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Long-term exposure to organochlorine pesticides and thyroid status in adults in a heavily contaminated area in Brazil☆,☆☆

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ABSTRACT

Organochlorine (OC) pesticides are endocrine disruptors altering the thyroid hormonal system. The aim of this study is to investigate the relationship between exposure to OC pesticides and thyroid status in adults from a rural area in Rio de Janeiro, Brazil, heavily contaminated with OC pesticides. A cross-sectional study was carried out in 303 men and 305 women >14 years old. Concentrations of 19 OC pesticides and levels of free thyroxine (T4), total triiodothyronine (T3), thyroid-stimulating hormone (TSH), anti-thyroperoxidase (TPOAb) and anti-thyroglobulin (TgAg) antibodies were analyzed in serum samples. Associations between OC pesticides concentrations and values of biochemical thyroid parameters were determined using multivariate regression models stratified by gender. Prevalence of subclinical hyperthyroidism and the presence of TPOAb antibodies were higher than those described for euthyroid populations elsewhere. After adjusting for confounders, total T3 levels were associated with lower concentrations of endosulphan 2 in men and with higher alpha-chlordane, *p,p′*-dichlorodiphenyl-trichloroethane (DDT), endosulphan 2, and methoxychlor in women. Levels of free T4 showed inverse association with beta-hexachlorocyclohexane (HCH) and *p,p′*-DDT in men, and were positively associated with hexachlorobenzene (HCB), heptachlor, *o,p′*-DDT, and *p,p′*-DDT in women. TSH levels were associated with higher beta-HCH in men. A positive association was observed between exposure methoxychlor in males and presence of TPOAb, but no association with TPOAb was found in women. These results suggest that OC pesticides can affect the thyroid system through gender-specific mechanisms that may differ among compounds. Further detailed investigations and health monitoring should be warranted for this population.

1. Introduction

Organochlorine (OC) pesticides are highly lipophilic compounds with endocrine-disrupting activity. Given the structural similarity to thyroid hormones (thyroxine [T4] and triiodothyronine [T3]), OC pesticides may mimic action of several hormones, including thyroid function, and even modulate the mechanisms and interfere with their binding to hormonal receptors (Langer et al., 2007; Meeker et al., 2007; Zoeller, 2007), potentially leading to thyroid dysfunction (Alvarez-Pedrerol et al., 2008; Chevrier et al., 2008).

Thyroid hormones in humans are essential for metabolic regulation and for maintaining normal cardiovascular, reproductive, and nervous system functions. Thyroid-stimulating hormone (TSH), produced by the adeno-hypophysis, regulates the thyroid synthesis and the balance between T3 and T4 serum levels (Giacomini et al., 2006). Although the consequences of subclinical changes in thyroid function are currently under active discussion (Boelaert and Franklyn, 2005; Surks et al., 2004), all factors associated with any alteration in thyroid hormone levels may be of concern.

Animal studies have shown associations between elevated concentrations of OC pesticides such as dichlorodiphenyltrichloroethane (DDT), its main metabolite *p,p′*-dichlorodiphenyldichloroethane

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(DDE), and hexachlorobenzene (HCB), with lower thyroid hormone levels or higher TSH levels (Boas et al., 2006). Human data also indicate that chronic exposure to certain OC pesticides may interfere with the activity or metabolism of thyroid hormones and TSH (Langer, 2010). Thus, several epidemiological studies examined associations between OC pesticide exposure, among other OC compounds, and circulating levels of free T4, total T4, total T3, and TSH in cohorts characterized by low or moderate OC exposure (Bloom et al., 2003; Meeker et al., 2007; Pelletier et al., 2002; Sala et al., 2001; Schell et al., 2004, 2008). The observed findings in humans are, however, still inconsistent.

The potential of OC compounds to impair immune responses and to trigger autoimmune disease is also a matter of growing concern (Schell et al., 2009). In this regard, serum positivity of anti-thyroid antibodies (anti-thyroperoxidase [TPOAb] and anti-thyroglobulin [TgAb]) is a useful biomarker of future thyroid dysfunction, since they are indicative of thyroid injury or inflammation. They are also useful as a diagnostic tool for autoimmune diseases such as hypothyroidism (Cho et al., 2011), chronic thyroiditis (Langer et al., 2007), and thyroid cancer (Kim et al., 2010).

In a previous investigation carried out among children in a rural village in Southeast Brazil with a history of OC contamination from an abandoned pesticide production plant, increased total T3 levels was associated with OC pesticide serum concentrations (Freire et al., 2012). The present study aimed to examine the relationship between exposure to several OC pesticides and thyroid status among adult residents in the same area.

2. Material and methods

2.1. Exposure set

Cidade dos Meninos is a rural village located in the county of Duque de Caxias, State of Rio de Janeiro, Southeast Brazil. In the late 1940s, a factory was set up in this village for the production of hexachlorocyclohexane (HCH) and the formulation and storage of other OC pesticides, such as DDT and hexachlorobenzene (HCB), to be used by the Ministry of Health in vector control programs in Brazil. The factory was deactivated in 1955 and closed in 1961, but the remaining pesticide products were abandoned outdoors in the vicinity of the factory (Ministério da Saúde, 2003). Since then, local population has been chronically exposed to high levels of several OC pesticides found in soil, water, and local food. In particular, high levels of HCH isomers, DDT and other OC pesticides were found in soil, and ground and superficial water, as well as in locally produced food items such as eggs, milk, fruits and vegetables (Ministério da Saúde, 2003). Consequently, commercialization of local fruits and vegetables, eggs, poultry, meat and dairy products were forbidden by federal sanitary authorities. In 2008, results from a risk assessment revealed that ingestion of local food and dermal absorption through contact with contaminated soil were the main routes of exposure to OC pesticides in residents in Cidade dos Meninos (Asmus et al., 2008).

2.2. Study population and design

From November 2003 to March 2004, a population-based survey was conducted in Cidade dos Meninos to investigate the levels of serum OC pesticide amongst their residents. Population participating in this survey has been previously described (Freire et al., 2012). Briefly, a total of 381 families (1346 subjects) recorded from the 2003 official family census conducted by the Ministry of Health were contacted. From these, 359 families agreed to participate in the study providing a serum sample from at least one of their members. Participation rate was 96% of the 1346 initially identified subjects, but an electricity blackout resulted in loss of 26% of serum samples, limiting the chemical analysis to 995 (74%) residents. Among these, 787 were older than 14 years. Several biochemical parameters, including levels of free T4, total T3, TSH, and anti-thyroid antibodies, were also determined in serum samples of participants.

Four participants were excluded from the present investigation because they had extremely high serum concentrations of pesticides (*i.e.* levels of beta-HCH were higher than 1000 ng/ml). Additional 175 individuals had missing information on thyroid parameters or important covariates. Thus, a cross-sectional analysis was conducted on 608 adults (303 men and 305 women) with completed information on study variables. The study was approved by the Ethics Committee of the National School of Public Health (CEP/ENSP no. 1268/2004), Oswaldo Cruz Foundation (Fiocruz) in Rio de Janeiro, Brazil, and a signed informed consent was obtained from all participants in the study.

2.3. Data collection

2.3.1. Questionnaire

A validated questionnaire designed by the International Agency of Research on Cancer, including information about sociodemographic characteristics and lifestyle, was completed by study subjects through face-to-face interviews. Four trained interviewers collected all data immediately after blood sample collection and without previous knowledge on participants' exposure status. Variables derived from the questionnaire and used in the present analysis are: age (years), ethnicity (recorded as white or non-white), length of time residing in Cidade dos Meninos (years), period of life in Cidade dos Meninos (recorded as residents whose pregnancy occurred in Cidade dos Meninos and had lived there since birth; residents that moved to the study area during childhood [*i.e.* 1–14 years old]; or residents that moved during adulthood [$>$ 14 years]), current alcohol use (any or none), cigarette smoking (recorder as never, ex-smoker, or current smoker), weight (kg), and height (cm). Women also provided information on breastfeeding history (any or none) and parity (as number of liveborn infants).

2.3.2. Laboratory analysis

Intravenous blood samples were collected under fasting conditions at the Primary Health Care Centre of Cidade dos Meninos. Blood samples were collected using a vacuntainer equipment and centrifuged at 2400*g* for 10 min to obtain sera, which were frozen at -20 °C until the analysis of pesticides and biochemical parameters. Concentration of OC pesticides was determined at the Laboratory of Toxicology of the Centre for Occupational and Human Ecology Health, Fiocruz. The analytical methodology, consisting of gas chromatography with electron-capture detection, has been previously described (Sarcinelli et al., 2003; Wolff et al., 1993). Target analytes were: HCH (alpha, beta and gamma isomers), HCB, chlordane (alpha and gamma isomers), *trans*-nonachlor, heptachlor, DDT metabolites (*p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT, and *p,p′*-DDD), endosulfan 1 and 2, aldrin, endrin, dieldrin, methoxychlor, and mirex. The limits of detection were 0.02 ng/ml for HCH isomers, *o,p′*-DDT, *p,p′*-DDT, *p,p′*-DDD, endosulfan, endrin, methoxychlor, and mirex; 0.009 ng/ml for *p,p′*-DDE; 0.008 ppb for alpha and gamma chlordane, *trans*-nonachlor, heptachlor, aldrin, and dieldrin; and 0.004 ng/ml for HCB.

Levels of total T3, free T4, TSH, TPOAb, and TgAb were measured in serum samples by chemiluminiscence assay using ELISA kit (Alka Tecnologia®, São Paulo, Brazil) at the Clinical Pathology Laboratory in the Cancer Hospital of the National Institute of Cancer, Rio de Janeiro. Laboratory reference values ranged between 0.89 and 1.76 ng/dl for free T4, in the range 60–181 ng/dl for total T3, and 0.35–5.5 mU/l for TSH. Antibody reference values were up to 35 U/ml for TPOAb and up to 40 U/ml for TgAb. Assay detection limit was 10 U/ml for TPOAb and 20 U/ml for TgAb. Concentrations of total cholesterol and triglycerides were determined by colorimetric enzymatic methods at the same laboratory and expressed in milligrams per deciliter.

2.4. Statistical analysis

Any individual concentration of an OC pesticide that was below the limit of detection was substituted with the midpoint value between zero and the limit of detection of each compound. Pesticide concentrations were treated as continuous variables since all of them but methoxychlor (which was categorized into values below and above the detection limit) were detected in more than 60% of the participants. Free T4, total T3, and TSH levels were untransformed because they closely approximated normality. The presence of TPOAb and TgAb were defined as having antibody levels equal to or above the respective assay detection limit. Because TgAb was below the limit of detection in 93% of men and 89% of women, only the presence of TPOAb antibodies was examined in bivariate or multivariate analyses.

Spearman bivariate correlation analysis, *t*-test, and non-parametric test were conducted to examine gender-stratified relationships between characteristics of study population and serum levels of free T4, total T3, TSH, TPOAb, and OC pesticides. Linear regression coefficients with their 95% confidence intervals were computed to determine the association between serum concentrations of each OC pesticide and levels of thyroid hormones and TSH, while controlling for confounders and stratifying by gender. Odds ratios were calculated to assess the risk of TPOAb presence associated with each OC pesticide. Further multivariate analysis of data was conducted stratifying the study population according to three different windows of exposure, *i.e.* residents that had lived in Cidade dos Meninos since birth; those moving to the study area during childhood; or residents that moved during adulthood.

Confounders were chosen on the basis of bivariate associations ($p < 0.10$) with thyroid status biomarkers and/or OC pesticides, and of previous literature on risk factors for OC exposure (*i.e.*, BMI, parity, and breastfeeding). Models also included the wet-weight serum levels of pesticides and the serum lipid content (towards inclusion of cholesterol and triglycerides levels as covariates). A significance level of

Table 1
Characteristics of adult population.

	Men	Women
Sample size	303	305
Age (years): mean (SD, range)	39 (17, 15–94)	39 (17, 15–92)
Ethnicity: *n* (%)		
White	93 (30.6)	94 (30.8)
Non-white	211 (69.4)	211 (69.2)
Years of residence: mean (SD, range)	23 (15, $<$1–61)	23 (14, $<$1–74)
Period of life in the study area: *n* (%)		
Born in the study area (*in utero* exposure)	82 (27.0)	89 (29.2)
Started living in childhood (1–14 years)	75 (24.7)	57 (18.7)
Started living in adulthood ($\geq$15 years)	147 (48.4)	159 (52.1)
BMI (kg/m^2): mean (SD, range)	24.9 (4.3, 15.0–39.7)	25.2 (5.8, 14.5–50.8)
Alcohol consumption: *n* (%)*		
No	142 (46.7)	233 (76.4)
Yes	162 (53.3)	72 (23.6)
Smoking habit: *n* (%)*		
Never	189 (62.2)	223 (73.1)
Ex-smoker	50 (16.4)	45 (14.8)
Current smoker	65 (21.4)	37 (12.1)
Parity: *n* (%)		
None	–	85 (27.9)
1	–	46 (15.1)
2	–	64 (21.0)
$\geq$3	–	110 (36.1)
Breastfeeding: *n* (%)		
Never	–	105 (34.4)
Ever	–	200 (65.6)
Total T3 (ng/dl): mean (SD, range)*	117 (25.8, 96.2–329)	124 (30.0, 86.4–162)
Free T4 (ng/dl): mean (SD, range)*	1.33 (0.23, 0.69–2.58)	1.30 (0.22, 0.90–1.53)
TSH (mU/l): mean (SD, range)	1.21 (0.92, 0.18–8.62)	1.20 (1.0, 0.12–11.3)
TPOAb (U/ml)		
Mean (SD, range)	18.8 (32.3, 10.0–399)	28.4 (101, 10.0–1000)
***n* (%) $\geq$ 10 U/ml[a]**	171 (56.3)	163 (53.4)
TgAb (U/ml)		
Mean (SD, range)	22.0 (16.2, 20.0–216)	41.5 (211, 20.0–3000)
***n* (%) $\geq$ 20 U/ml[a]**	21 (6.9)	34 (11.1)
Cholesterol (mg/dl): mean (SD, range)	175 (68, 37–399)	188 (50.4, 57–385)
Triglycerides (mg/dl): mean (SD, range)	127 (108, 29–798)	102 (57.6, 17–431)

SD: standard deviation; BMI: body mass index; TPOAb: anti-thyroperoxidase antibody; TgAb: anti-thyroglobulin antibody.

[a] Assay detection limits.

* $p < 0.05$ in χ^2 test or student *t*-test.

0.05 was established. SPSS version 17.0 (SPSS Inc., Chicago, IL, US) and STATA version 10.0 (Corporation, College Station, Texas) were used for the analyses.

3. Results

Characteristics of study population and thyroid parameters are described in Table 1. Both men and women had a mean age of 39 years (standard deviation, 17 years), had lived in Cidade dos Meninos for an average time of 23 years, had a BMI around 25 kg/m^2, and were mostly non-whites. Half of the study population moved to the study area during adulthood, and most men and women were non-smokers, although men were more likely to smoke than women ($p=0.005$). Half of the men reported drinking alcohol, while only 25% of women were alcohol consumers ($p<0.001$). There were a number of women (36%) that had given birth to 3 or more children, and much of them (66%) had breastfed anytime.

Total T3 levels were above the reference range (181 ng/dl) in 2 (0.7%) men and 11 (3.6%) women, and were below the reference (60 ng/dl) in 1 male (0.3%) and 1 female (0.3%). Free T4 level was elevated ($>$1.76 ng/dl) in 7 (2.3%) males and 5 (1.6%) females, and was low ($<$0.89 ng/dl) in 6 (2%) men and 6 (2%) women. Except for one hyperthyroid woman who had elevated total T3 and TSH below the reference, all these individuals had normal TSH levels (*i. e.* in the range 0.35–5.5 mU/l). Among participants with normal free T4 and total T3 levels, 2 (0.6%) men and 1 (0.3%) woman had elevated TSH, figuring subclinical hypothyroidism, while 11 men (3.6%) and 22 (7.2%) women had subclinical hyperthyroidism. TPOAb was above the laboratory reference value (35 U/ml) in 12 (3.9%) men and 15 (4.9%) women, while TgAb was above the reference (40 U/ml) in 5 (1.6%) men and 11 (3.6%) women (data not shown).

Prevalence of any level of TPOAb $\geq$ 10 U/ml was 56.3% in men and 53.4% in women. Gender differences were observed in thyroid hormone levels, *i.e.* women had significantly higher total T3 ($p=0.005$), but lower free T4 levels ($p=0.04$) than men (Table 1).

Bivariate analysis between thyroid hormones and characteristics of study population showed statistically significant negative correlations between total T3 levels and age and years of residence, both in men and women (Table S1, Supplementary material). Spearman coefficients (r) of these correlations ranged from -0.16 (men's years of residence) to -0.28 (women's age). Also, total T3 was lower among female ex-smokers compared to never smokers and among women with 3 or more children compared to none. Free T4 levels were negatively correlated with age ($r=-0.23$) and years of residence ($r=-0.11$) in men, and with BMI in women ($r=-0.11$). In addition, free T4 was significantly lower among men moving to Cidade dos Meninos after childhood ($\geq$15 years), ex-smoker males and non-white females. Levels of TSH were positively correlated with women's BMI, and were significantly lower among non-white women and current male smokers compared to never smokers.

Men with TPOAb levels $\geq$10 U/ml (7% of which had positive TPOAb) were older ($p<0.01$) than those with non-detected TPOAb (Table S2, Supplementary material and Fig. 1). The presence of TPOAb was also higher in older women ($>$49 years) and in those in the age range of 20–34 years (Fig. 1), but the difference was not significant. Also, women with detected TPOAb were less likely to smoke ($p=0.02$) (Table S2). When TSH levels were examined according to TPOAb prevalence and age (Fig. 2), it was observed that mean TSH level was higher ($p=0.03$) among older men ($>$64 years) with presence of TPOAb, compared to men with non-detected TPOAb. The same was seen among women 20–34 years old but, contrary to men, older women with presence of TPOAb had lower TSH mean level ($p=0.04$) than those with non-detected TPOAb. Statistically significant differences in TPOAb presence were not observed according to other characteristics of study population.

Serum *p,p′*-DDE and beta-HCH showed the highest prevalences ($>$99%), followed by gamma-HCH, alpha-HCH, *p,p′*-DDT, and dieldrin ($>$90%), both in men and women (Table 2). The highest median concentrations in both sexes were observed for *p,p′*-DDE, beta-HCH, *p,p′*-DDT, alpha-HCH, and aldrin. Methoxychlor was the compound with the lowest prevalence ($<$40%). Except for HCB, aldrin, and methoxychlor, most correlations between pesticide concentrations were positive and statistically significant (data not shown). Fig. S3 (Supplementary material) illustrates correlations between beta-HCH and *p,p′*-DDE among males ($r=0.68$) and females ($r=0.72$), which were amongst the pesticides showing the highest prevalence and serum levels. Concentrations of a large number of OC pesticides were associated with age, years of

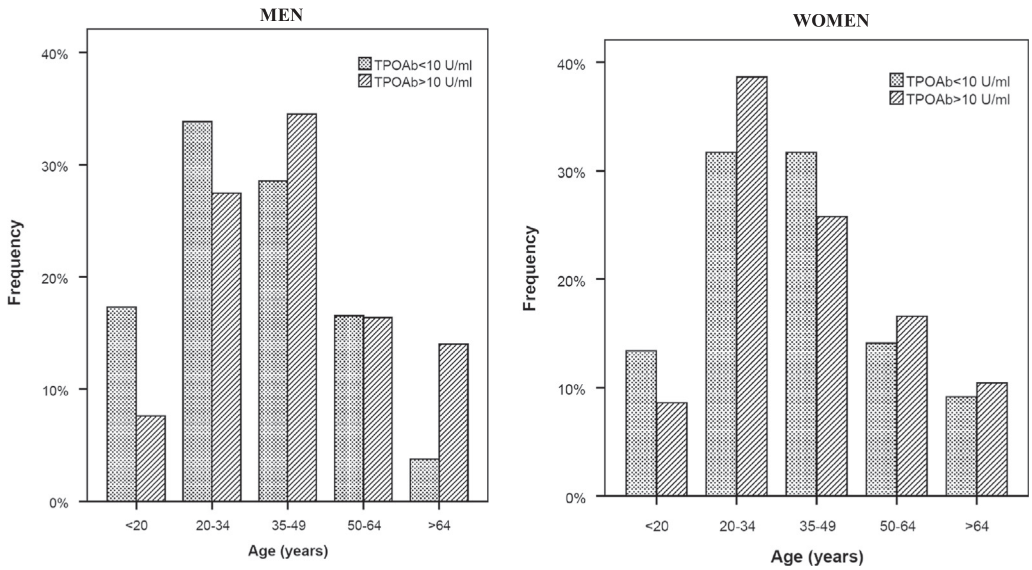

Fig. 1. Prevalence of anti-thyroperoxidase antibody (TPOAb) according to age.

residence, BMI, and ethnicity, both in men and women (data not shown). For instance, beta-HCH serum levels in men were positively correlated with age, years of residence, and BMI (*r* ranging between 0.18 and 0.26, $p<0.05$), and were significantly higher among non whites. In females, most pesticides were additionally associated with smoking, and much of them were associated with parity.

All multivariate models were adjusted for age, years of residence in Cidade dos Meninos, ethnicity, BMI, cholesterol, and triglycerides serum content (Tables 3 and 4). Women's models were additionally adjusted for smoking, parity, and breastfeeding. Several significant associations were observed between OC pesticide serum concentrations and levels of thyroid hormones, which differed between sexes. Exposure to endosulfan 2 was significantly associated with decreased total T3 in men (Table 3), while it was positively associated with such hormone in women (Table 4). In addition, increased total T3 levels were significantly associated with higher concentrations of methoxychlor, alpha-chlordane, and *p*,*p*′-DDT in women. In men, free T4 levels were inversely and significantly associated with beta-HCH and *p*,*p*′-DDT, and borderline negative associations were observed with alpha-HCH and aldrin (Table 3). Conversely, elevation in free T4 in women was significantly associated with serum *o*,*p*′-DDT, heptachlor, *p*,*p*′-DDT, and HCB, and was marginally associated with higher *p*,*p*′-DDE (Table 4). Increased levels of TSH in men were significantly associated with beta-HCH and marginally associated with *p*,*p*′-DDE. Evidence for association between OC pesticides and TSH was not found among women.

When multivariate analysis was conducted stratifying by window of exposure (using the same potential confounders but “years of residence”), results in men were somehow inconsistent (data not shown). Thus, increased total T3 was positively and significantly related to exposure to alpha-chlordane and negatively associated with gamma-HCH in males born in Cidade dos Meninos and among those that moved to the study area during childhood, respectively. However, the aforementioned statistically significant association between beta-HCH and *p*,*p*′-DDT and lower free T4, as well as the association between beta-HCH and higher TSH, were found among men moving to Cidade dos Meninos during adulthood. In contrast, all significant associations with thyroid hormones in females were positive and most of them were observed in women born in Cidade dos Meninos, *i.e.*, total T3 was associated with beta-HCH, *o*,*p*′-DDT, and *p*,*p*′-DDT; free T4 was related to heptachlor, *p*,*p*′-DDE, *o*,*p*′-DDT, and endrin; and TSH was associated with heptachlor, *o*,*p*′-DDT, endosulfan 1, endrin, and dieldrin. In addition, alpha-chlordane showed a statistically significant association with elevated total T3 among women moving to Cidade dos Meninos during adulthood.

Regarding TPOAb, associations with OC were weak and inconsistence. Men with detected methoxychlor had a statistically significant 2-fold increased risk estimate for presence of TPOAb antibodies (Table 3). Such a risk estimate was 3-fold significantly increased in men that did not live in Cidade dos Meninos during childhood (data not shown). Among females, aldrin was associated with the presence of TPOAb, but the risk estimate was increased only by 1% (Table 4).

4. Discussion

In this cross-sectional study of adult residents of a rural village with high environmental levels of OC pesticides, biochemical parameters of thyroid function (free T4, total T3, TSH, TPOAb, and TgAb) were, in general, within the reference values. However, differences in hypo- and hyperthyroidism prevalences between this and non-exposed populations elsewhere were noted (Bahn et al., 2011; Hollowell et al., 2002; Vanderpump and Tunbridge, 2002). Low and high free T4 levels were equally prevalent in the present study, while high T3 was more prevalent than low levels. The prevalence of TSH below the reference value (embracing subclinical and clinical hyperthyroidism) was 5.6%, while only 0.5% of participants had increased TSH (embracing clinical and subclinical hypothyroidism). In contrast, other studies in iodine-replete populations of similar mean age as that in Cidade dos Meninos have reported higher prevalence of hypothyroidism than hyperthyroidism (Hollowell et al., 2002; Tunbridge et al., 1977). For its part, the American Association of Clinical Endocrinologists in its 2011 guideline reported a national prevalence of hyperthyroidism of approximately 1.2% (being 0.5% overt and 0.7% subclinical) (Bahn et al., 2011), which is about 5 times lower than what was found in adult population in the present study.

In comparing our results to those other studies that have examined OC pesticides and thyroid hormone status, our findings in men are quite coherent with published findings. In general, epidemiological studies in adults have found no association or have found inverse relationships between these OC pesticides and

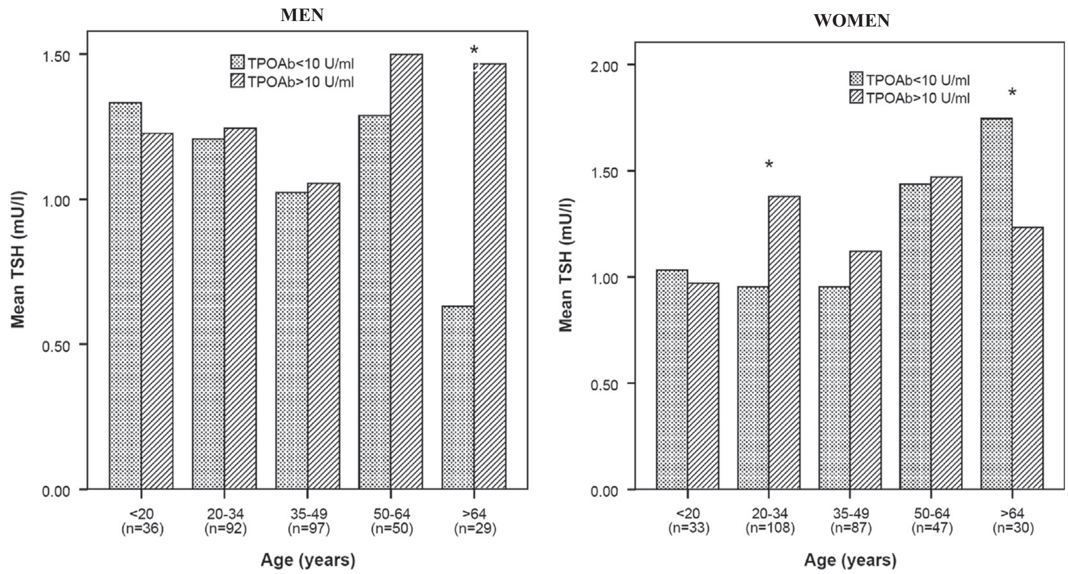

Fig. 2. Mean concentration of thyroid-stimulating hormone (TSH) according to prevalence of anti-thyroperoxidase antibody (TPOAb) and age in men and women; $^{*}p < 0.05$.

Table 2
OC pesticide concentrations in serum (ng/ml).

Pesticides	Men		Women	
	% > LD	Median (P25; P75)	% > LD	Median (P25; P75)
Alpha-HCH	95.5	2.52 (0.97; 0.65)	94.7	2.60 (1.01; 6.03)
Beta-HCH	99.1	6.00 (2.08; 15.4)	97.0	6.98 (2.81; 17.6)
Gamma-HCH	96.0	0.95 (0.44; 2.21)	95.8	0.97 (0.37; 2.22)
HCB	88.0	0.33 (0.14; 0.63)	87.8	0.37 (0.17; 0.67)
Alpha-chlordane	73.8	0.23 (< LD; 0.51)	74.4	0.27 (< LD; 0.60)
Gamma-chlordane	66.3	0.16 (< LD; 0.44)	67.3	0.16 (< LD; 0.39)
***Trans*-nonachlor**	82.5	0.32 (0.16; 0.77)	83.3	0.38 (0.20; 0.78)
Heptachlor	71.2	0.31 (< LD; 0.89)	71.9	0.35 (< LD; 0.77)
***p,p′*-DDE**	99.8	8.32 (2.86; 21.9)	98.4	9.64 (3.45; 28.9)
***o,p′*-DDT**	60.0	0.30 (< LD; 0.89)	63.5	0.42 (< LD; 1.10)
***p,p′*-DDT**	93.7	3.09 (0.94; 6.96)	93.1	3.20 (1.03; 7.59)
***p,p′*-DDD**	80.9	0.61 (0.19; 1.34)	82.0	0.66 (0.21; 1.41)
Endosulfan 1	63.0	0.22 (< LD; 0.46)	62.6	0.22 (< LD; 0.42)
Endosulfan 2	61.9	0.23 (< LD; 0.71)	63.8	0.24 (< LD; 0.69)
Aldrin	86.5	1.89 (0.73; 11.0)	88.5	2.44 (0.77; 14.1)
Endrin	87.4	0.63 (0.24; 1.48)	87.1	0.58 (0.25; 1.51)
Dieldrin	90.2	0.61 (0.28; 1.27)	90.1	0.56 (0.24; 1.21)
Mirex	68.3	0.47 (< LD; 0.97)	71.6	0.41 (< LD; 1.03)
Methoxychlor	32.5	< LD (< LD; 0.28)	38.7	< LD (< LD; 0.34)

LD: Limit of detection; P25, P75: 25th and 75th percentiles.

levels of T4 and T3 (Langer, 2010). Thus, serum HCB was inversely related to total T4 (Sala et al., 2001) and free T4 (Schell et al., 2008) in adults and adolescents living in areas with high OC pollution, respectively. Nevertheless, free T4 was not associated with HCB in the first study (Sala et al., 2001) nor was with *p,p′*-DDE in the latter (Schell et al., 2008). An inverse association between total T4 and HCB but not *p,p′*-DDE was found in a small sample of sportsmen (Bloom et al., 2003). Lowered T3 was also associated with HCB exposure in a small study among 16 obese men, which found no significant relation with *p,p′*-DDE or DDT (Pelletier et al., 2002). No association between HCB, DDT, or *p,p′*-DDE and thyroid hormones were reported in adult populations elsewhere (Hagmar et al., 2001; Persky et al., 2001; Rylander et al., 2006; Turyk et al., 2006, 2007). No statistically significant associations were found between thyroid hormone levels and other OC pesticides, such as mirex, beta-HCH, *trans*-nonachlor, and oxychlordane in other populations (Bloom et al., 2003; Pelletier et al., 2002; Schell et al., 2008).

Women in the present study showed several positive associations between serum concentrations of OC pesticides and thyroid hormone levels, which have been rarely reported in the literature (Meeker et al., 2007; Rádiková et al., 2008). One study investigating 341 adult men observed a positive association of serum *p,p′*-DDE with total T3 and free T4 (Meeker et al., 2007). For their part, Rádiková et al. (2008) found some striking results in a study that examined the thyroid status of more than 2000 people living in two areas, one of which was an area with heavy OC pollution and a background pollution area. Using PCB serum level as indicator of OC exposure, they found a positive association between OC exposure and total T3 and free T4, a weak negative association with TSH, and a positive relation with thyroid volume (Rádiková et al., 2008). In contrast, inverse relationships have been reported in pregnant women between levels of HCB in plasma and total T3 (Takser et al., 2005) and between serum *p,p′*-DDE and free T4 (Lopez-Espinosa et al., 2009).

In Cidade dos Meninos, positive associations between exposure to *p,p′*-DDT and total T3, and between *p,p′*-DDT, *p,p′*-DDE, or *o,p′*-DDT and free T4 were found in women. Furthermore, they showed positive associations of endosulphan 2, methoxychlor, alpha-chlordane, heptachlor, and HCB with total T3 or free T4. Interestingly, significant positive associations between OC pesticides and thyroid hormones were observed only among women who were born in the village, which seems to be in accordance with results of a similar analysis among children (≤ 14 years) living in the study area. In this age stratum, a consistent trend of increasing total T3 with higher levels of exposure to OC pesticides was reported (Freire et al., 2012). The authors suggested that the thyroid system in children with long-term OC exposure could respond comparably as adults in similar conditions. Inconsistent findings in men according to windows of exposure could be explained by lack of statistical power when using smaller population strata (*i.e.* participants born in Cidade dos Meninos represent about 30% of the study population).

Regarding TSH, conflicting findings on the relationship with OC pesticides have been reported in several adult cohort studies (Langer, 2010). Among studies reporting significant associations with TSH, inverse relationships were found with exposure to *p,p′*-DDE in three studies (Langer et al., 2006; Lopez-Espinosa et al., 2009; Meeker

Table 3
Adjusted association between OC pesticide serum concentrations and thyroid parameters in men.

Pesticides (ng/ml)	Total T3 (ng/dl)		Free T4 (ng/dl)		TSH (mU/l)		TPOAb ≥ 10 U/ml	
	β	95% CI	*β*	95% CI	*β*	95% CI	OR	95% CI
Alpha-HCH	0.04	−0.14; 0.23	−0.002	−0.003; 0.00	−0.003	−0.01; 0.004	0.99	0.97; 1.01
Beta-HCH	0.06	−0.04; 0.15	**−0.003**	**−0.005; −0.001**	**0.003**	**0.001; 0.007**	1.00	0.99; 1.01
Gamma-HCH	0.23	−0.51; 0.98	−0.006	−0.012; 0.001	−0.01	−0.04; 0.01	0.95	0.87; 1.03
HCB	−0.24	−0.61; 0.12	−0.002	−0.005; 0.002	−0.004	−0.02; 0.01	0.83	0.65; 1.06
Alpha-chlordane	−0.28	−3.31; 2.76	−0.01	−0.04; 0.01	−0.04	−0.15; 0.07	0.85	0.64; 1.14
Gamma-chlordane	−0.53	−3.64; 2.58	−0.004	−0.03; 0.02	−0.05	−0.17; 0.06	0.66	0.41; 1.01
***Trans*-nonachlor**	−0.20[a]	−3.96; 3.56	−0.01[a]	−0.04; 0.02	−0.08[a]	−0.22; 0.05	1.01[a]	0.75; 1.35
Heptachlor	−0.65	−1.96; 0.67	−0.009	−0.02; 0.003	−0.03	−0.08; 0.02	0.92	0.82; 1.03
***p,p′*-DDE**	−0.003	−0.08; 0.07	−0.001	−0.002; 0.00	0.003	0.00; 0.005	1.00	0.99; 1.01
***o,p′*-DDT**	0.49	−1.17; 2.15	−0.004	−0.02; 0.01	−0.03	−0.09; 0.03	0.92	0.80; 1.07
***p,p′*-DDT**	0.14	−0.18; 0.46	**−0.003**	**−0.006; −0.001**	−0.004	−0.01; 0.01	1.02	0.99; 1.05
***p,p′*-DDD**	−1.01	−2.77; 0.75	−0.009	−0.02; 0.006	−0.03	−0.10; 0.03	0.99	0.86; 1.15
Endosulfan 1	−0.95	−5.21; 3.30;	−0.02	−0.06; 0.01	−0.08	−0.23; 0.07	0.83	0.57; 1.21
Endosulfan 2	**−2.27**	**−4.55; −0.01**	−0.006	−0.03; 0.01	−0.06	−0.14; 0.03	0.95	0.78; 1.14
Aldrin	0.05	−0.05; 0.15	−0.001	−0.002; 0.00	0.001	−0.003; 0.005	1.00	0.99; 1.01
Endrin	−0.40	−1.75; 0.95	−0.002	−0.01; 0.01	−0.03	−0.07; 0.02	0.95	0.85; 1.06
Dieldrin	−0.003	−1.62; 1.61	−0.01	−0.03; 0.002	−0.03	−0.09; 0.03	0.88	0.75; 1.03
Mirex	−0.92[b]	−2.97; 1.12	−0.01[b]	−0.03; 0.006	−0.07[b]	−0.14; 0.005	0.95[b]	0.81; 1.13
Methoxychlor[c]	1.88[a]	−4.63; 8.40	0.001[a]	−0.06; 0.06	−0.02[a]	−0.25; 0.22	**2.19**[a]	**1.26; 3.76**

β: Linear regression coefficient; CI: confidence interval; OR: odds ratio for TPOAb levels ≥ 10 U/ml compared to levels < 10 U/ml. All coefficients and odds ratios are adjusted for age, ethnicity, years of residence in Cidade dos Meninos, body mass index, cholesterol and triglycerides serum content. The bold values indicate $p < 0.05$.

[a] Additionally adjusted for smoking.

[b] Additionally adjusted for smoking and alcohol consumption.

[c] Methoxychlor was treated as a dichotomous variable, *i.e.* levels below and above the limit of detection.

Table 4
Adjusted association between OC pesticide serum concentrations and thyroid parameters in women.

Pesticides (ng/ml)	Total T3 (ng/dl)		Free T4 (ng/dl)		TSH (mU/l)		TPOAb ≥ 10 U/ml	
	β	95% CI	*β*	95% CI	*β*	95% CI	OR	95% CI
Alpha-HCH	0.24	−0.10; 0.58	0.002	−0.001; 0.004	0.004	−0.007; 0.02	0.99	0.97; 1.02
Beta-HCH	0.08[a]	−0.04; 0.22	0.001[a]	0.00; 0.002	−0.001[a]	−0.005; 0.004	1.00[a]	0.99; 1.01
Gamma-HCH	0.64	−0.54; 1.84	0.003	−0.006; 0.01	0.01	−0.03; 0.05	0.99	0.90; 1.08
HCB	1.11	−1.40; 3.63	**0.02**	**0.001; 0.04**	−0.03	−0.11; 0.06	0.93	0.76; 1.12
Alpha-chlordane	**5.66**	**1.12; 10.21**	0.01	−0.03; 0.04	0.03	−0.13; 0.19	0.93	0.67; 1.29
Gamma-chlordane	2.54	−3.81; 8.90	0.004	−0.04; 0.05	0.07	−0.16; 0.29	1.16	0.72; 1.87
***Trans*-nonachlor**	2.23	−3.19; 7.65	0.006	−0.03; 0.05	−0.03	−0.22; 0.16	0.84	0.57; 1.26
Heptachlor	0.54	−1.24; 2.32	**0.02**	**0.003; 0.03**	0.01	−0.05; 0.08	1.01	0.89; 1.15
***p,p′*-DDE**	−0.02	−0.09; 0.04	0.001	0.00; 0.001	−0.001	−0.003; 0.002	1.00	0.99; 1.01
***o,p′*-DDT**	1.34	−1.13; 3.82	**0.02**	**0.007; 0.04**	0.01	−0.07; 0.10	1.04	0.86; 1.24
***p,p′*-DDT**	**0.40**	**0.06; 0.73**	**0.003**	**0.001; 0.005**	0.002	−0.01; 0.01	1.01	0.99; 1.04
***p,p′*-DDD**	1.17[a]	−1.07; 3.42	0.007[a]	−0.01; 0.02	0.01[a]	−0.07; 0.09	1.08[a]	0.92; 1.28
Endosulfan 1	3.39	−2.46; 9.24	0.007	−0.04; 0.05	0.15	−0.05; 0.35	1.06	0.69; 1.64
Endosulfan 2	**3.54**	**0.12; 6.95**	0.004	−0.02; 0.03	−0.06	−0.18; 0.06	1.11	0.86; 1.43
Aldrin	−0.02[a]	−0.14; 0.10	0.001[a]	−0.001; 0.001	0.001[a]	−0.003; 0.005	**1.01**[a]	**1.00; 1.02**
Endrin	0.62	−1.39; 2.61	0.01	−0.004; 0.03	−0.02	−0.09; 0.05	1.06	0.91; 1.23
Dieldrin	0.74	−1.18; 2.67	0.006	−0.009; 0.02	0.02	−0.05; 0.08	1.06	0.91; 1.24
Mirex	1.12[a]	−0.25; 2.46	0.001[a]	−0.01; 0.01	−0.007[a]	−0.05; 0.04	0.94[a]	0.85; 1.05
Methoxychlor[b]	**8.54**	**1.69; 15.41**	−0.01	−0.06; 0.04	−0.08	−0.32; 0.16	1.39	0.84; 2.30

β: Linear regression coefficient; CI: confidence interval; OR: odds ratio for TPOAb levels ≥ 10 U/ml compared to levels < 10 U/ml. All coefficients and odds ratios are adjusted for age, ethnicity, years of residence in Cidade dos. The bold values indicate $p < 0.05$.

[a] Additionally adjusted for alcohol consumption.

[b] Methoxychlor was treated as a dichotomous variable, *i.e.* levels below and above the limit of detection.

et al., 2007), with HCB in one study (Langer et al., 2006), and a positive relation with *p,p′*-DDE was reported in one study (Rylander et al., 2006). In contrast, serum levels of HCB were no associated with TSH in adults living in the vicinity of an OC compound factory (Sala et al., 2001).

Several positive associations were found between TSH and OC pesticide in women born in Cidade dos Meninos, as well as with beta-HCH in all men, as reported in newborns in Spain (Alvarez-Pedrerol et al., 2008; Ribas-Fitó et al., 2003). Finding in men in the present study could be explained by the significant reduction of free T4 associated to beta-HCH exposure, which might have stimulated compensatory reactions, resulting in increased TSH levels. It is noteworthy to mention that HCH isomers present specific toxicological properties compared with other OC pesticides (ATSDR, 2005), but limited information is available on their specific thyroid effects. In addition, because no relation was seen with any of the remaining pesticides, the possibility that the association between beta-HCH and TSH was due to chance cannot be ruled out.

Anti-thyroid antibodies prevalence rises with age (Antonelli et al., 2006) and depends on the sensitivity of the method employed. Population-based studies among euthyroid individuals using high-sensitivity assays (*i.e.* limits of detection of 0.5, 1.1, and

0.3 U/ml) reported prevalences of detected TPOAb ranging from 8.0% to 14.4% in men and from 14.6% to 25.8% in women (Hollowell et al., 2002; Prentice et al., 1990; Zöphel et al., 2003). In general, using immunoassays with higher sensitivity, the prevalence of detectable levels of anti-thyroid antibodies reaches 10% to 12% of the healthy population, being greater in women (Vanderpump, 2011). Using an assay with a low sensitivity (*i.e.* TPOAb detection limit of 10 U/ml), the present study found a prevalence of TPOAb of 56% in men and 53% in women, which is over 6 times higher than that observed in healthy controls in a study with the same detection limit (Mariotti et al., 1990). Such a high prevalence may be indicative of an immune alteration that could have been triggered by exposure to endocrine-disrupting OC pesticides in residents in Cidade dos Meninos. However, the fact that TPOAb and TgAb levels were above the reference values (*i.e.* 35 U/ml and 40 U/ml, respectively) in less than 5% of adults prevents us from concluding that this population is at higher risk for autoimmune thyroid disease than the general population.

There is increasing evidence that the positivity of thyroid antibodies in euthyroid individuals indicates an autoimmune thyroid disease component that may lead to the development of thyroid dysfunction (Roti et al., 1992; Strieder et al., 2003). Thus, positive TPOAb has been associated with elevated TSH and risk of hypothyroidism, particularly in older individuals (O'Leary et al., 2006; Strieder et al., 2003). Accordingly, such a direct relationship between TPOAb detection, TSH and age were observed in men in Cidade dos Meninos, but not in women. Regarding association with environmental exposures, little evidence exists for the potential of OC compounds to impair immune responses in humans (Rádiková et al., 2008; Shell et al., 2009). Rádiková et al. (2008) observed a statistically significant higher prevalence of positive TPOAb (above 37 U/ml) in people living in a highly OC polluted area compared to residents in a background area, particularly in men. Also, elevated TPOAb levels were observed in young adults with higher *p*,*p*′-DDE, mirex, and HCB exposure levels (Schell et al., 2009). Except for methoxychlor, any relation between anti-thyroid antibodies and OC exposure could not be confirmed in adult population from Cidade dos Meninos.

There are several postulated mechanisms for the effect of OC compounds on thyroid system, which may affect thyroid hormone metabolism and alter circulating thyroid hormone levels (Boas et al., 2006). These mechanisms are most likely to manifest as altered T4 and TSH levels due to compensatory production/secretion of T4 or TSH to maintain homeostasis. The majority of T3 in humans is derived from the enzymatic removal of an iodine atom from T4 by deiodinases (Nussey and Whitehead, 2001; Vander et al., 1998), signifying that, if the deiodination mechanism is intact, a decrease in T3 should also be accompanied by a decrease in T4. Also, altered thyroid hormone levels give rise to increased or decreased TSH secretion from the anterior pituitary through negative feedback, so studies that observe altered levels of thyroid hormones should also see inversely altered levels of TSH. Thus, while findings in the present study relating to men were expected, the positive associations between certain OC pesticides and total T3 or free T4 observed in women suggest an alternative mechanism that affects thyroid hormone levels. Total T3 is bound to albumin and thyroxine-binding globulin (TBG), which is known to increase with estrogen exposure such as oral contraceptive use or pregnancy (Tahboub and Arafah, 2009). Total thyroid hormone levels were positively related to endocrine disruptors with estrogen-like activity, providing an explanation for the increased total T3 levels in women other than the direct thyroid action. Hence, some mechanisms of thyroid disruption by OC compounds may be then gender specific, according to previous observations that the likelihood of thyroid dysfunction appears to be greater in females (Lee et al., 2007; O'Leary et al., 2006).

It is now thought that environmental chemicals have very complex interactions with the thyroid hormone receptor, producing complex effects on hormone signaling (Zoeller, 2005). Hence, it is reasonable that mechanisms explaining the positive associations with total T3 and free T4, not necessarily accompanied by an inverse association with TSH, are unclear at this time. OC compounds may affect the deiodination of thyroid hormones by inducing or increasing type 3 deiodinase expression leading to increased T3 degradation; or may bind to thyroid hormone binding proteins (thyroid-binding globulin, transthyretin, and/or albumin) which may alter circulating T4 and T3 levels. Findings in women in the present study may suggest an effect of OC pesticides on one or more of these mechanisms, which might be more marked among women exposed to such chemicals during a critical time window. Because all OC pesticides analyzed presented high residuals levels in the study population, their interaction may also occur. In this case, it is likely that the major toxicological effect prevails, different to situations of exposure to single compounds or exposure to multiple chemicals at different doses.

Studies on adverse health effects associated with small changes in thyroid hormones in humans are limited (Boelaert and Franklyn, 2005; Surks et al., 2004). Most changes found in the present study may not be clinically important at the individual level except for the increased clinical and subclinical thyroid hyperfunction observed in the present population, compared to other studies. However, due to the chronic exposure to high levels of these OC compounds in Cidade dos Meninos, together with the increased detection of anti-thyroid antibodies, there may be persistent consequences related to subtle changes in thyroid function.

The main limitation of the present study is its cross-sectional design, which prevents to ascertain whether OC pesticide exposures are causally related to outcomes of thyroid status. It should be noted that about 50% of the study population had been living in Cidade dos Meninos for, at least, the 20 past years, and 75% for 10 years or more, periods wherein they were very probably exposed to high concentrations of OC pesticides as well. Hence, although serum pesticide measurement did not precede thyroid data, thyroid disruption by OC pesticide is a plausible explanation for the observed significant associations. The high prevalence of subclinical hyperthyroidism found in the present population could be explained by exogenous causes of lowered TSH others than environmental exposure to thyroid-disrupting chemicals. For instance, the use of slimming pills was found to decrease TSH levels in a large sample of Brazilian women (Sichieri et al., 2007). In the present study, low TSH levels were more prevalent in women (7.2%), but men also showed an increased prevalence of low TSH (3.6%) compared to other studies. The lack of this and other information, such as use of thyroid medications, represents a limitation to the study. In addition, no analytical data on free T3 and total T4 were available, which would have provided a better understanding of the thyroid status of the study population.

The present study has several strengths. First, it is so far the first investigation performed in Latin America analyzing the long-term effects of endocrine disrupting chemicals on thyroid function among adults. Second, the present results are based on a large population sample, *i.e.* a total of 608 adults, which allowed us to stratify the analysis by gender and explore potential sex-specific associations. Third, body concentrations of 19 OC pesticides were determined, many of which have been scarcely or never studied elsewhere in relation to human thyroid status. In addition, epidemiological research on the relation between OC exposure and thyroid function has rarely assessed effects on immune response, as measured by levels of thyroid antibodies in this investigation.

5. Conclusions

Prevalence of subclinical hyperthyroidism and the presence of TPOAb antibodies in adult residents in Cidade dos Meninos were higher than those described for euthyroid populations elsewhere. Exposure to certain OC pesticides was associated with lower levels of thyroid hormones in men, whereas positive associations were found for exposure to some pesticides in women. These results suggest that OC pesticides can affect the thyroid system through gender-specific mechanisms that may differ among compounds. Further detailed investigations and health monitoring should be warranted for this population.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2013.09.001.

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