Impaired Semen Quality Associated With Environmental DDT Exposure in Young Men Living in a Malaria Area in the Limpopo Province, South Africa

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ABSTRACT: The pesticide DDT [1,1,1-trichloro-2,2-bis(chlorodiphenyl)ethane] is 1 of the 12 persistent organic pollutants (POPs) under negotiation at the Stockholm Convention to restrict or ban their production and use because of their toxicity, resistance to breakdown, bioaccumulation, and potential for being transported over long distances. DDT has estrogenic potential, and the main metabolite, p,p’-dichlorodiphenyl-dichloroethylene (p,p’-DDE), is a potent antiandrogen. In response to mounting evidence on the endocrine-disrupting influence of environmental chemicals on human health, this epidemiological study was initiated to test the hypothesis that nonoccupational exposure to DDT affects male reproductive parameters. In a cross-sectional study, healthy male subjects (n = 311) between 18 and 40 years (23 ± 5) of age were recruited from 3 communities in an endemic malaria area in which DDT is sprayed annually. A semen analysis according to World Health Organization (WHO) standards was performed. The Hamilton Thorne Computer Assisted Sperm Analysis (CASA) system was simultaneously used to determine additional sperm motility parameters. Blood plasma samples were assayed for p,p’-DDT and metabolites as a measure of exposure. The exposure levels were expressed as lipid-adjusted p,p’-DDT and p,p’-DDE values. The mean p,p’-DDT and p,p’-DDE concentrations were 90.23 μg/g (±102.4) and 215.47 μg/g (±210.6), respectively. The multivariate linear regression analyses indicated that mean CASA motility was lower with a higher p,p’-DDE concentration (β = −0.02, P = .001) and the CASA parameter beat cross-frequency (BCF) was higher with a higher p,p’-DDT concentration (β = 0.01, P = .000). There was also a statistically significant positive association between percent sperm with cytoplasmic droplets and p,p’-DDT concentration (β = 0.0014, P = .014). The ejaculate volume (mean 1.9 ± 1.33 mL) was lower than the normal range (≥2.0 mL) according to WHO, and a significant decrease with increasing p,p’-DDE values was seen for both square root–transformed volume (β = −0.0003; P = .024) and count (β = −0.003; P = .04). Although there were no associations between either p,p’-DDT or p,p’-DDE concentrations and the rest of the seminal parameters, the incidence of teratozoospermia (99%; normal sperm <15%) was high. Twenty-eight percent of the study group presented with oligozoospermia (<20 × 10^6 sperm/mL), which had a significant positive association with p,p’-DDE (odds ratio [OR] = 1.001, P = .03). There was a significant positive association between participants with asthenozoospermia (32%) and p,p’-DDT (OR 1.003, P = .006) and p,p’-DDE (OR 1.001, P = .02). The results imply that nonoccupational exposure to DDT is associated with impaired seminal parameters in men. The high exposure levels of p,p’-DDT and p,p’-DDE are of concern because these levels could have far-reaching implications for reproductive and general health.

Key words: Seminal parameters, organochlorine pesticides, p,p’-DDT, p,p’-DDE, POPs, spermatozoa, CASA.


The Stockholm Convention resulted from a decision made in 1995 by the United Nations Environment Programme Governing Council (UNEP) to develop a legally binding instrument to control certain chemicals. The convention initially targeted 12 chemicals known as persistent organic pollutants (POPs), arguing that those chemicals pose major and increasing threats to human health and the environment (UNEP, 1995). The Stockholm Convention on POPs became legally binding on May 17, 2004. The Convention is a global multilateral agreement with the aim of protecting human and environmental health from the effects of exposure to specific POPs. Restricting the use and production of these chemicals or banning them will, when the measures of the convention are successfully implemented, reduce the hazards posed by these pollutants. Although South Africa ratified the Convention on September 4, 2002 (Bouwman, 2004), it has
applied for exemption as far as the use of DDT [1,1,1-trichloro-2,2-bis(chlorodiphenyl)ethane] for malaria vector control is concerned.

POPs are organic compounds that, to a varying degree, resist photolytic, biological, and chemical degradation. These compounds are often halogenated and characterized by low water solubility and high lipid solubility. They are also semivolatile, enabling them to move long distances in the atmosphere before deposition occurs. POPs, which are noted for their persistence and bioaccumulative characteristics, include DDT, dieldrin, toxaphene, chlordane, and several industrial chemical products and byproducts, including polychlorinated biphenyls (PCBs), dioxins, and furans (UNEP, 2006).

DDT and similar stable chlorinated compounds can be transported via air, rivers (Rawn et al, 1999; Buehler et al, 2004), and ocean currents (Bidleman et al, 1995) over long distances and have been detected in the Antarctic and other areas, far from their production sites or regions of use (Bouwman, 2004). While DDT is targeted by the treaty, exemptions are available for countries that are still using DDT to combat malaria. The treaty mobilizes much needed funding to help countries shift to safer alternatives for malaria control, which has drawn attention and resources to the ongoing and long-ignored tragedy of malaria, particularly in Africa. A small group of United States conservatives continues to push to re-establish DDT as a “safe” chemical for use against malaria, despite a clear decision by the international community that DDT should be targeted for ultimate elimination (Pesticide Action Network North America). All these factors have made the continued use of DDT for malaria vector control in South Africa, Africa, and all the other countries that have applied for exemption a matter of global interest.

A number of reports have indicated that, in addition to being toxic, organochlorine pesticides, including DDT and its metabolites, might act as endocrine disruptors (Turusov et al, 2002). Endocrine-disrupting chemicals can be defined as compounds that influence normal hormone functions, generally causing adverse effects (Goddahn and Duffy, 2003). Technical-grade DDT is a mixture of \( p,p'-\text{DDT} \) (≈85%), \( o,p'-\text{DDT} \) (≈15%), and \( o,o'-\text{DDT} \) (trace amounts), with both \( p,p'-\text{DDT} \) and \( o,p'-\text{DDT} \) having estrogenic activity. \( p,p'-\text{Chlorodiphenylchlorohydroxyene (p,p'-DDE)}, a persistent metabolite of \( p,p'-\text{DDT} \), is a widespread environmental contaminant (Turusov et al, 2002). The \( p,p'-\text{DDE} \) isomer is antiandrogenic by inhibitory binding to androgen receptors (Rogan and Chen, 2005) and has been shown to inhibit the action of testosterone (Kelce et al, 1995; Danzo, 1997; Bhatia et al, 2005). The hypothesis has been advanced that \( p,p'-\text{DDE} \) interacts in an additive or multiplicative way with other endocrine-disruptive environmental pollutants (Turusov et al, 2002). Serum levels of \( p,p'-\text{DDE} \) are an integrated measure of internal dose, reflecting exposure from all sources over the previous years (Hauser et al, 2003). Reproductive disorders were among the first adverse effects linked to organochlorine exposure (Beard, 2005).

Reproductive abnormalities attributed to DDE exposure after a major pesticide spill in 1980 were found in reptiles inhabiting Lake Apopka in Florida in the 1990s. The types of deformities found were ambiguous gonads (ovotestes) in turtles and abnormal sex hormone levels, poorly organized testes, and small penises in male alligators (Guillette et al, 1995). The Great Lakes fish found to be contaminated with organochlorine compounds such as PCBs, dioxins, DDT metabolites, and dibenzofuran have exhibited reproductive and other endocrine abnormalities. Wildlife (birds, turtles, and mammals) that have consumed these fish have also exhibited various abnormalities, which include impaired reproduction, same-sex pairing, feminization, ambiguous genitalia, and reduced fertility (Colborn et al, 1993, 1996; Fry, 1995). In mice, the uterotrophic effect of DDT increased the weight of the uterus and the development of a pseudouterus (Morozova et al, 1997). A permanent, functional male-to-female sex reversal following a single exposure of eggs to \( o,p'-\text{DDT} \) was observed in medaka fish (Edmunds et al, 2000; Turosov et al, 2002). There is also evidence that DDT acts as a promoter of mammary tumors in rats and that it can inhibit gap junctional intercellular communication (Snedeker, 2001). Other evidence of hormone-disrupting effects of DDT and its metabolites has included reproductive defects and eggshell thinning in avian species (Fry, 1995; Snedeker, 2001). DDT or \( p,p'-\text{DDE} \) might alter sex hormone metabolism, reducing available testosterone to tissues (Guillette et al, 1995).

In humans, a trend in decreasing human sperm count might have occurred in several European regions during the last 50 years (Irvine, 1994; Auger et al, 1995; Toft et al, 2004). The decrease in sperm count is paralleled by a rise in the trend of testicular cancer and malformations of the male reproductive organs such as hypospadias and cryptorchidism (Toppari et al, 1996). Skakkebaek et al (2001) presented a hypothesis that poor semen quality, testicular cancer, cryptorchidism, and hypospadias are all indicative of 1 underlying entity—testicular dysgenesis syndrome (TDS)—with an origin in fetal life. The cause of TDS is unclear, but owing to the rapid temporal changes in symptoms over the last few decades, it is suspected to be at least partly linked to environmental and lifestyle factors. In addition, genetic polymorphisms or aberrations might render some individuals particularly susceptible to potential environmental disruptors (Bay et al, 2006). Because technical-
grade DDT comprises estrogenic molecules and because its major metabolite is a potent antiandrogen, it has been hypothesized that exposure to DDT is involved in the increase in male reproductive tract anomalies (Guillette et al, 1995; de Jager et al, 2006).

In Africa, indoor residual spraying of DDT has become part of the national Roll Back Malaria strategic plan in several countries (Hougard et al, 2002; Rogan and Chen, 2005). In South Africa, DDT is sprayed in the low-altitude parts of Limpopo Province, Mpumalanga Province, and KwaZulu Natal. Currently, of the approximately 40 million people in South Africa, 10%, or 4 million, live in a malaria risk area (Rogan and Chen, 2005). This puts the inhabitants of the rural communities in these areas at risk of being exposed to high concentrations of DDT and DDE. The exposure occurs through inhalation (indoor air spraying of dwellings and outdoors), dermal contact (soil and house dust), and ingestion of contaminated foods and water. In South Africa, information on the health effects of environmental DDT exposure is not available. In light of the above discussion, this study aims to assess the effects of nonoccupational exposure to DDT and semen parameters in young healthy men in a rural area in the Limpopo Province, South Africa, where DDT is still sprayed.

Materials and Methods

Study Design and Population

In a cross-sectional study design, the participants were volunteer, nonoccupationally exposed Venda men. The participants recruited were between 18 and 40 years old and had been living in the communities for at least a year. Participants were excluded if they presented with a history of testicular trauma, orchitis, urinary infection, sexually transmitted diseases, use of hormonal medication, or exposure to known gonadotoxins or had neuropsychiatric disorders.

Study Area

The Limpopo Province is situated in the northeastern corner of South Africa and is divided into 6 districts, with the study area lying within the Vhembe district. After consultation with the regional Department of Health and Social Development, 3 rural communities, Dididi, Tshiulungoma, and Tshikhudini, near Thoyohandou were selected from a malaria endemic area. The housing in these communities consists of traditional mud dwellings with thatch (grass) roofs or brick and cement houses. DDT is sprayed inside unpainted brick, cement, and mud houses annually, but not inside the painted houses.

Recruitment and Sampling

The Ethics Committee of the Faculty of Health Sciences, University of Pretoria (Reference 43/2003) and the Limpopo Provincial Government’s Department of Health approved the study protocol July 11, 2002. An initial visit to the proposed study area took place in October 2003. The project team approached the village Chiefs and Elders for permission to address the community about the proposed study. Meetings were held at all 3 villages to inform the residents about the study and the procedures that would be followed. A representative was selected from each village (Dididi, Tshiulungoma, and Tshikhudini) to assist with the recruitment of participants. The representative was also trained to assist with the study questionnaire. After being properly informed, any man who volunteered to participate and met the inclusion criteria was included in the study.

The Tshilidzini Hospital near Thoyohandou was used as a central laboratory point. Samples were collected between November 2003 and July 2005. The participants produced semen samples in specially provided rooms adjacent to the onsite laboratory. In addition to the semen samples, blood samples were collected and all participants signed an informed consent form and completed a questionnaire. During this period, 362 participants were recruited, of which 51 were unable to produce a semen sample or did not meet the inclusion criteria.

Questionnaire

The questionnaire included questions on general health history, DDT exposure source (whether houses were sprayed with DDT for malaria control or not), diet, fertility history, and other potential spermatotoxic exposures. Exposures studied included physical agents (exposure to heat or radiation and history of testicular trauma), biological agents (genitourinary tract infections, history of STDs, orchitis, and epididymitis), and chemical agents (exposure to recreational and occupational drugs, pollutants, other pesticides, or any other chemical agent, as well as smoking and drinking habits).

Exposure Assessment

Blood samples were collected from each participant. The samples were centrifuged at 670 × g for 10 minutes at room temperature. Plasma was stored at −20°C on site and then transferred to a −70°C freezer until analyzed. The Agricultural Research Council, Veterinary Institute, Residue Laboratory in Pretoria, South Africa, determined DDT and its metabolites with the use of a Shimatzu GCMS-QP2010 (Shimatzu, Tokyo Japan). Concentrations of DDT compounds in the plasma were expressed on a lipid-adjusted basis (µg/g). The detection limit for p,p′-DDT and p,p′-DDE was 0.02 µg/g lipid adjusted. Total cholesterol and triglycerides were determined by enzymatic methods and the total plasma lipid concentration was calculated according to the formula proposed by Rylander et al (2006).

Semen Analyses

Semen samples were obtained from 311 participants after the prescribed 3-day period of sexual abstinence. Semen specimens were produced by masturbation directly into a sterile wide-mouthed container. The semen sample was then incubated at 37°C until liquefied. Trained researchers performed semen
analyses and additional andrological tests according to the standards and procedures of the World Health Organization (WHO, 1999), and quality control (QC) procedures were adhered to (European Society for Human Reproduction and Embryology [ESHRE], 1998; WHO, 1999).

After liquefaction, the following seminal physical characteristics were assessed: appearance, liquefaction, viscosity, ejaculate volume, and semen pH (Mortimer, 1994). Sperm concentration was determined with a hemocytometer (WHO, 1999). Sperm motility was assessed manually on a wet preparation according to the WHO (1999) motility classification. This classification uses the class a through d sperm progression rating (where a indicates rapid progressive motility and d, immotile sperm; Nordic Association of Andrology, European Society of Human Reproduction and Embryology — Special Interest Group on Andrology, 2002; WHO, 1999). The viable sperm were assessed by the eosin-nigrosin method (Mortimer, 1994). The presence of leukocytes, erythrocytes, bacteria, and agglutinates was also noted. The presence of immunoglobulin on the sperm surface was assessed with the IgG test (SperMar test) on all fresh samples with motility <40%. Immunological infertility can be considered when 50% or more of the motile sperm have IgG antibodies. Sperm morphology slides were stained by the Papanicolaou method and scored according to the WHO (1999) classification. The morphology assessment was performed by the same technologist in the Andrology laboratory at the University of Pretoria. This laboratory ensures that the technologists follow strict quality control (QC) and quality assurance (QA) procedures. The Andrology laboratory also takes part in an international external QC program with the European Society for Human Reproduction and Embryology, and all observations fall within ±1 SD of the reference results.

**Computer-Assisted Sperm Analysis**

Sperm motility was further evaluated with a Hamilton Thorne sperm motion analyzer (HTM-IVOS, Version 12; Beverly, Mass) at 60 Hz. Twelve microliters of semen were placed into Leja slides (Leja, SC 20-01-C; Calicom Trading [PTY] Ltd, Johannesburg, South Africa) with a chamber depth of 20 μm. Thirty frames were captured for analysis; a minimum of 150 sperm were analyzed in duplicate at 37°C (Schrader et al, 1992; Mortimer and Fraser, 1996). Samples having an estimated count of more than 40 × 10⁶ spermatozoa/mL were diluted with cell-free seminal plasma from the same individual. The percentages of motile sperm, progressive motility, linear velocity, and curvilinear velocity were measured.

**Statistical Analyses**

Exploratory data analysis was conducted on the final database to detect missing or outlier values. Tabulation and graphical univariate analysis was done to describe the distribution of each variable and identify the necessary transformation to normalize variables. In each case, after exploring several transformations and the raw form, the set closer to normal distribution was used in linear regression analysis. The distribution of variables describing sperm morphology—head, midpiece, and tail defects percentage—required negative binomial regression analysis. Information obtained from the questionnaire, as well as the participant’s p,p′-DDT and p,p′-DDE levels, was compared between different categories by analysis of variance or regression analyses. Bivariate analyses with regression models were conducted between the different reproductive outcomes and questionnaire variables to determine the risk factors and to identify confounding factors. Multivariate models were examined to evaluate the effect of DDE/DDT in the different reproductive outcomes. A saturated multivariate model was produced for each dependent variable (semen parameter), including all independent variables with P ≤ .15 in the bivariate analyses. A manual stepwise elimination was used until every variable in the multivariable model had P ≤ .05 or was capable of altering the other coefficients by at least 10%. The p,p′-DDT and p,p′-DDE plasma levels were used as continuous as well as categorical variables in multivariate analysis. All final regression models were adjusted by age. Final model sensitivity to individual observations was done by plotting the residuals vs fitted values, leverage vs normalized residuals squared, and residuals vs predicted values. The dfbetas statistic was also estimated. Models were tested without detected influential observations with dfbetas > 2√n.

**Results**

The mean age of the participants was 23 ± 4.7 years (mean ± SD). Participants were Venda men from a rural area and a low socioeconomic status who had never been occupationally exposed to the pesticide DDT. A selection bias affecting results is not probable because participants were not aware of the study hypothesis. Because the study design controlled for sexual abstinence time and this variable was not significant, there was no need to control for it in analysis. Other explored exposures did not prove to be sufficiently present or intense to cause sperm alterations.

The mean serum concentration of p,p′-DDT was 529.67 ± 617.7 μg/L, and the mean lipid-adjusted p,p′-DDT concentration was 90.23 ± 102.4 μg/g (Table 1). The p,p′-DDE level had a mean serum concentration of 1259.10 ± 1297.0 μg/L. When expressed as a lipid-adjusted concentration, the mean p,p′-DDE concentration was 215.47 ± 210.6 μg/g (Table 1). The source of p,p′-DDT or p,p′-DDE exposure was found to be statistically significantly higher between participants whose houses were sprayed with DDT (n = 249) (i.e., mud and thatch roof dwellings) when compared with those whose houses were not sprayed (n = 48; p,p′-DDE P = .000; p,p′-DDT P = .000; Table 1).

The distribution of the semen parameters and their age-adjusted regression associations with the serum lipid p,p′-DDT and p,p′-DDE are shown in Table 2. Diagnostic tests on final regression models showed an
The significant negative influential observation for one subject that, by itself, consistently and considerably altered the statistical significance, but not the coefficients. Although \( p,p' \)-DDT and \( p,p' \)-DDE and semen results were measured correctly in this case, the subject was excluded because he was not representative of the studied population. Excluding him only changed previously borderline significant associations. Volume and count \( P \) values with and without the subject in the final models changed from .05 to .02 and from .1 to .04, respectively. Participants showing a significant positive association with continuous \( p,p' \)-DDT levels were the round cells (beta = 0.0013, \( P = .000 \)) and the cytoplasmic droplets (beta = 0.0014, \( P = .014 \)). The significant negative

### Table 1. Exposure data indicating the \( p,p' \)-dichlorodiphenyl-dichloroethene (\( p,p' \)-DDE) and \( p,p' \)-1,1,1-trichloro-2,2-bis(chlorodiphenyl)ethane (\( p,p' \)-DDT) serum levels (\( n = 303 \))

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Mean (±SD)</th>
<th>Median</th>
<th>Minimum*</th>
<th>Maximum</th>
<th>Mean Houses Sprayed (±SD)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p,p' )-DDE (µg/L)</td>
<td>1259.10 (1297.0)</td>
<td>697.0</td>
<td>ND</td>
<td>6621.0</td>
<td>529.7 (658) 1409.8 (1339)</td>
</tr>
<tr>
<td>( p,p' )-DDE (µg/g), lipid adjusted</td>
<td>215.47 (210.6)</td>
<td>134.0</td>
<td>ND</td>
<td>997.0</td>
<td>99.5 (123) 239.0 (215)†</td>
</tr>
<tr>
<td>( p,p' )-DDT (µg/L)</td>
<td>529.67 (617.7)</td>
<td>249.0</td>
<td>ND</td>
<td>2644.0</td>
<td>167.0 (339) 602.4 (630)</td>
</tr>
<tr>
<td>( p,p' )-DDT (µg/g), lipid-adjusted</td>
<td>90.23 (102.4)</td>
<td>46.0</td>
<td>ND</td>
<td>519.0</td>
<td>30.5 (58) 101.9 (104)†</td>
</tr>
</tbody>
</table>

* ND indicates nondetectable (detection limit = 0.02 µg/g).
† Yes indicates that a participant’s house was sprayed with DDT within the last year. Six participants did not know.
†† \( P = .000 \).

### Table 2. Distribution of seminal parameters and age-adjusted regression associations* with serum lipid \( p,p' \)-1,1,1-trichloro-2,2-bis(chlorodiphenyl)ethane (\( p,p' \)-DDT) and \( p,p' \)-dichlorodiphenyl-dichloroethylene (\( p,p' \)-DDE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Mean (±SD)</th>
<th>Median</th>
<th>Beta 95% CI</th>
<th>Beta 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Semen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mL)†</td>
<td>303</td>
<td>1.88 (1.3)</td>
<td>1.5</td>
<td>-0.0003</td>
<td>-0.0006, -0.00004</td>
</tr>
<tr>
<td>Total count (mL ejaculate)‡</td>
<td>295</td>
<td>101.6 (159.3)</td>
<td>59</td>
<td>-0.003</td>
<td>-0.006, -0.0002</td>
</tr>
<tr>
<td>Sperm concentration (10⁶/mL)†</td>
<td>296</td>
<td>51.76 (48.2)</td>
<td>39</td>
<td>-0.0003</td>
<td>-0.0020, 0.0014</td>
</tr>
<tr>
<td>pH§</td>
<td>300</td>
<td>7.46 (0.3)</td>
<td>7.5</td>
<td>0.0033</td>
<td>-0.0226, 0.0291</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>298</td>
<td>48.14 (21.1)</td>
<td>55</td>
<td>-0.2807</td>
<td>-1.099, 0.5379</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>299</td>
<td>50.1 (15.8)</td>
<td>57</td>
<td>-1.56</td>
<td>-63.90, 60.74</td>
</tr>
<tr>
<td>(sum of grades a + b)§</td>
<td>299</td>
<td>49.26 (20.7)</td>
<td>43</td>
<td>0.0006</td>
<td>-0.0002, 0.0013</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>286</td>
<td>54.13 (21.8)</td>
<td>59</td>
<td>-0.6571</td>
<td>-1.756, 0.4417</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>282</td>
<td>4.13 (2.70)</td>
<td>4</td>
<td>0.0006</td>
<td>-0.0003, 0.0004</td>
</tr>
<tr>
<td>Head defects (%)</td>
<td>282</td>
<td>95.13 (3.3)</td>
<td>96</td>
<td>-0.00009</td>
<td>-0.00007, 0.00005</td>
</tr>
<tr>
<td>Neck/midpiece defects (%)</td>
<td>282</td>
<td>15.01 (5.4)</td>
<td>15</td>
<td>-0.00009</td>
<td>-0.0003, 0.00011</td>
</tr>
<tr>
<td>Tail defects (%)</td>
<td>282</td>
<td>12.92 (7.7)</td>
<td>11</td>
<td>-0.0002</td>
<td>-0.0005, 0.0001</td>
</tr>
<tr>
<td>Round cells (10⁶/mL)†</td>
<td>291</td>
<td>1.13 (1.3)</td>
<td>1</td>
<td>0.0005</td>
<td>0.0002, 0.0008</td>
</tr>
<tr>
<td>Cytoplasmic droplets (%)†</td>
<td>282</td>
<td>11.47 (6.5)</td>
<td>10</td>
<td>0.0005</td>
<td>-0.0008, 0.0010</td>
</tr>
<tr>
<td>Teratozoospermic index†</td>
<td>282</td>
<td>1.39 (0.1)</td>
<td>1.4</td>
<td>8.94 × 10⁻⁶</td>
<td>-0.00003, 0.00005</td>
</tr>
<tr>
<td><strong>CASA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average path velocity (VAP, µm/s)¶</td>
<td>241</td>
<td>36.51 (15.2)</td>
<td>36.0</td>
<td>-0.0065</td>
<td>-0.0154, 0.0024</td>
</tr>
<tr>
<td>Straight-line velocity (VSL, µm/s)¶</td>
<td>241</td>
<td>26.98 (13.0)</td>
<td>26.5</td>
<td>-0.0006</td>
<td>-0.0014, 0.0001</td>
</tr>
<tr>
<td>Amplitude of lateral head displacement (ALH)¶</td>
<td>241</td>
<td>2.54 (0.8)</td>
<td>2.5</td>
<td>-0.0001</td>
<td>-0.0003, 0.00003</td>
</tr>
<tr>
<td>Beat cross-frequency (BCF)¶</td>
<td>239</td>
<td>28.68 (6.3)</td>
<td>29.0</td>
<td>0.0064</td>
<td>0.0028, 0.0100</td>
</tr>
<tr>
<td>Mean motility (%)¶</td>
<td>240</td>
<td>48.53 (18.6)</td>
<td>52.0</td>
<td>-0.0175</td>
<td>-0.0283, -0.0068</td>
</tr>
</tbody>
</table>

* Dependent variables were transformed when required to normalize their distribution for linear regression analysis. Negative binomial regression was used for morphology parameters because of their distribution. CI indicates confidence interval.
† Square root transformation.
†† Cubed transformation.
§ Squared transformation.
|| Reciprocal transformation.
¶ No transformation (raw.).
Table 3. Oligozoospermia, asthenozoospermia, and teratozoospermia distribution and their age-adjusted association to lipid-adjusted p,p'-dichlorodiphenyl-dichloroethylene (p,p'-DDE) and p,p'-1,1,1-trichloro-2,2-bis(chlorodiphenyl)ethane (p,p'-DDT)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Classification (%)</th>
<th>Odds Ratio</th>
<th>P</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligozoospermia (&lt;20 million sperm/mL)</td>
<td>295</td>
<td>72</td>
<td>28</td>
<td>DDE, 1.001 .03</td>
</tr>
<tr>
<td>Asthenozoospermia (&lt;50% grades a + b motility)</td>
<td>285</td>
<td>68</td>
<td>32</td>
<td>DDT, 1.003 .006</td>
</tr>
<tr>
<td>Teratozoospermia (&lt;15% normal morphology)</td>
<td>291</td>
<td>0.5</td>
<td>99.5</td>
<td>*</td>
</tr>
</tbody>
</table>

* Not computable because of variable’s distribution.

associations with continuous p,p'-DDT levels were volume (square root–transformed, beta = −0.0003, P = .024) and count (square root–transformed, beta = −0.003; P = .04). Semen volume and sperm count (both square root–transformed) were also significantly reduced when the first 3 quartiles of lipid-adjusted p,p'-DDE (0–345 μg/g) were compared with the fourth quartile (346–997 μg/g): volume beta = −0.16, P = .01; sperm count beta = −1.16, P = .04. The CASA parameters that had a statistically significant negative association with p,p'-DDT were straight-line velocity (VSL; beta = −0.002, P = .03), amplitude of lateral head displacement (ALH; beta = −0.003, P = .03), and beat cross-frequency (BCF; beta = −0.01, P = .000). The CASA mean motility (cubed) had a significant negative association with p,p'-DDE (beta = −0.02, P = .001; Table 2). In comparing the first 3 p,p'-DDE quartiles against the fourth, the coefficient for cubed motility was found to be −8.79 (P = .001).

The participants' semen characteristics were classified according to dichotomous abnormal semen categories (WHO, 1999), and these were expressed as percentages (Table 3). Of all the participants, 28% were classified with oligozoospermia, 99.5% with teratozoospermia, and 32% with asthenozoospermia. The distribution and crude regression of the dichotomous abnormal semen categories indicated by the odds ratio (OR) showed that those participants with oligozoospermia were significantly associated with p,p'-DDE (OR 1.001, P = .03; Table 3). The distribution of oligozoospermia was statistically significantly associated with the lipid-adjusted p,p'-DDE percentile concentrations, as is shown in the Figure. The distribution of asthenozoospermia was also significantly associated with the lipid-adjusted p,p'-DDT percentile concentrations (OR 1.003, P = .006) and p,p'-DDE (OR 1.001, P = .02). The graph shows that the higher the p,p'-DDE concentration, the greater the incidence of oligozoospermia and asthenozoospermia (Figure).

**Discussion**

The finding that both p,p'-DDT and p,p'-DDE values were statistically significantly higher in men living in sprayed houses than in men from nonsprayed houses (102.0 μg/g p,p'-DDT and 239.0 μg/g p,p'-DDE vs 31.0 μg/g p,p'-DDT and 100.0 μg/g p,p'-DDE, P = .0000) highlighted an important route of exposure, which could be an indication that indoor residual spraying contributes to increased exposure to DDT.

The mean lipid-adjusted p,p'-DDT levels (90.23 ± 102.4 μg/g) and p,p'-DDE levels (215.47 ± 210.6 μg/g) in the nonoccupationally exposed population of this study can be considered very high when compared with another study in the same province. Dalvie et al (2004a,c) assessed the reproductive effects of long-term DDT exposure in malaria vector-control workers (n = 47) in Limpopo Province. The mean lipid-adjusted p,p'-DDT levels in that study were 26.1 ± 13.7 μg/g and the mean p,p'-DDE levels were 65.0 ± 48.8 μg/g. Those levels are almost 3.5 times lower than those found in this study, which is to be expected because the malaria vector-control workers wear protective clothing and take the necessary safety precautions when working with DDT. In a similar nonoccupational exposure study done
in 2000–01 by de Jager et al (2006) in Chiapas, Mexico, the \( p,p'\)-DDE level (45 ± 32 \( \mu g/g \)) was found to be almost 5 times lower than in this study; however, this was after DDT had been phased out by 2000. It is known that \( p,p'\)-DDE concentration in lipids is used as a surrogate for chronic exposure to technical DDT, a mixture that comprises estrogenic compounds such as \( o,p'\)-DDT and \( p,p'\)-DDT and the androgen antagonist \( p,p'\)-DDE (de Jager et al, 2006). The high \( p,p'\)-DDT level in this study indicates current acute exposure to DDT and the high \( p,p'\)-DDE levels indicate chronic long-term exposure. These levels are much higher than the levels in the above-mentioned studies that found reproductive effects because of DDT exposure (Ayotte et al, 2001; Dalvie et al, 2004a,c; de Jager et al, 2006). The levels in this study are supported by lower semen volume, total sperm count, progressive motility, and viability with higher levels of \( p,p'\)-DDT. Higher levels of \( p,p'\)-DDE also resulted in lower semen volume, total sperm count, progressive motility, and viability.

To assess reproductive function, a basic semen analysis was carried out and CASA motility parameters were evaluated as well. This included semen volume, pH, and viscosity. The epididymal epithelium is androgen dependent and has both absorptive and secretory functions. The epididymal plasma in which the sperm are suspended within the epididymis is also secreted by the epididymal epithelium. It is a complex fluid that changes along the length of the epididymis. The spermatozoa experience a series of sophisticated microenvironments that regulate their maturation (Mortimer, 1994). Under physiological conditions, the various components of the ejaculate originate in different parts of the male reproductive tract and are emitted in a definite order (Mann and Lutwak-Mann, 1982). The vesicular fluid is the last fraction of semen ejaculated and contributes up to 70% of the ejaculate volume, whereas the prostate contributes the other 30% (Mortimer, 1994). It has been hypothesized that toxicants or their metabolites can act directly on accessory glands by altering the quality or quantity of their secretions and that this could influence semen volume (Mann and Lutwak-Mann, 1982; Pant et al, 2004). Because both the prostate and seminal vesicles are also androgen-dependent organs (Mann and Lutwak-Mann, 1982), the antianstrogen properties of \( p,p'\)-DDE could have an influence on the functions of the organs. This could account for the mean semen volume (1.88 ± 1.3 mL), which was slightly lower than the WHO (1999) reference value and similar to the Chiapas study (mean volume = 1.84 mL; de Jager et al, 2006). The study showed a strong and significant negative association between \( p,p'\)-DDE and volume; a similar association was found in the Chiapas study. When \( p,p'\)-DDE was analyzed by quartiles, results showed that the expected semen volume of the most exposed men (\( p,p'\)-DDE = 346–997 \( \mu g/g \)) would be approximately 1.38 mL. Pant et al (2004) showed that \( p,p'\)-DDE and \( p,p'\)-DDD (\( p,p'\)-DDD = 1.1 dichloro-2,2 bis \( (p\)-chlorophenyl)ethane) were higher in the semen of infertile men compared with fertile men. The levels of gamma-galactosidase and acid phosphatase activity were also lower in infertile men, whereas the high fructose level observed could suggest “non-utilization of the enzyme by sperms due to some biochemical defects” (Pant et al, 2004:213), although this was not studied. It would have been in the interest of the study to assess the accessory gland markers such as fructose and \( \alpha\)-glucosidase, but unfortunately, because of the low volume, this could not be done.

Although the mean total sperm count was within the WHO (1999) reference range, there was a significant negative association with \( p,p'\)-DDT and \( p,p'\)-DDE. Analyzing \( p,p'\)-DDE by quartiles showed that the expected sperm count in the highest quartile (\( p,p'\)-DDE = 346–997 \( \mu g/g \)) would be approximately 56.3 million. The participants were divided into oligo- and normozoospermic categories according to WHO (1999). The crude regression associations showed a statistically significant positive dose-dependent association between participants with oligozoospermia (28%) and \( p,p'\)-DDE concentration (OR 1.001, \( P = .03 \)). This indicates that participants with high concentrations of \( p,p'\)-DDE are at risk of presenting with oligozoospermia. In support, the distribution of oligozoospermia shows that the higher the \( p,p'\)-DDE concentration, the greater the incidence of oligozoospermia. This trend is similar to the findings of a study by Rozati et al (2002), who suggested that there was a significant deterioration in semen parameters, including sperm count in infertile men with PCBs in their seminal plasma, compared with the control group. This trend is also in agreement with a report citing an inverse correlation of PCBs and sperm motility in men with oligozoospermia (Bush et al, 1986).

There were negative associations between the progressive motility, total motility, and viability and the \( p,p'\)-DDT and \( p,p'\)-DDE concentrations. Some animal data suggest that \( p,p'\)-DDE might be hormonally active and therefore would adversely affect semen parameters. The compounds that readily pass the blood-testis barrier might directly affect spermatogenesis (Hauser et al, 2003). Effects at the mitotic or meiotic level could lead to decreased sperm production, whereas the targeting of the postmeiotic processes and epididymal sperm maturation might lead to impaired sperm motility (Hauser et al, 2003). Despite a mean motility within the WHO normal range (WHO, 1999), 32% (\( n = 285 \)) of the participants
presented with asthenozoospermia (>$50\%$ a + b or >25\% grade a). Although there was no significant association between sperm motility and $p,p'$-DDE, the distribution of asthenozoospermia shows that the higher the $p,p'$-DDE concentration the greater the incidence of asthenozoospermia. The multivariate logistic regressions OR showed a statistically significant positive dose-dependent association between participants with asthenozoospermia and $p,p'$-DDT concentrations (OR 1.003, $P = .006$) and $p,p'$-DDE concentrations (OR 1.001, $P = .02$). This result was similar to a finding in a study by Hauser et al (2003), which showed that PCB-138, which is also an organochlorine compound, was inversely associated with sperm motility and morphology.

The mean CASA motility (48.53\% ± 18.6\%) compared well with the manual mean motility (50.1\% ± 15.8\%). CASA is being used in reproductive toxicology because some of the motility parameters are sensitive to toxins (ESHRE, 1998). The significant association with $p,p'$-DDE and the cubed CASA mean motility (beta = $-0.02$, $P = .001$) in this study compares well to a study by Hirano et al (2001), which compared the CASA parameters of “good” (fertilization rate > 50\%) and “poor” (fertilization rate ≤ 50\%) fertilization groups.

The poor fertilization group was found to be 48.9\% ± 22.1\% as opposed to the good fertilization group (59.9\% ± 16.5\%; Hirano et al, 2001). This indicates that the fecundity of this exposed Limpopo population might be compromised. CASA parameters showing a significant negative association with $p,p'$-DDT were the VSL (beta = $-0.002$, $P = .03$), ALH (beta = $-0.0003$, $P = .03$), and BCF (beta = $-0.01$, $P = .000$). The VSL (26.98 μm/s) and ALH (2.54 μm) values in this study are similar to those in a study by Guo et al (2000), which found that the VSL (25.4 μm/s) and ALH (2.9 μm) were lower in a PCB-exposed population than in an unexposed population (VSL = 33.0 μm/s, ALH = 3.3 μm). The BCF is useful in determining changes in the flagellar beat pattern (Mortimer, 2000). The negative association with $p,p'$-DDT indicates that higher levels of DDT cause an increase in the flagellar beat pattern with an adverse effect on sperm motility. In the study by Guo et al (2000), the BCF was lower (17.4 Hz) for participants prenatally exposed to PCBs and dibenzofurans but was comparable to this study (BCF = 29 Hz). These findings were similar to those in animals, in which in utero exposure to similar toxic levels of these chemicals reduced daily sperm production and increased the percent abnormal sperm (Faqui et al, 1998; Guo et al, 2000). The findings of this study indicate that DDT exposure could have a negative effect on sperm motility. Sperm motility is commonly believed to be one of the most important characteristics correlated with fertility (Eimers et al, 1994; Hirano et al, 2001).

This study population had a high percentage of round cells ($1.13 ± 1.3 \times 10^6$/mL), which showed a statistically significant positive association with both $p,p'$-DDT (beta = 0.0013, $P = .000$) and $p,p'$-DDE (beta = 0.0005, $P = .000$). Indications are that round cells occur frequently in infertile patients and are associated with poor semen quality (Arata de Bellabarba et al, 2000). A study carried out in Austria showed a significant increase in round cells in the semen of smokers compared with nonsmokers (Trummer et al, 2002). In this study, smoking was taken into account as a possible confounder and did not affect the outcome of the data.

During spermatogenesis, spermatids are transformed into sperm by different processes, including condensation and structural shaping of the cell nucleus and the formation of the flagellum. Disruption at this stage of development can cause impairment of sperm condensation, motility, and morphology (Parvinen, 1998; de Jager et al, 2006). A significant proportion of the sperm in each sample might be morphologically abnormal, but if the proportion is above 5\%, it could account for impaired fertility (Menkveld et al, 1990). WHO (1999) states that below 15\% normal forms, the fertilization rates in vitro will be reduced. The study investigating exposure to DDT in malaria vector-control workers had a mean normal morphology score of 2.5\% ± 1.8\%, with 84\% of the morphology scores being below the WHO (1992) and Tygerberg strict criteria. The study was carried out in Austria showed a significant decrease in normal morphology (Rozati et al, 2002).

During spermiation, residual cytoplasm is shed from the neck of the mature spermatid and a small residual cytoplasmic droplet remains attached to the testicular sperm, which is lost through epididymal transit and sperm maturation (Hess et al, 2001). The mean prevalence of cytoplasmic droplets is 2.2\% (Belsey et al, 1980; Mortimer, 1994), whereas in this study, it was 11.5\%. Fisher et al (1998) demonstrated that neonatal exposure of rats to diethylstilboestrol (DES) caused permanent distention of the rete testis and efferent ducts, with loss of epithelial height through adulthood. Sharpe (1998) argues that, owing to the loss of the apical portion of the cell, the endocytic apparatus might be
dysfunctional. This could imply that, similar to DES, prenatal DDT (estrogenic) exposure might have affected the development of these epididymal cells and subsequently could contribute to the high number of cytoplasmic droplets.

Not only are cytoplasmic droplets associated with immature spermatocytes, their presence is correlated with oxidative damage (Mortimer, 1994; Gergely et al., 1999; Chantler and Abraham-Peskir, 2004). A study by Gomez et al (1996) correlated a specific morphological defect of human sperm with reactive oxygen species (ROS). The residual cytoplasm present in the midpiece of the human sperm revealed a significant correlation between excess residual cytoplasm in the midpiece and the enhanced generation of ROS. The study by Aziz et al (2004) supported this finding and additionally showed a positive correlation of ROS with percent sperm with cytoplasmic droplets (8%) and tail defects (12%). This study showed a statistically significant positive association between cytoplasmic droplets (11%) and p,p'-DDT (beta = 0.0014, P = .014); although there was no association with the tail defects (13%), these percentages were similar to those in the study by Aziz et al (2004).

Oxidative stress or ROS generation can be induced in the testes by exposure to common xenobiotics, such as nonylphenol and dioxin (Aitken et al., 2004; Chitra and Mathur, 2004). Subchronic exposure to DDT is associated with an increase in free radical generation by lipid peroxidation (Koner et al., 1998). It is well known that ROS generation impairs sperm motility (Aitken et al., 1998). This means that there is a strong possibility of increased ROS generation in this exposed group of men, which will influence morphology and sperm motility parameters negatively.

The etiology of TDS is suspected to be related to genetic, environmental, or both factors, including endocrine disruptors. Both p,p'-DDT and p,p'-DDE are considered to be hormonally active, with p,p'-DDT having estrogenic activity via binding and activation of the estrogen receptor and p,p'-DDE being antiandrogenic (Kelce et al., 1995). Irrespective of the exact mechanism, which remains to be elucidated in these cases, either reduced testosterone production by Leydig cells (via DDT’s estrogenic suppression of the hypothalamic-pituitary-testicular axis) or by impeded androgen action (via DDE’s effect on the androgen receptor), the physiological consequence would be impaired Sertoli cell function (Pavrin, 1998). The primary role of these cells is to support spermatogenesis (de Jager et al., 2006). The increased serum p,p'-DDT and p,p'-DDE levels could be exerting an effect on the Sertoli cells, preventing normal spermatogenesis and resulting in abnormal sperm function as observed in this study.

Conclusions

The data on seminal parameters from 311 participants makes this study one of the largest to look at the effects of DDT exposure. The study found evidence that indicated that nonoccupational exposure to p,p'-DDT and its metabolite p,p'-DDE has an effect on seminal parameters of young men living in the Limpopo Province. The mean age of the participants was 23 years, which is young when compared with similar studies. This is of concern because it has been found that semen volume, motility, and morphology decrease with age (Kidd et al., 2001). It is impossible to know what the effects will be on the fertility potential of this population in 5 to 10 years with the continued use of DDT for malaria vector control. This data and the Mexico data (de Jager et al., 2006), together with the well-documented effects of DDT on animals (Toppuri et al., 1996), should provide sufficient evidence to elicit concern about the effect of this pesticide and its metabolite p,p'-DDE on human health.

Long-term exposure to small amounts of organochlorine contaminants leads to the accumulation of considerable burdens in animal and human tissue (de Jager et al., 2006). The young are the most vulnerable. It is not necessarily the amount of DDT to which the mother is exposed during pregnancy that is critical but rather her lifetime exposure and bioaccumulation that determines the level of exposure of the fetus and breast-fed infant (Longnecker et al., 2000; Korrick et al., 2001). Many of the infants in these rural areas in South Africa are breast fed. Other effects could occur; in a study by Longnecker et al (2002), there was a modest to moderate association in boys with maternal levels of DDE greater than or equal to 85.6 μg/L with the development of cryptorchidism, hypospadias, and polythelia. Sunyer et al (2005) found that prenatal exposure to DDE residues (geometric mean in cord serum: 1.06 μg/L) might contribute to the development of asthma. Prenatal exposure to DDT and, to a lesser extent, DDE was associated with neurodevelopmental delays during early childhood (Eskenazi et al., 2006).

Of concern in this nonoccupationally exposed population is that these high levels of p,p'-DDT and p,p'-DDE appear to have adverse effects on the seminal parameters, supporting the findings by de Jager et al (2006). The elevated levels of exposure indicate a high degree of chronic exposure and imply an urgent need for continued epidemiologic studies in the Limpopo Province to determine the potential adverse effects of these pesticides. These findings are not only applicable to Limpopo Province, but also to other malaria areas in South Africa, Africa, and other parts of...
the world where DDT is used for malaria control. The feasibility of cost-effective and environmentally safe alternative methods for pest control needs to be considered.

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