and methods

2.1. Study design and population

A cross-sectional study was conducted between 2012 and 2013 aiming to investigate reproductive and endocrine effects of pesticides in adult agricultural workers in the South of Brazil. A random sample of farmers and farm family members was selected from the agricultural population of Farroupilha, a small town in Serra Gaúcha region. Assuming a participation rate of around 90% and at least 3 adults per household, 90 residences were randomly selected from the list of rural households of the municipal agriculture office to reach the estimated sample size of 220 individuals. All persons aged 18–69 years living in the selected households were personally invited to participate in the study, representing a total of 301 subjects. Among these, 21 (7%) refused to participate. Farm owners working in farm work for less than one year and their respective family members were excluded from the study (5 persons), leaving a final sample of 275 individuals.

The study was approved by the Ethics Committee of the National School of Public Health, Oswaldo Cruz Foundation (ENSP/FIOCRUZ), and written informed consent was obtained from all participants.

2.2. Data collection

2.2.1. Questionnaire

Trained interviewers administered a structured questionnaire to participants through face-to-face interviews at farmers' residences. The questionnaire contained more than 200 questions, divided into the following sections: demographics, occupation, lifestyle, agricultural work practices, pesticide use, health status, medical and reproductive history. Variables used in the present study were: gender, age (continuous and grouped into 18–30, 31–45, 46–60, and >60 years), years of education (continuous and categorized as ≤ 8, 9–11, and 12 or more years), marital status (married; others), annual household income (categorized as ≤ 10, 11–20, 24–50, and >50 thousands of Brazilian reais), place of birth (Farroupilha; other place), smoking habit (never smoked; ex-smoker; current smoker), lifetime smoking (categorized as 0, 1–9, and 10 or more years), regular alcohol intake in the last 30 days (no; yes), practiced physical activity regularly in the last 3 months (no; yes), current weight (kg) and height (cm), history of thyroid disease (no; yes), and family history of thyroid disease in first-degree relatives (no; yes). Body mass index (BMI) was calculated by dividing weight in kg by height in meters squared and categorized as lower than 25 kg/m^2 (eutrophic) and equal to or greater than 25 kg/m^2 (overweight or obese).

The following information on agricultural work practices and pesticide use was obtained from the questionnaire: current occupation (farmer; non farmer), self-reported pesticide exposure (not exposed/rarely exposed; low exposure; high exposure), years of farming activities (categorized as <1, 1–10, 11–25, 26–50, and >50), years mixing or applying pesticides (categorized as ≤ 1, 2–10, and >10), frequency of mixing or applying pesticides (categorized as <5, 5–39, 40–59, and ≥ 60 days per year), season of interview and blood sample collection (low pesticide use season: from March to September; high pesticide use season: from April to August), use of full personal protective equipment (PPE) (yes; no), current use of any pesticide (no; yes), and total number of pesticides currently used (categorized as none, 1, 2 or more). In addition, participants were asked to report on their current and former use of specific pesticides from a list of 18 commercial products. This list was obtained from the Brazilian Entity for Technical Assistance and Rural Extension (EMATER) and included the pesticides most commonly used in the study area (Table S1). Participants were also asked about the use of pesticides not included in this list. Active ingredients in commercial products were then grouped into chemical and functional classes, *i.e.*: herbicides, insecticides, fungicides, OP insecticides, dithiocarbamate fungicides, carbamates, synthetic pyrethroids, and others chemical classes. Lifetime years of overall pesticide use and for each functional and chemical class were calculated as the difference between starting and finishing dates of use, regardless of simultaneous use of different pesticides of the same class (for example, if mancozeb and carbendazim were used for 10 years, from 2000 to 2010, it was assumed 10 years of fungicide use). Cumulative use of pesticides was categorized as never use, 1–20, and more than 20 years.

2.2.2. Laboratory analysis

An intravenous blood sample (15 mL) was collected from each participant by a trained nurse after a 12-h overnight fast at farmers' residences. Plasma and serum were separated from whole blood by centrifugation. Specimens were stored at -20 °C in vacutainer tubes containing EDTA and delivered to the laboratories responsible for pesticide and biochemical analysis.

Levels free T4 (FT4), total T3 (TT3), and TSH were measured in serum samples of 15, 30 and 50 mL, respectively, by electrochemiluminescence immunoassay using Roche® kit. Normal laboratory reference range was 0.93–1.70 ng/dL for FT4 and 0.24-0.37 ng/dL for TT3. Regarding TSH, normal reference range was 0.30–4.30 µUI/mL for subjects aged 18–60 years, and 0.40–5.80 µUI/mL for those older than 60 years. Samples with hormone values outside reference ranges were run in duplicate. Serum concentrations of total cholesterol and triglycerides (in mg/dL) were determined by colorimetric enzymatic methods. Estimates of