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Alterations in male reproductive hormones in relation to environmental DDT exposure

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ABSTRACT

DDT [1, 1, 1-trichloro-2,2-bis (*p*-chlorophenyl)-ethane] compounds are used for indoor residual spraying (IRS) to control malaria mosquitoes. DDT is an endocrine disruptor chemical in experimental conditions, but little is known of adverse effects related to living conditions with continual uptake across a time span by all possible means of exposure. Based on estrogenic and/or anti-androgenic effects found in animal studies, we hypothesized that chronic DDT/DDE exposures in men may be associated with changes in male reproductive hormones. We tested this hypothesis by compared the magnitude and direction of associations between DDT and DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene) concentrations and male reproductive hormones in samples collected from IRS and non-IRS areas.

We sampled a cross-section of 535 men (aged 18–40 years). Men living in IRS villages had significantly higher DDT and DDE concentrations compared with men from non-IRS villages. Men with DDT or DDE uptake (as reflected in detectable plasma concentrations) had significantly higher total-, free and bio-available testosterone (T), and lower follicle stimulating hormone (FSH) concentrations; lower luteinizing hormone (LH) concentrations were only evident with DDT uptake. To establish a dose-dependent effect, four sub-categories were defined. Men with the highest DDT (74–519 μg/g) and DDE (173–997 μg/g) concentrations had significantly higher total-, free and bio-available T, and lower FSH concentrations compared with subjects with non-detectable isomer concentrations. Estradiol concentrations were significantly higher in men with DDT and DDE concentrations in both the third (DDE: 27–172 μg/g; DDT: 5–73 μg/g) and fourth (DDE: 173–997 μg/g; DDT: 74–519 μg/g) categories. Men from IRS villages were significantly more likely to have higher total and bioavailable T as well as higher estradiol concentrations OR = 2.5 (95% CI 1.2, 3.2); OR 2.5 (95% CI 1.6, 4.0) and OR = 2.3 (95% CI 1.3, 4.1) compared to men from non-IRS villages, after controlling for age, BMI, personal use of pesticides, and smoking.

Men living in IRS villages with life-long exposure (17.6 (± 6) years) at the current residence with multiple exposure modalities incurred the highest degree of physiological imbalance over and above circulating isomer concentrations. Further studies are needed to elucidate the health implications of these findings.

Abbreviations: AR, Androgen receptor; BDL, Below detection limit; BFRs, Brominated flame retardants; BMI, Body mass index; b-T, Bioavailable testosterone; CYP19, Aromatase; DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene; DDT, 1, 1, 1-trichloro-2,2-bis (*p*-chlorophenyl) ethane; DHT, Dihydrotestosterone; E_2, Estradiol; ECL, ElectroChemi-Luminescence; ER, Estrogen receptors; FSH, Follicle stimulating hormone; f-T, Free testosterone; HBCD, Hexabromocyclododecane; HPT, Hypothalamic-pituitary-testicular; IRS, Indoor residual spraying; LH, Luteinizing hormone; LOD, Limit of detection; PBDEs, Polybrominated diphenyl ethers; PCBs, Polychlorinated biphenyls; POPs, Persistent organic pollutants; SHBG, Sex hormone-binding globulin; SHSPH, School of Health Systems and Public Health; t-T, Total Testosterone; T, Testosterone; ΣDDT, Sum of DDT isomers

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1. Introduction

DDT is an insecticide sprayed onto the inside walls of homes for indoor residual spraying (IRS) to control malaria mosquitoes in several endemic countries and areas (WHO, 2011). The DDT used for spraying (termed 'technical DDT') contains 65%–80% of the active insecticidal ingredient, 1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane (*p,p′*-DDT) and 15%–21% of the less insecticidal 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl) ethane (*o,p′*-DDT) (Bouwman, 2004). DDT bio-accumulates in fatty tissue as the metabolite 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (*p,p′*-DDE) (referred to as DDE) (ATSDR, 2002; Smith, 1991). Both DDT and DDE are persistent organic pollutants (POPs) and are hormonally active substances (ATSDR, 2002).

In vitro, DDT and DDE impact on various biochemical and physiological processes, including estrogen receptors (ER) and androgen receptors (AR), which ultimately influence complex hormonal regulatory systems. Technical DDT (mixture of *p,p′*- and *o,p′*-isomers) is estrogenic, but has less agonist activity than estradiol as monitored by ER-positive cell lines (Chen et al., 1997; Dees et al., 1997) and also stimulates ERα- and ERβ-mediated transcription (Lemaire et al., 2006). DDE competitively binds with the androgen receptor and blocks androgen-induced transcription (Kelce et al., 1995; Kelce and Wilson, 1997). DDE also stimulates aromatase (CYP19) activity in cultures of human ovarian (Younglai et al., 2004) and endometrial cells (Holloway et al., 2005), thereby significantly increasing local estradiol (E_2) concentrations through elaboration from testosterone.

In vivo DDT exposure to adult male (Krause, 1977) and juvenile rats (Rhouma et al., 2001) resulted in lower testosterone concentrations and inconsistent follicle-stimulating hormone (FSH) and luteinizing hormone (LH) concentrations, but others reported reduced dihydrotestosterone (DHT), increased estradiol (E_2) and no significant changes in testosterone, LH and FSH concentrations (O'Connor et al., 2002). In vivo DDE exposure resulted in significantly increased estradiol and T, but decreased FSH concentrations (O'Connor et al., 2002). Multigenerational in vivo reproductive toxicity studies using DDT in mice (Tarjan and Kemeny, 1969; Turusov et al., 1973), rats (Ottoboni, 1969, 1972) and dogs (Ottoboni et al., 1977) were not contributing to a clearer understanding of DDT effects. Collectively, the in vitro and in vivo evidence supports the estrogen-like properties of DDT (Metcalf and Nicola, 1995) and the AR antagonist properties of DDE (ATSDR, 2002; Danzo, 1997).

The epidemiologic associations of DDT and DDE exposure with male sex hormones in humans are inconsistent and the comparison of findings is confounded by differences in exposure concentrations, duration, pathways/routes and study populations (Ayotte et al., 2001; Blanco-Muñoz et al., 2012; Bonde et al., 2008; Cocco et al., 2004; Dalvie et al., 2004a; Hagmar et al., 2001; Haugen et al., 2011; Martin Jr et al., 2002; Rignell-Hydbom et al., 2004). Most reported studies arise from the United States and Europe, where the use of DDT has been banned since the late 1970s, such that exposures were low and indirect. In men from IRS-related exposure, Mexican men with non-occupational DDT exposure had significant correlation between DDE concentrations and the ratio of bioavailable to total testosterone (Ayotte et al., 2001). Workers spraying DDT in the Limpopo province of South Africa had positive correlations between both E_2 and t-T concentrations and ΣDDT isomers (Dalvie et al., 2004b). Young men living in an IRS area had impaired semen quality associated with environmental DDT exposure (Aneck-Hahn et al., 2007) and weak associations with a high incidence of sperm with DNA breaks (De Jager et al., 2009).

To the best of our knowledge, the impact of concurrent exposure to both DDT and DDE on male reproductive hormones (hypothalamic-pituitary-testicular (HPT) hormones); has not been reported in animal models or of men living in a currently IRS area. Based on in vivo and in vitro evidence that DDT is estrogenic and the metabolite, DDE is anti-androgenic, men in an IRS area may be simultaneously exposed to a mixture of DDT and DDE and experience estrogenic, anti-androgenic effects or a combination. We tested the hypothesis that exposure to DDT has anti-androgenic and/or estrogenic effects and changes in reproductive hormone concentrations. We compared male reproductive hormones and DDT and DDE concentrations in samples collected from men living in IRS and non-IRS areas to determine the difference in DDT and DDE concentrations and whether these DDT and DDE concentrations were associated with changes in male reproductive hormones.

2. Materials and methods

2.1. Study design and population

This cross-sectional, observational study was part of a larger ongoing study that commenced in 2003 to evaluate the effects of indoor residual spraying (IRS) on the reproductive health of young men from Limpopo, South Africa.

2.2. Study area

The Limpopo Province is situated in the north-eastern corner of South Africa and is divided into five districts, including the Vhembe District. The Vhembe district is a malaria-endemic area where housing comprises traditional mud dwellings with thatch (straw grass) roofs or brick and cement houses. The inside of unpainted brick, cement, and daub houses are sprayed annually with DDT to control malaria mosquitos. DDT is usually not sprayed on painted surfaces.

The participants were from rural IRS villages or from nearby non-IRS villages in the Thulamela Local Municipality. The decision to spray villages depends on the Department of Health, based on the number of malaria cases. Volunteers comprised men, between 18 and 40 years old, living in these communities. Full detail on the recruitment and questionnaire was reported elsewhere (Aneck-Hahn et al., 2007; De Jager et al., 2009). Participants from sprayed and non-sprayed villages volunteered, but we excluded men who had lived in study villages for less than a year, those younger than 18 or older than 40 years, those with neuropsychiatric disorders or who appeared intoxicated.

All participants provided informed consent and were interviewed using a structured questionnaire, which detailed their general history, personal use of insecticides, diet, smoking and drinking habits, illegal substance use, exposure to other insecticides and fertility history. Physical measurements included participants' weight and height and the body mass index (BMI) was calculated. Blood samples were collected at the Tshilidzini Hospital and Thohoyandou Health Care Centre. The Limpopo Provincial Government's Department of Health and Social Development (July 11, 2002) and the Ethics Committee of the Faculty of Health Sciences, University of Pretoria (UP) (Reference 43/2003) approved the study.

2.3. Biochemical analyses

Data were collected during each of six five-day visits to the study area (between November 2003 and October 2007). We collected venous blood samples from participants between 08:00–10:00. The samples were centrifuged at 670 × *g* for 10 min at room temperature and stored in 500 μL aliquots at −20 °C on site and during transport. At the UP laboratory, samples were stored at −80 °C until analyzed. Hormones (abbreviation and kit catalog number) measured, in serum with the Cobas® 6000 analyser (Roche Products (Pty) Ltd. Diagnostics Division using ECL (ElectroChemi-Luminescence) immunometric detection, were: Luteinizing hormone (LH; 11732234122); Follicle-stimulating hormone (FSH; 11775863122), estradiol (E_2; 03000079190); total testosterone (t-T; 05200067160), and human sex hormone-binding globulin (SHBG; 03052001160). We measured serum albumin on the general automated platform to calculate bioavailable testosterone (b-T) and free testosterone (f-T) using the calculator available at http://www.issam.ch/freetesto.html, following Vermeulen's formula.