Exposure to DDT from Indoor Residual Spraying and biomarkers of inflammation among reproductive-aged women from South Africa

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Highlights

- Assessed the effect of DDT exposure (via IRS) on immune markers among reproductive-aged women
- Plasma DDE and DDT were positively associated with pro-inflammatory biomarkers
- Associations with plasma DDE and DDT were not consistent across all immune markers assessed
- Study participants had higher concentrations IgG than US black females

Abstract

Background. Evidence from animal studies suggests that DDT and DDE can adversely affect immuno-competence while human data are less conclusive. We aimed to assess the association of plasma concentrations of DDT and DDE with biomarkers of inflammation among reproductive-aged women residing in homes sprayed with DDT through Indoor Residual Spraying (IRS).

Methods. This study included 416 women from the Study of Women and Babies, South Africa (2010-2011). DDT, DDE, and biomarkers of inflammation (immunoglobulins A, G and M, interleukins 1β, 6, and 8, tumor necrosis factor-α, C-reactive protein, serum amyloid-A, intercellular adhesion molecule-1, vascular cell adhesion molecule-1) were quantified in plasma. Linear regression was used to assess associations of DDT and DDE with each natural log-transformed biomarker. Models were adjusted for age, body mass index, parity, income, and season; beta estimates were expressed as percent differences.

Results. Compared to women with the lowest plasma concentrations of DDT and DDE, those with the highest concentrations of both compounds had higher levels IL-1β, IL6, and TNF- α. While associations were statistically significant for both DDT and DDE, the magnitude of the associations was slightly stronger for DDT. Compared to women in the lowest quintile of DDT, women in the highest quintile were estimated to have 53.0% (95%CI: 21.7%, 84.4%), 28.1% (95%CI: 6.4%, 49.8%), and 26.6% (95%CI: 12.0%, 41.1%) higher levels of IL-1β, IL6, and TNF- α, respectively.

Conclusions. Our results suggest that increased plasma concentrations of DDT and DDE resulting from exposure to IRS may increase concentrations of pro-inflammatory biomarkers among reproductive-aged women in South Africa.

Key words: Africa, DDE, DDT indoor residual spraying, immune biomarkers, reproductive-aged women.

Abbreviations:

p,p'-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene

p,p'-DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane

I-CAM1, Intercellular Adhesion Molecule 1

IgA, immunoglobulin A

IgG, immunoglobulin G

IgM, immunoglobulin M

IL-1β, Interleukin-1β

IL-6, Interleukin-6

IL-8, Interleukin-8

IQR, interquartile range

IRS, Indoor residual spraying

SAA, Serum Amyloid A

TNF-α, Tumor Necrosis Factor-α

V-CAM1, Vascular Cell Adhesion Molecule 1

Running title: Exposure to *p,p'*-DDE from IRS and immune markers

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Ethics approval and consent to participate: All woman gave their written consent before participation. The study was approved by the institutional review boards at the University of Pretoria (IRB number: 76/2009), South Africa and at the National Institute of Environmental Health Sciences (IRB number: 09-E-N115), National Institutes of Health, Department of Health and Human Services, USA.

Competing interests: The authors declare that they have no competing interests.

INTRODUCTION

Generally, results from animal studies suggest that exposure to DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) and its main breakdown product, DDE (1,1-Dichloro-2,2-bis(p-chlorophenyl)ethylene), can adversely affect an animal's ability to produce a normal immune response (ATSDR 2002). However, human studies are less conclusive regarding DDT's immunosuppressive effects or inappropriate immune-stimulation, although the potential for adverse effects on the human immune system cannot be disregarded (Corsini et al. 2008; Eskenazi et al. 2009; WHO 2011a). Alterations of cell-mediated immune response includes decreased production of cytokines (*i.e.,* tumor necrosis factor-α [TNF-α], interleukin [IL]-10, and IL-2) as well as increased production of pro-inflammatory cytokines (*i.e.,* TNF-α, IL-1β and IL-6) *in vitro;* greater serum concentrations of TNF-α and IL-10; and lower plasma concentrations of IL-4 in relation to DDE or DDT exposure (Bilrha et al. 2003; Cardenas-Gonzalez et al. 2013; Corsini et al. 2013; Gascon et al. 2013; Martin et al. 2019a; Martin et al. 2019b). Decreased cell counts *(e.g.,* T-cells, white blood cells, lymphocytes, and neutrophils) have also been related to DDE exposure (Hermanowicz et al. 1982; Karmaus et al. 2005; Nagayama et al. 2007; Schaalan et al. 2012). Among the few human studies that evaluated humoral response, measured by immunoglobulin (Ig) concentrations, increasing IgA and IgM (Karmaus et al. 2005; Vine et al. 2000; Vine et al. 2001) were observed with high levels of DDE; whereas, increasing and decreasing IgG (Cooper et al. 2004; Karmaus et al. 2005) have been reported in relation to DDE. However, most of these studies have largely reported null findings (Cooper et al. 2004; Dewailly et al. 2000; Karmaus et al. 2005; Vine et al. 2000; Vine et al. 2001) for some of these immunoglobulins in relation to DDE exposure. These inconsistencies in study findings might be related to heterogeneity of exposure levels, sample sizes, and ages of the participants.

DDT is an organochlorine pesticide that was extensively applied to crops and used to eradicate malaria and other diseases (starting from the 1950's) around the world; its widespread use ended around 2001 when the Stockholm Convention on Persistent Organic Pollutants (POPs) was implemented (Rogan and Chen 2005; WHO 2011a). Present use of DDT is restricted to vector-borne disease control, particularly in malaria-endemic regions, including some countries in Africa (Eskenazi et al. 2009; Rogan and Chen 2005) where the World Health Organization (WHO) has approved the use of DDT for indoor residual spraying (IRS) (WHO 2006). Thus, identifying potential health risks associated with DDT in countries where IRS is ongoing provides valuable information when reassessing the need to continue use of DDT for vector-borne diseases control. Our objective was to assess the association of plasma concentrations of DDT and DDE with biomarkers of inflammation among reproductive-aged women residing in homes sprayed with DDT through IRS for malaria control.

MATERIAL AND METHODS

We analyzed data collected at baseline from the South African Study of Women and Babies (SOWB), which has been described elsewhere (Whitworth et al. 2014). Briefly, the SOWB was carried out in 2010-2011 and enrolled 427 women from eight villages in the Limpopo Province, South Africa. Women were eligible if they were: 20 to 30 years of age, not using contraception, with regular menstrual periods, not pregnant at the enrollment, without previous difficulty getting pregnant, planning to reside in the same village during the study period, and without a previous pregnancy in the study. All woman gave their written informed consent before participation. Women provided a blood specimen collected in EDTA (Ethylenediaminetetraacetic acid) tubes at recruitment; the samples were kept in coolers with

cold packs at the collection site and shipped the same day to the field office (~5 miles) where plasma was extracted and immediately stored at -20°C. Frozen samples were shipped on a weekly basis to the University of Pretoria in specialized freezers powered by the transportation vehicle, where they remained frozen at -80°C until shipment for analysis.

The study was approved by the institutional review boards at the University of Pretoria (IRB number: 76/2009), South Africa and at the National Institute of Environmental Health Sciences (IRB number: 09-E-N115), National Institutes of Health, Department of Health and Human Services, USA. For the present analysis, we excluded women without blood samples (n=1) and those who reported ever being told by doctor, nurse or staff member at a clinic or hospital as having and HIV infection (n=2). Additionally, eight women who reported smoking cigarettes or other items (n=8) in the past 12 months were excluded, leaving a sample size of 416 women for statistical analysis.

DDT and DDE

Concentrations of *p,p'*-DDT and *p,p'*-DDE were measured using gas chromatography-mass spectrometry (GC-MS) at the Institute National de Sante Publique du Quebec (INSPQ), Canada in plasma samples collected from women at enrollment. A detailed description of the analytical methods are described elsewhere (Whitworth et al. 2014). The limit of quantification (LOQ) was 0.02 µg/L for both DDT and DDE; values below the LOQ were assigned a value equal to one half of the LOQ. The between-assay coefficient of variation was 5% for DDT (at 1.9 µg/L) and 7.8% for DDE (at 9.4 µg/L). Total plasma lipids were calculated based on triglycerides (TG, mg/dL) and total cholesterol (TC, mg/dL) measured using standard enzymatic methods, with the following equation: $TL = 1.3*(TG + TC) + 90$ mg/dL (Rylander et al. 2006).

Concentrations of plasma *p,p′*-DDE and *p,p′*-DDT were expressed as nanograms per gram of lipid (ng/g).

Biomarkers of inflammation

Biomarkers of inflammation were quantified from plasma samples at the National Institute of Environmental Health Sciences (NIEHS) using reagents from Meso Scale Discovery (MSD, Gaithersburg, MD). Interleukin (IL)-1β, IL-4, IL-6, IL-8, and tumor necrosis factor-α (TNF-α) were determined (pg/ml) with the human pro-inflammatory II 4-plex ultrasensitive kit. C-reactive protein (CRP), serum amyloid A (SAA), vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) were determined (mg/L) with the human vascular injury panel II kit. Immunoglobulins (Ig) A, G, and M (mg/dL) were determined with the isotyping Panel-1 human non-primate kit. All assays were conducted following the protocols from the manufacturer; quantitative measures of the analytes in these samples were obtained by measuring the intensity of the emitted light. The within- and between-batch coefficients of variation (CV) of the analytical method were all $<5\%$. These biomarkers of inflammation had right-skewed distributions and were natural log transformed before the analyses.

Covariates

Information on women's sociodemographic, economic, health, and reproductive history; lifestyle; as well as detailed characteristics of households and IRS were obtained at enrollment during in-person interviews conducted by specially trained personnel. The questionnaire was translated into Tshivenda and back to English, certified by professional translators, and pretested to assure accurate comprehension and cultural appropriateness. Education was reported as the highest degree completed; total monthly family income was recorded in Rands and categorized in

quartiles. While a detailed section on alcohol consumption was included in the questionnaire, few women reported ever drinking alcohol (n=67), thus, we only report ever having an alcoholic drink. A composite variable for ever having an infection was generated from a list of infections self-reported by the women during the interview (*i.e.,* tuberculosis, malaria, syphilis, gonorrhea, herpes, and other sexually transmitted diseases) as few woman reported ever having each specific infection. A total of 61 women reported having any of the above listed infections; the most common infection reported was herpes (n=33). Height and weight were measured three times by specially trained personnel at the local clinics during enrollment. Body mass index (BMI, kg/m^2) was calculated from the averaged weight in kilograms divided by averaged height in meters squared; cut points from the WHO were used to identify overweight (BMI 25.0-29.9 kg/m^2) and obese (BMI ≥30.0 kg/m^2) women (WHO 2011b). Only few women (n=14) were underweight (BMI <18.5 kg/m^2) in the present study and therefore were included in the normal BMI group (BMI <25.0 kg/m^2) for analysis.

The eight villages from which women were recruited for the SOWB included those where IRS spraying occurred as well as villages where no IRS occurred. Moreover, individual homes within IRS villages may have been sprayed with DDT or with other pesticides (primarily pyrethroids). The identification of households in IRS villages sprayed with DDT was based on the housing characteristics that best predicted women's DDT concentrations. Detailed information about the construction of this variable has been published previously (Whitworth et al. 2014). Based on our previous work, women were classified into three groups: 1) non-IRS households (*i.e.,* women living in villages where IRS did not occur, n=170); 2) non-DDT IRS households (*i.e.,* women living in an IRS village and in a home with a low likelihood of DDT spraying, n=129); and 3) DDT IRS households (*i.e.,* women living in an IRS village and in a home with a high likelihood of DDT spraying, n=117).

Statistical analyses

The relationship of *p,p'*-DDT and *p,p'*-DDE with each biomarker of inflammation (log transformed) was assessed separately with linear regression models. Because all outcomes were natural log transformed before modeling, the beta estimate for the exposure represents the percent difference in concentrations of the outcome between the exposure groups being compared. Plasma concentrations of DDT and DDE (ng/g) were modeled in quintiles; the cut-points for DDT were: 61.26 (20th centile); 155.76 (40th centile); 377.95 (60th centile); and 1,120.90 (80th centile). The cut-points for DDE were: 345.04 (20th centile); 972.76 (40th centile); 2,011.87 (60th centile); and 3,633.32 (80th centile). Linearity of both DDE and DDT with each outcome (log-transformed) were confirmed in unadjusted and fully adjusted models using the Stata module *nlcheck* (Ben 2008). For all models, a 1-df (degree of freedom) trend test was conducted by coding DDE or DDT concentration as the median level within each quintile.

All regression models were adjusted for age (years), BMI (kg/m^2: <25.0, 25.0-29.9, and 30.0+), parity, household monthly income (Rands), and season of sample collection. These variables were selected *a priori* based on directed acyclic graphs (DAGs) as the minimal sufficient adjustment set for confounding (Greenland et al. 1999; Textor et al. 2011). To assess potential residual confounding from variables not included in the DAG (*i.e.,* education, alcohol consumption, and use of medication), we implemented a stepwise backwards selection starting with all variables in the model and using change-in-estimate as the selection criterion (Greenland 1989). Variables that changed the coefficients by >10% were alcohol consumption (IgM, IL-1β, and IL-6 models), medication use in the past 24 hours (IL-6 model), and education (TNF-α model).

In sensitivity analyses, we assessed the potential interaction between IRS with our DDE and DDT exposure variables because, as mentioned, some households were likely sprayed with pesticides other than DDT; a *p-interaction* <0.20 was defined as relevant *a priori*. We also conducted sensitivity analyses excluding women who reported having diabetes (n=1) or high blood pressure (n=7), given that metabolic abnormalities and cardiovascular disease might be related to chronic inflammation (Gonzalez-Quintela et al. 2008; Ridker et al. 2000). We assessed the effect of adjustment for ever having an infection on the associations between DDE or DDT exposure and each biomarker of inflammation. Finally, we assessed the association of the sum of DDT+DDE with each outcome. All statistical analyses were conducted using STATA (release 15.1; StataCorp, College Station, TX, USA).

RESULTS

Characteristics of the women are presented in Table 1. Median age was 24 years; less than one-fifth (16.8%) of the women reported having a high school diploma and ever having consumed an alcoholic drink (16.1%); 79.5% had had a previous child and a similar proportion (78.4%) reported ever breastfeeding. A little more than a half of the women (59.1%) lived in households with IRS, which is expected as the original study intentionally included both IRS and non-IRS villages. Almost half (48.1%) of the women were overweight or obese; and very few reported taking any medication in the previous 24 hours from enrollment (5.8%). The median plasma concentrations of *p,p'*-DDT and *p,p'*-DDE among women in this study were 238.41 ng/g lipid (IQR: 695.53) and 1,528.95 ng/g lipid (IQR: 2,497.37), respectively. As expected, concentrations of DDE (median: 2,442.7; IQR: 3,134.4) and DDT (median, 705.9; IRQ: 1,312.5) were significantly higher (*p-value*<0.01) among women living in houses with DDT-IRS than

those from houses with non-DDT IRS (DDE: 2,110.0; IQR: 2,334.2 and DDT: 336.4; IQR: 639.5) and from unsprayed houses (DDE: 485.5; IQR: 1,443.1 and DDT: 84.1, IQR: 210.2).

The distributions and geometric means (GM) of biomarkers of inflammation across all participants are shown in Table 2. GM were similar across quintiles of DDE (*p-value*$\geq$*0.37*) and DDT (*p-value*$\geq$0.15) exposure (data not shown). GMs of all biomarkers of inflammation were similar among women from unsprayed and IRS sprayed dwellings; although IgA was slightly higher whereas IgM and CRP were slightly lower among women living in IRS households compared to women living in non IRS households, nonetheless, such differences were not statistically significant (Supplemental Table 1).

Crude results showed no clear associations between DDE exposure and each biomarker of inflammation (Supplemental Table 2). In adjusted models, positive trends for IL-1β, IL-6, and TNF-α across quintiles of DDE (*p-trends* <0.05) were observed. Compared to women from the lowest quintile, those in the highest quintile had greater concentrations of IL-1β (adjusted β [aβ]= 46.3%; 95%CI: 15.6, 76.9), IL-6 (aβ= 21.4%; 95%CI: 0.4, 42.4), and TNF-α (aβ= 14.9%; 95%CI: 0.5, 29.4). Greater concentrations of IL-8 were observed among women in the third (aβ= 28.4%; 95%CI: 4.3, 52.4) and top (aβ=24.7%; 95%CI: 0.0, 49.3) quintile compared to women from the lowest quintile of DDE exposure. No clear associations were observed between DDE exposure and the other biomarkers of inflammation (immunoglobulins, CRP, SAA, I-CAM and V-CAM) in adjusted models (Table 3).

Unadjusted associations between DDT exposure and each biomarker of inflammation are shown in Supplemental Table 3; similar to our DDE findings, no clear association emerged. After adjustment, we also observed positive trends for IL-1β, IL-6 and TNF-α across quintiles of DDT (*p-trends*<0.05) (Table 4). Higher concentrations of IL-1β were observed among women from the third (aβ= 31.8%; 95%CI: 1.8, 61.7), fourth (aβ= 33.7%; 95%CI: 3.6, 63.7), and fifth (aβ=

53.0%; 95%CI: 21.7, 84.4) quintiles compared to those in the lowest quintile of DDT exposure (Table 4). Having the same reference group for DDT, women in the top quintile had also greater concentrations of IL-6 (aβ= 28.1%; 95%CI: 6.4, 49.8) and TNF-α (aβ= 26.6%; 95%CI: 12.0, 41.1). Consistent with our DDE results, we found no evidence that DDT exposure was related to the other biomarkers of inflammation (immunoglobulins, CRP, SAA, I-CAM and V-CAM) in adjusted models ; the lower concentrations of CRP observed among women in the third quintile of DDT exposure (aβ= -49.5%; 95%CI: -98.3, -0.8) appears to be an isolated finding.

In sensitivity analysis, our results remain unchanged after excluding women that reported having diabetes (n=1) or high blood pressure (n=7) (data not shown). Further adjustment for ever having an infection did not result in a change of our overall conclusions (data not shown). No interaction was observed between IRS with our DDE or DDT exposures (all *p-interaction*>0.20). Results examining associations between the sum of DDT+DDE and biomarkers of inflammation were also similar (data not shown).

DISCUSSION

Overall, our results provide little evidence of consistent associations between plasma concentrations of DDT or DDE and altered immune response or inflammation among reproductive aged women living in areas with active IRS spraying for malaria control. We did observe isolated associations between DDE and DDT with increased concentrations of pro-inflammatory biomarkers IL-1β, IL-6, and TNF-α, though our estimates were imprecise as shown by the wide confidence intervals.

Concentrations of IgA and IgM among women in the present study (Table 2) were within the range of those reported for healthy US black females aged 20 to 40 years; nonetheless, IgG

tended to be higher among our participants (5th, and 95th percentiles from US healthy black females: 900.5 and 2,396 mg/dL) (Maddison et al. 1975). We also observed somewhat higher levels of CRP (medians: 7.6 vs. 2.7 mg/L), IL-6 (medians: 2.2 vs. 1.3 pg/ml), SAA (medians: 15.3 vs. 5.2 mg/L) and I-CAM (GM: 0.83 vs. 0.32 mg/L) in our sample relative to those reported for adult US black and Caucasians females (Ridker et al. 2000; Woloshin and Schwartz 2005); no data was available to directly compare our results with only US back females.

Very few earlier studies have assessed the association of non-occupational exposure to DDE with immunoglobulins and cytokines measured in the present study, none of these however, assessed its relation to DDT (Corsini et al. 2013; Gascon et al. 2013). Prior studies have reported equivocal results regarding associations between DDE and some immunoglobulins (*i.e.,* IgA, IgG and IgM) in various populations, including US adults living near a pesticide dump site (Vine et al. 2000; Vine et al. 2001), male African American farmers (Cooper et al. 2004), and German children (Karmaus et al. 2005). As in these prior studies, we did not observe clear associations between exposure to DDE and immunoglobulin concentrations in the present study. Our results are in agreement with a previous study that reported no associations of DDE (measured in breast-milk) with immunoglobulin concentrations (*i.e.,* IgA, IgG and IgM), though this was a study among Inuit infants (Dewailly et al. 2000).

Unlike earlier studies that reported decreased *in vitro* production of TNF-α with prenatal exposure to DDE and lower serum levels of TNF-α in relation to lactational exposure to a mix of eight contaminants including DDT (Bilrha et al. 2003; Schaalan et al. 2012), we did observed greater levels of TNF-α with increasing levels of DDE and DDT in the present study. Increased *in vitro* production of TNF-α, IL-1β and IL-6 in peripheral blood mononuclear cells with very high levels of DDE exposure was reported previously (Cardenas-Gonzalez et al. 2013; Martin et al. 2019a; Martin et al. 2019b). Our results are in line with a recent study that reported a slight

increase in TNF-α secretion in peripheral blood mononuclear cells from healthy adult women in relation to DDE and DDT exposure (Dominguez-Lopez et al. 2017). Additionally, in a study of 4-year old Spanish children, positive, albeit not statistically significant, associations were observed between DDE measured in cord blood and IL-6, IL-8, and TNF-α (Gascon et al. 2014); which are in agreement with our results.

Taken together, previous epidemiologic studies show little consistency regarding the association between DDE exposure and plasma biomarkers of inflammation. These inconsistencies however, might be related to different samples sizes across the studies (n= 72 to 360), different ages of the participants (1 to 88 years), and possibly the diverse range of exposure. Women in the present study had higher levels of DDE exposure (median: 5.3 ng/mL; IQR, 9.5) compared to earlier studies, in which the median concentrations of DDE in plasma or whole blood ranged from 0.32 to 4.05 ng/mL (Bilrha et al. 2003; Karmaus et al. 2005; Vine et al. 2000; Vine et al. 2001). In only one previous study, among US male African American farmers, DDE concentrations were higher (median: 7.7 ng/mL) (Cooper et al. 2004) than observed among women in the present study. On the other hand, most prior studies included male and female participants; thus, the effect of having only females in the present study is unknown, though heterogeneity by sex was generally not assessed in these prior studies. Other alterations of the immune system reported in association with high levels of DDT and DDE in adults include an increase in plasma interleukin-4 (IL-4) and decrease in IL-2 (Daniel et al. 2002) and mitogen proliferation (Vine et al. 2000; Vine et al. 2001). These results also points towards the potential modulatory effect of DDT on the immune response, which cannot be disregarded.

A limitation of the present study, like many previous studies (Bilrha et al. 2003; Cooper et al. 2004; Karmaus et al. 2005; Vine et al. 2000; Vine et al. 2001), is inherent to its cross-sectional nature. Biomarkers of inflammation were measured at the same time of the exposure, thus

temporality cannot be established nor can we infer a causal relationship; however, given that DDE has a half-life of approximately 10 years in humans (Longnecker 2005), it is likely that exposure preceded the outcome in this case. In addition, our study included relatively young, healthy women because the parent study (SOWB) was designed to assess the association between DDT exposure and pregnancy outcomes (e.g., clinically recognized pregnancy loss) (Whitworth et al. 2014); thus, our results have limited generalizability. A strength of the study, however, is the standardized determination of biomarkers of inflammation. Laboratory personnel were unaware of subjects' DDT and DDE concentrations. Although random errors in measurement of both biomarkers of inflammation or DDT and DDE may have occurred, these would be non-differential in nature. While the clinical relevance of our findings is not clear, it is possible that changes in inflammatory cytokine concentrations could be related to more frequent infections. Nonetheless, our results remain similar after adjusting for previous infections, although only a few women reported ever having infections (n=61). Given the lack of detailed information on infections (*e.g.,* frequency and date of diagnosis) however, residual confounding remains an issue in the present study; our composite variable of ever having infections was only related marginally to IL-8, but not to other biomarkers of inflammation in fully adjusted models of DDE and DDT (data not shown).

Conclusions

Our results based on reproductive-aged women living in areas with ongoing DDT-IRS suggest that increasing plasma concentrations of DDE and DDT might be associated with higher plasma concentrations of pro-inflammatory biomarkers, suggesting some degree of chronic inflammation. However, given the nature of our study, findings should be replicated in other settings.

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Authors' contributions:

LACU conceive the hypothesis, analyzed and interpreted all participant data, and drafted the manuscript. KWW was involved in drafting the manuscript and revising it critically for important intellectual content from early stages. GST and REW analyzed the samples for immune markers and helped with the interpretation. RB, JIA, MOK, GST, and REW provided critical revisions of the manuscript for important intellectual content. All authors read and approved the final manuscript.

REFERENCES

ATSDR. 2002. Toxicological profile for ddt, dde, and ddd. Atlanta, GA:Agency for Toxic Substances and Disease Registry.

Ben J. 2008. Nlcheck: Stata module to check linearity assumption after model estimation. Part S456968:Boston College Department of Economics. doi: DOI:

Bilrha H, Roy R, Moreau B, Belles-Isles M, Dewailly E, Ayotte P. 2003. In vitro activation of cord blood mononuclear cells and cytokine production in a remote coastal population exposed to organochlorines and methyl mercury. Environmental health perspectives 111:1952-1957. doi: 10.1289/ehp.6433

Cardenas-Gonzalez M, Gaspar-Ramirez O, Perez-Vazquez FJ, Alegria-Torres JA, Gonzalez-Amaro R, Perez-Maldonado IN. 2013. P,p'-dde, a ddt metabolite, induces proinflammatory molecules in human peripheral blood mononuclear cells "in vitro". Exp Toxicol Pathol 65:661-665. doi: 10.1016/j.etp.2012.07.006

Cooper GS, Martin SA, Longnecker MP, Sandler DP, Germolec DR. 2004. Associations between plasma dde levels and immunologic measures in african-american farmers in north carolina. Environmental health perspectives 112:1080-1084.

Corsini E, Liesivuori J, Vergieva T, Van Loveren H, Colosio C. 2008. Effects of pesticide exposure on the human immune system. Hum Exp Toxicol 27:671-680. doi: 10.1177/0960327108094509

Corsini E, Sokooti M, Galli CL, Moretto A, Colosio C. 2013. Pesticide induced immunotoxicity in humans: A comprehensive review of the existing evidence. Toxicology 307:123-135. doi: 10.1016/j.tox.2012.10.009

Daniel V, Huber W, Bauer K, Suesal C, Conradt C, Opelz G. 2002. Associations of dichlorodiphenyltrichloroethane (ddt) 4.4 and dichlorodiphenyldichloroethylene (dde) 4.4

blood levels with plasma il-4. Arch Environ Health 57:541-547. doi: 10.1080/00039890209602086

Dewailly E, Ayotte P, Bruneau S, Gingras S, Belles-Isles M, Roy R. 2000. Susceptibility to infections and immune status in inuit infants exposed to organochlorines. Environmental health perspectives 108:205-211.

Dominguez-Lopez P, Diaz-Cueto L, Aguilar-Rojas A, Arechavaleta-Velasco F. 2017. Synergistic effect of ddt and its metabolites in lipopolysaccharide-mediated tnf-alpha production is inhibited by progesterone in peripheral blood mononuclear cells. J Biochem Mol Toxicol 31. doi: 10.1002/jbt.21907

Eskenazi B, Chevrier J, Rosas LG, Anderson HA, Bornman MS, Bouwman H, et al. 2009. The pine river statement: Human health consequences of ddt use. Environmental health perspectives 117:1359-1367. doi: 10.1289/ehp.11748

Gascon M, Morales E, Sunyer J, Vrijheid M. 2013. Effects of persistent organic pollutants on the developing respiratory and immune systems: A systematic review. Environ Int 52:51-65. doi: 10.1016/j.envint.2012.11.005

Gascon M, Sunyer J, Martinez D, Guerra S, Lavi I, Torrent M, et al. 2014. Persistent organic pollutants and children's respiratory health: The role of cytokines and inflammatory biomarkers. Environ Int 69:133-140. doi: 10.1016/j.envint.2014.04.021

Gonzalez-Quintela A, Alende R, Gude F, Campos J, Rey J, Meijide LM, et al. 2008. Serum levels of immunoglobulins (igg, iga, igm) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. Clinical and experimental immunology 151:42-50. doi: 10.1111/j.1365-2249.2007.03545.x

Greenland S. 1989. Modeling and variable selection in epidemiologic analysis. Am J Public Health 79:340-349.

Greenland S, Pearl J, Robins JM. 1999. Causal diagrams for epidemiologic research. Epidemiology 10:37-48.

Hermanowicz A, Nawarska Z, Borys D, Maslankiewicz A. 1982. The neutrophil function and infectious diseases in workers occupationally exposed to organochloride insecticides. Int Arch Occup Environ Health 50:329-340. doi: 10.1007/bf00377829

Karmaus W, Brooks KR, Nebe T, Witten J, Obi-Osius N, Kruse H. 2005. Immune function biomarkers in children exposed to lead and organochlorine compounds: A cross-sectional study. Environ Health 4:5. doi: 10.1186/1476-069X-4-5

Longnecker MP. 2005. Invited commentary: Why ddt matters now. American journal of epidemiology 162:726-728. doi: 10.1093/aje/kwi277

Maddison SE, Stewart CC, Farshy CE, Reimer CB. 1975. The relationship of race, sex, and age to concentrations of serum immunoglobulins expressed in international units in healthy adults in the USA. Bulletin of the World Health Organization 52:179-185.

Martin TJ, Gabure S, Maise J, Snipes S, Peete M, Whalen MM. 2019a. The organochlorine pesticides pentachlorophenol and dichlorodiphenyltrichloroethane increase secretion and production of interleukin 6 by human immune cells. Environ Toxicol Pharmacol 72:103263. doi: 10.1016/j.etap.2019.103263

Martin TJ, Maise J, Gabure S, Whalen MM. 2019b. Exposures to the environmental contaminants pentachlorophenol and dichlorodiphenyltrichloroethane increase production of the proinflammatory cytokine, interleukin-1beta, in human immune cells. J Appl Toxicol 39:1132-1142. doi: 10.1002/jat.3798

Nagayama J, Tsuji H, Iida T, Nakagawa R, Matsueda T, Hirakawa H, et al. 2007. Immunologic effects of perinatal exposure to dioxins, pcbs and organochlorine pesticides in japanese infants. Chemosphere 67:S393-398. doi: 10.1016/j.chemosphere.2006.05.134

Ridker PM, Hennekens CH, Buring JE, Rifai N. 2000. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342:836-843. doi: 10.1056/NEJM200003233421202

Rogan WJ, Chen A. 2005. Health risks and benefits of bis(4-chlorophenyl)-1,1,1-trichloroethane (ddt). Lancet 366:763-773. doi: 10.1016/S0140-6736(05)67182-6

Rylander L, Nilsson-Ehle P, Hagmar L. 2006. A simplified precise method for adjusting serum levels of persistent organohalogen pollutants to total serum lipids. Chemosphere 62:333-336. doi: 10.1016/j.chemosphere.2005.04.107

Schaalan MF, Abdelraouf SM, Mohamed WA, Hassanein FS. 2012. Correlation between maternal milk and infant serum levels of chlorinated pesticides (cp) and the impact of elevated cp on bleeding tendency and immune status in some infants in egypt. J Immunotoxicol 9:15-24. doi: 10.3109/1547691X.2011.606432

Textor J, Hardt J, Knuppel S. 2011. Dagitty: A graphical tool for analyzing causal diagrams. Epidemiology 22:745. doi: 10.1097/EDE.0b013e318225c2be

Vine MF, Stein L, Weigle K, Schroeder J, Degnan D, Tse CK, et al. 2000. Effects on the immune system associated with living near a pesticide dump site. Environmental health perspectives 108:1113-1124.

Vine MF, Stein L, Weigle K, Schroeder J, Degnan D, Tse CK, et al. 2001. Plasma 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (dde) levels and immune response. American journal of epidemiology 153:53-63.

Whitworth KW, Bornman RM, Archer JI, Kudumu MO, Travlos GS, Wilson RE, et al. 2014. Predictors of plasma ddt and dde concentrations among women exposed to indoor residual spraying for malaria control in the south african study of women and babies (sowb). Environmental health perspectives 122:545-552. doi: 10.1289/ehp.1307025

WHO. 2006. Indoor residual spraying: Use of indoor residual spraying for scaling up global malaria control and elimination: Who position statement. Geneva, Switzerland:Geneva: Global Malaria Programme World Health Organization.

WHO. 2011a. Ddt in indoor residual spraying: Human health aspects. Geneva, Switzerland:World Health Organization.

WHO. 2011b. Waist circumference and waist-hip ratio: Report of a who expert consultation. . Geneva, Switzerland:WHO Press

Woloshin S, Schwartz LM. 2005. Distribution of c-reactive protein values in the united states. N Engl J Med 352:1611-1613. doi: 10.1056/NEJM200504143521525

Table 1. Characteristics of 416 reproductive-aged women from the South African Study of Women and Babies (SOWB), 2010-2011.

Characterístics	n	%
Age (years)		
20-22	157	37.7
23-25	125	30.0
26-28	91	21.9
29-30	43	10.3
Education (years)		
≤ 11	217	52.2
12	129	31.0
> 12	70	16.8
Body mass index (kg/m^2)		
<25.0	216	51.9
25.0 - 29.9	123	29.6
30.0+	77	18.5
Parity		
Nulliparous	85	20.4
1	206	49.5
2+	125	30.0
Ever consumed alcohol	67	16.1
Monthly household income (Rands)		
<1,250	103	24.8
1,250 - 1,999	103	24.8
2,000 - 3,000	110	26.4
>3,000	100	24.0
Indoor residual spraying		
Non IRS household	170	40.9
Non-DDT IRS household	129	31.0
DDT IRS household	117	28.1
Season when sample was drawn		
Winter	170	40.9
Spring	134	32.2
Summer	112	26.9
Any medication use in previous 24 hours	24	5.8

Table 2. Plasma concentrations of biomarkers of inflammation[a] among reproductive-aged women from the South African Study of Women and Babies (SOWB), 2010-2011 (n=416), by selected percentiles.

	Geometric means	Percentiles						
		5th	10th	25th	50th	75th	90th	95th
IgA	193.92	111.00	123.00	154.00	196.00	249.00	293.00	341.00
IgG	1589.48	1133.50	1207.90	1341.00	1533.85	1801.00	2125.50	2723.40
IgM	166.26	92.70	101.10	126.35	159.50	217.65	298.70	347.70
IL-1β	0.56	0.06	0.15	0.30	0.62	1.35	2.47	3.24
IL-6	2.22	0.47	0.69	1.23	2.23	4.02	7.04	9.82
IL-8	2.14	0.45	0.57	1.06	2.04	4.62	8.76	11.04
TNF-α	8.80	3.03	3.89	5.82	8.48	13.82	20.79	25.28
CRP	7.15	0.41	0.77	2.64	7.60	23.50	62.80	93.88
SAA	14.98	1.47	2.77	5.97	15.29	39.47	97.86	170.00
I-CAM	0.83	0.27	0.33	0.51	0.78	1.32	2.19	2.93
V-CAM	1.17	0.40	0.47	0.66	1.18	1.92	2.86	3.76

Abbreviations: CRP, C Reactive Protein; Ig, immunoglobulins; I-CAM1, Intercellular Adhesion Molecule-1; IL-1β, Interleukin-1β; IL-6, Interleukin-6; IL-8, Interleukin-8; SAA, Serum Amyloid A; TNF-α, Tumor Necrosis Factor-α; V-CAM1, Vascular Cell Adhesion Molecule-1.

[a] IgA, IgG and IgM: mg/dL; IL-1β, IL-6, IL-8, and TNF-α: pg/ml; CRP, I-CAM, SAA and V-CAM: mg/L.

Table 3. Percent change in plasma concentrations of biomarkers of inflammation in relation to quintiles[a] of *p,p'*-DDE (ng/g lipids) exposure among 416 reproductive-aged women from the South African Study of Women and Babies (SOWB).

Biomarkers[b] (*nat log*)	Adjusted[c] coefficients (95% CI)			
	Quintile 2	Quintile 3	Quintile 4	Quintile 5
IgA	0.7 (-10.3, 11.6)	4.0 (-7.1, 15.0)	2.9 (-8.2, 14.0)	-0.8 (-12.2, 10.5)
IgG	1.2 (-6.8, 9.3)	5.4 (-2.7, 13.5)	4.2 (-3.9, 12.4)	5.4 (-2.9, 13.8)
IgM	-0.7 (-13.3, 11.9)	1.8 (-10.9, 14.5)	4.9 (-7.8, 17.7)	8.7 (-4.4, 21.8)
IL-1β	16.9 (-12.6, 46.4)	16.6 (-13.1, 46.4)	23.4 (-6.4, 53.3)	46.3 (15.6, 76.9)
IL-6	9.8 (-10.6, 30.2)	-1.1 (-21.6, 19.5)	14.8 (-5.8, 35.3)	21.4 (0.4, 42.4)
IL-8	11.5 (-12.3, 35.3)	28.4 (4.3, 52.4)	20.4 (-3.7, 44.5)	24.7 (0.0, 49.3)
TNF-α	-1.3 (-15.2, 12.5)	1.2 (-12.9, 15.2)	10.0 (-4.0, 24.1)	14.9 (0.5, 29.4)
CRP	-14.0 (-62.3, 34.3)	-16.4 (-65.2, 32.4)	-9.7 (-58.5, 39.2)	-17.6 (-67.6, 32.4)
SAA	5.2 (-37.1, 47.6)	-15.0 (-57.7, 27.8)	-4.2 (-47.0, 38.7)	-21.3 (-65.1, 22.5)
I-CAM	-6.8 (-29.2, 15.6)	4.1 (-18.6, 26.7)	5.4 (-17.3, 28.1)	-8.1 (-31.3, 15.1)
V-CAM	-1.8 (-24.5, 20.8)	1.3 (-21.5, 24.1)	3.9 (-18.9, 26.8)	-10.4 (-33.8, 13.0)

Abbreviations: CRP, C Reactive Protein; Ig, immunoglobulins; I-CAM, Intercellular Adhesion Molecule-1; IL-1β, Interleukin-1β; IL-6, Interleukin-6; IL-8, Interleukin-8; SAA, Serum Amyloid A; TNF-α, Tumor Necrosis Factor-α; V-CAM, Vascular Cell Adhesion Molecule-1.

[a] Cut-points: quintile-1, ≤345.04 (reference); quintile-2, 345.05 to 972.76; quintile-3, 972.77 to 2,011.87; quintile-4, 2,011.88 to 3,633.32; and quintile-5, >3,633.32 ng/g lipids.

[b] IgA, IgG and IgM: mg/dL; IL-1β, IL-6, IL-8, and TNF-α: pg/ml; CRP, I-CAM, SAA and V-CAM: mg/L

[c] Adjusted for age, BMI, parity, income, and season of sample collection. IgM, IL-1β, and IL-6 models were additionally adjusted for alcohol consumption; IL-6 was additionally adjusted medication use in the past 24 hours; and TNF-α additionally adjusted for education. *P-trend* <0.05 for IL-1β, IL-6 and TNF-α models.

Table 4. Percent change in plasma concentrations of biomarkers of inflammation in relation to quintiles[a] of *p,p'*-DDT (ng/g lipids) exposure among 416 reproductive-aged women from the South African Study of Women and Babies (SOWB).

Biomarkers[b] (*nat log*)	Adjusted[c] coefficients (95% CI)			
	Quintile 2	Quintile 3	Quintile 4	Quintile 5
IgA	2.0 (-8.9, 12.9)	-1.3 (-12.5, 9.8)	1.0 (-10.1, 12.1)	2.4 (-9.1, 13.9)
IgG	-1.6 (-9.6, 6.4)	2.4 (-5.8, 10.6)	-0.7 (-8.9, 7.5)	5.4 (-3.0, 13.9)
IgM	9.6 (-2.9, 22.1)	4.6 (-8.3, 17.4)	5.4 (-7.5, 18.2)	3.0 (-10.4, 16.4)
IL-1β	25.9 (-3.3, 55.1)	31.8 (1.8, 61.7)	33.7 (3.6, 63.7)	53.0 (21.7, 84.4)
IL-6	16.8 (-3.5, 37.1)	14.5 (-6.3, 35.2)	19.9 (-0.9, 40.7)	28.1 (6.4, 49.8)
IL-8	14.9 (-8.9, 38.7)	14.6 (-9.7, 39.0)	13.2 (-11.1, 37.5)	23.7 (-1.5, 48.8)
TNF-α	6.0 (-7.7, 19.7)	9.7 (-4.3, 23.7)	11.9 (-2.2, 25.9)	26.6 (12.0, 41.1)
CRP	6.4 (-41.2, 54.0)	-49.5 (-98.3, -0.8)	7.1 (-41.6, 55.7)	-23.2 (-73.6, 27.1)
SAA	20.5 (-21.5, 62.5)	-19.9 (-62.8, 23.1)	-2.0 (-44.9, 40.9)	8.9 (-35.5, 53.3)
I-CAM	9.5 (-12.7, 31.8)	0.6 (-22.2, 23.3)	17.1 (-5.6, 39.9)	1.4 (-22.2, 25.0)
V-CAM	12.1 (-10.4, 34.5)	-6.1 (-29.0, 16.9)	8.0 (-14.9, 30.9)	0.0 (-23.8, 23.7)

Abbreviations: CRP, C Reactive Protein; Ig, immunoglobulins; I-CAM, Intercellular Adhesion Molecule 1; IL-1β, Interleukin-1β; IL-6, Interleukin-6; IL-8, Interleukin-8; SAA, Serum Amyloid A; TNF-α, Tumor Necrosis Factor-α; V-CAM, Vascular Cell Adhesion Molecule.

[a] Cut-points: quintile-1, ≤61.26 (reference); quintile-2, 61.27 to 155.76; quintile-3, 155.77 to 377.95; quintile-4, 377.96 to 1,120.90; and quintile-5, >1120.90 ng/g lipids.

[b] IgA, IgG and IgM: mg/dL; IL-1β, IL-6, IL-8, and TNF-α: pg/ml; CRP, I-CAM, SAA and V-CAM: mg/L.

[d] Adjusted for age, BMI, parity, income, and season of sample collection. IgM, IL-1β, and IL-6 models were additionally adjusted for alcohol consumption; IL-6 was additionally adjusted medication use in the past 24 hours; and TNF-α additionally adjusted for education. *P-trend* <0.05 for IL-1β, IL-6 and TNF-α models.