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Reduced Seminal Parameters Associated With Environmental DDT Exposure and *p,p′*-DDE Concentrations in Men in Chiapas, Mexico: A Cross-Sectional Study

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ABSTRACT: In response to mounting concerns about the endocrine-disrupting influence of environmental chemicals on human health, this epidemiological study was initiated to test the hypothesis that nonoccupational exposure to the estrogenic pesticide 1,1,1-trichloro-2,2-bis(chlorodiphenyl)ethane (DDT) affects male reproductive parameters. One hundred and sixteen men aged 27 years (SD = 8.2) living in malaria endemic-areas in Chiapas (Mexico), where DDT was sprayed until 2000, participated in a cross-sectional study. Semen analyses were conducted according to World Health Organization methods and a quality control program was followed. DDT exposure was defined as the level of blood plasma *p,p′*–dichlorodiphenyl dichloroethylene (DDE), the major metabolite of DDT. The *p,p′*-DDE concentration adjusted for total lipids was 100 times higher than that reported for nonexposed populations at 45 plus or minus 32 μg/g (mean ± SD). Crude regression analysis showed that several sperm motion parameters, including the percentage of motile sperm, decreased with higher *p,p′*-DDE concentrations ($\beta = -8.38$; $P = .05$ for squared motility), and the percentage of sperm with morphological tail defects increased with higher plasma *p,p′*-DDE concentration ($\beta = 0.003$; $P = .017$). Insufficient sperm chromatin condensation was observed in 46.6% of participants, and the most severe category of incomplete DNA condensation was also positively correlated with *p,p′*-DDE concentration ($r = .223$; $P = .044$). Therefore, nonoccupational exposure to DDT, as assessed by plasma *p,p′*-DDE concentrations, is associated with poorer semen parameters in men, indicating adverse effects on testicular function and/or the regulation of reproductive hormones. Previously, a causal role of environmental toxicants in human male infertility has been lacking because observed effects have been the result of unusually high exposures, either occupationally or as a result of industrial accidents, resulting in unprecedented controversy (reviewed by Cheek & McLachlan, Environmental hormones and the male reproductive system. *J Androl.* 1998;19:5). This is the first epidemiological study demonstrating effects after nonoccupational exposures to DDT. Based on these findings, the effect of DDT on male reproductive health should not be ignored.

Key words: Pesticide, organochlorine, spermatozoa, sperm motility, sperm morphology, chromatin.

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The pesticide 1,1,1-trichloro-2,2-bis(chlorodiphenyl) ethane (DDT) is one of the 12 persistent organic pollutants that was under negotiation for the worldwide treaty, the Stockholm Convention, which considered whether to ban or restrict the production or use of these pollutants as result of their toxicity, resistance to breakdown, bioac-

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cumulation potential, and potential to be transported over long distances. Initially, DDT was used in the 1940s to control wartime typhus and agricultural pests, and it then was popular on a worldwide basis as a measure to control malaria vectors, until its deleterious effects on wildlife led to a ban on routine DDT use in many countries in the 1970s. Despite restrictions throughout much of North America and Europe and the limited availability of alternatives, DDT continues to be used, mainly for malaria vector control (Smith, 1999). Some countries still use DDT, as vector species eradicated by DDT 30 years ago have become resistant to pyrethroid and have made a comeback, resulting in a sharp increase in malaria cases (Bouwman, 2000).

In the 1970s, the Mexican government launched an intense effort to control malaria in several areas of the country (Lopez-Carrillo et al, 1996). As a result of the activities related to the Malaria Control Program, the number of cases has shown an impressive decrease from 140 000 in 1986 to less than 7000 in 1996. This reduction was due to an intense public health program based on the combined effect of case location and treatment and on the massive use of DDT in in-house spraying within the endemic areas. Between 1971 and 1973, this program consumed approximately 226 000 tons of DDT, with an estimated 500 g per household used (Lopez-Carrillo et al, 1996). In 1997, however, the Mexican government initiated a program to phase out all uses of DDT, and phase out was completed in the year 2000 (Yanez et al, 2002).

Numerous reports indicate that DDT has estrogenic effects (Sonnenschein and Soto, 1998). Technical-grade DDT is a mixture of *p,p′*-DDT (approximately 85%), *o,p′*-DDT (approximately 15%), and *o,o′*-DDT (trace amounts), with both *p,p′*-DDT and *o,p′*-DDT having estrogenic activity. *p,p′*–dichlorodiphenyl dichloroethylene (DDE), a persistent metabolite of *p,p′*-DDT, is a widespread environmental contaminant (Turusov et al, 2002). There is evidence that *p,p′*-DDE is an androgen receptor antagonist (Kelce et al, 1995), and the hypothesis has been advanced that *p,p′*-DDE may interact in an additive or multiplicative way with other environmental endocrine-disrupting pollutants (Turusov et al, 2002). Chronic exposure alters sexual steroid hormone homeostasis; *p,p′*-DDE induced the expression of hepatic aromatase in adult male rats (You et al, 2001) and also impaired sexual development in male rat pups following exposure in utero (You et al, 1998). Guillette et al (1995) reported abnormal ovarian morphology and increased estradiol concentrations in female juvenile alligators, as well as abnormal testicular germ cells, decreased serum testosterone levels, and small phalli in males exposed to dicofol and DDT. These findings have led experts to consider DDT and its metabolites to be endocrine disruptors that are able to promote hormone-dependent pathologies (Kavlock et al, 1996).

In humans, the increasing incidence of testicular anomalies in male infants and the apparent decline of sperm quality in adults noted in some regions of the world (Toppari et al, 1996) have been hypothetically attributed to the introduction of estrogenic chemicals, such as DDT, into the environment (Sharpe, 1995). Such disorders of the male reproductive tract are also compatible with androgen receptor blockade. Since technical-grade DDT comprises estrogenic molecules and because its main metabolite is a potent antiandrogenic agent, it has been hypothesized that exposure to DDT is involved in the increase in male reproductive tract anomalies (Guillette et al, 1995). However, direct evidence that these compounds can induce adverse effects on reproductive function in men is still lacking. A pilot study in Chiapas revealed very high levels of exposure to DDT compounds in 24 young men (Ayotte et al, 2001). The mean concentration of lipid *p,p′*-DDE was 300-fold greater than in Canadian women exposed to background environmental levels (Dewailly et al, 1996; Lebel et al, 1998), and *p,p′*-DDE concentration was inversely correlated to both semen volume and sperm count (Ayotte et al, 2001). Thus, the objective of this project was to assess nonoccupational biological exposure to DDT in a large sample of the Mexican male population in Chiapas and to use standard sperm parameters and specialized sperm function tests to test the hypothesis that DDT exposure is associated with reduced male reproductive health.

Materials and Methods

Study Area

Six rural communities with a history of high DDT exposure from the malaria-endemic region of Chiapas, Mexico, were identified (La Libertad, Cuauhtemoc, El Dorado, Hidalgo, Ignacio López Rayon, and Miguel Aleman). The mean altitude is 187 m above sea level, with an annual rainfall of 650 mm and mean annual temperature of 25°C. DDT was sprayed inside residents' homes once or twice a year until 1997 and also during the 1997 hurricane, when everything was sprayed.

Study Design and Population

The study design was cross-sectional. A sample of volunteer, nonoccupationally exposed men was selected. The participants recruited were between 18 and 40 years old and had been living in the study communities for at last a year. Participants had to meet all the inclusion criteria of the study and were excluded if they presented with history of any of the following: diagnosed infertility, important testicular trauma, orchitis, urinary infection, sexually transmitted diseases, use of hormonal medication, exposure to known gonadotoxins, use of sauna baths, or history of neuropsychiatric disorders.

Recruitment and Sampling

Between October 2000, and February 2001, study team members visited the villages and held informative meetings with authorities and potential participants. The study was identified as the ''DDT and male health'' study, and the hypothesis of the study was not disclosed. During the first appointment, all participants were informed about the procedures and had to provide consent. A male nurse assisted in completion of the questionnaires on an individual level. The 150-question questionnaire was administered in Spanish and included general information, identification data, exposure assessment, fertility background, previous diseases and treatments, and hormonal characteristics.

The study's research protocol was approved by the Human Subjects Committee of the National Institute of Public Health of Mexico. All participants gave their informed consent and received a detailed explanation of the study and procedures used as well as counseling on how to reduce DDT exposure.

Exposure Assessment

A venous blood sample of about 10 mL was drawn from a cubital vein of each individual. Blood samples were centrifuged at 1500 × *g* for 10 minutes at 37°C. Plasma was stored at −70°C until analyzed.

p,p′-DDE and *p,p′*-DDT were determined by the Quebec Toxicology Center (Institut National de Santé Publique du Québec, Ste-Foy, Quebec, Canada) using a Hewlett-Packard (5890) Series II gas chromatograph (Hewlett-Packard, Palo Alto, Calif) equipped with dual-capillary columns and 2 electron-capture detectors. Detection limits were *p,p′*-DDE 0.02 μg/L and 0.03 μg/L for *p,p′*-DDT. Each series of analyses included 3 calibration standards, 1 plasma blank, and 1 quality control standard. The between-day precision of the method (coefficient of variation) was 4.3% for *p,p′*-DDE and 8.5% for *p,p′*-DDT, and recoveries were greater than 90% for both compounds. Concentrations of DDT compounds in plasma were expressed on a lipid basis (μg/g lipids). Total and free cholesterol, phospholipids, and triglycerides were determined by enzymatic methods and the total plasma lipid concentration calculated according to the formula of Phillips et al (1989).

Semen Analyses

Semen samples were obtained from 133 subjects after a prescribed 3-day period of sexual abstinence. Semen specimens were produced by masturbation directly into a sterile plastic container in a room specially provided for the purpose, which was located adjacent to the laboratory. The time of collection, days of abstinence, and any medical problems having occurred in the last month before collection were noted. The semen sample was incubated at 37°C until liquefied. Semen analyses and andrological tests were performed in a blind manner by trained researchers, according to the standards and procedures of the World Health Organization (WHO, 1999). Analyses were done in duplicate.

After liquefaction, seminal physical characteristics (appearance, contamination, liquefaction and viscosity, odor, ejaculate volume, and semen pH) were assessed (Mortimer, 1994). Sperm motility was assessed on the wet preparation, using the class a–d sperm progression rating (a = rapid progressive motility; d = immotile sperm; WHO, 1999; Nordic Association of Andrology, European Society of Human Reproduction and Embryology–Special Interest Group on Andrology, 2002). Sperm concentration was determined using a hemocytometer (WHO, 1999). The presence of leukocytes, erythrocytes, and spontaneous agglutinates was noted. The percentage of viable sperm was assessed using the eosin-nigrosin method (Mortimer, 1994). The immunoglobulin G (IgG) mixed antiglobulin reaction (MAR) test was used in all fresh samples with more than 40% motility (WHO, 1999). The diagnosis of immunological infertility is probable when 50% or more of the motile sperm have IgG antibodies (WHO, 1999). The percentage of abnormal sperm and the average number of defects per spermatozoa (the teratozoospermia index, or TZI) were assessed using the Papanicolaou method; the same technologist reviewed all samples (Mortimer, 1994). Morphology scoring (WHO, 1999) was performed at the University of Pretoria and was included in the quality control program.

Computer-Assisted Sperm Analysis

Sperm motility was additionally evaluated with a Hamilton-Thorne sperm analyser (HTM-2030; 6.4A software, Beverly, Mass) at 30 Hz. A Makler counting chamber (Sefi-Medical Instruments, Haifa, Israel) was used and dilutions were made to about 40 × 10^6 sperm/mL in Earles Balanced Salt Solution (EBSS; Sigma E-2888, Chemical Co, St Louis, Mo). The percentage of motile sperm, progressive motility, linear velocity, and curvilinear velocity were all analyzed in duplicate. For each 5-μL sample, 20 fields and a minimum of 150 sperm were selected and automatically analyzed at 37°C (Schrader et al, 1992; Mortimer and Fraser, 1996).

Sperm Chromatin Condensation Test

The Aniline Blue staining method was used to detect sperm chromatin condensation. One hundred sperm per sample were categorized and scored. The normal value is less than 25% 2+ (whole sperm head is slightly stained—light blue) and 3+ (whole sperm head is stained—dark blue) of 100 sperm. Slides were stained and evaluated according to standard methods and criteria (Foresta et al, 1992).

Alpha-Glucosidase Activity

Alpha-glucosidase was assayed as a biochemical marker for epididymal function (Cooper et al, 1990; Fourie and Bornman, 1994). The principle of the assay is the hydrolysis of the glycocidic bond in the synthetic substrate *p*-nitrophenol-α-D-glucopyranoside by the α-glucosidase enzyme, to form the reaction products glucopyranoside and *p*-nitrophenol. *p*-Nitrophenol was determined in an alkaline solution by spectrophotometry at 404 nm.

Androgen Receptor Genotype by DNA Analysis of Coding CAG Repeats

We also considered that polyglutamine polymorphism of the androgen receptor gene could be associated with semen parameters and/or any relationship among these parameters and *p,p′*-DDE. Therefore, the participants of this study were also genotyped for AR (CAG)n polymorphism.

After DNA purification using QIAGEN whole-blood plates (QIAGEN, Inc, Mississauga, Canada), the AR (CAG)n trinucleotide repeats were analyzed by polymerase chain reaction amplification using radiolabeled oligonucleotides followed by migration in high-resolution gel electrophoresis in a 6% denaturing polyacrylamide gel. Genotyping of the (CAG)n repeats was performed according to Tilley et al (1989). Internal controls included 3 homozygous individuals with a sequenced gene that had, respectively, 19, 22, and 25 CAG repeats. Autoradiograms were interpreted independently by 3 readers who were blinded to the status (case vs control) of the samples studied. Agreement between readers was calculated at 95%. To ensure no gel-to-gel drift in genotyping from one series to another, a control sample was included in triplicate in each series of analyses and was used to calibrate the readings from each gel/autoradiogram to the others. Also, random samples from each series of analyses were independently reanalyzed to validate the allocation of genotypes. Concordance between the initial readings and the replicate set was calculated at 98%. The smallest (CAG)n allele had 11 triplets and the largest observed had 28 triplets. The allele distribution was bimodal, one major mode comprised the two most frequent alleles of the (CAG)n repeat polymorphism in this sample, namely 23 and 24 CAG repeats (each with an allele frequency of 0.21). The third most frequent allele had 22 repeats (allele frequency of 0.18). A minor mode was observed at 18 CAG repeats (allele frequency 0.06). Given that the (CAG)n repeat is preceded by another exon (CAA), also coding for a glutamine, the total number of consecutive glutamine residues in AR alleles of 24 (CAG) repeats, for instance, is 25 glutamine residues. In this report, we will only use the number of (CAG)n repeats to describe AR alleles, unless noted otherwise. The number of CAG repeats was used as a continuous independent variable in all analyses, given that males have only one copy of the androgen receptor (located on chromosome X) and that the efficacy of the androgen receptor may vary according to the length of the (CAG)n repeat (Giguere et al, 2001).

Statistical Analyses

The final database was checked and exploratory data analysis was used to clean and test consistency of the database. Tabulation and graphical univariate analyses were done to describe the distribution of each variable and to identify the necessary transformation for the analyses.

Since there were 92 men who had 2 semen samples and 24 men who only had 1 semen sample, *t* tests were done to assess whether and when both types of participants could be combined into a single group. The mean semen parameters of the 92 men with 2 samples were tested against the semen parameters of the 24 with a single sample and against the 116 with either 1 or 2 samples.

Information obtained by questionnaire as well as participants' serum organochlorine levels was compared between different categories using analysis of variance or regression analysis. Pearson correlations were conducted between each transformed continuous seminal parameter and *p,p′*-DDE levels. Spearman correlations were also tested in nontransformed variables. There were no major differences between these correlations, except for variables that were transformed to their reciprocal. Thus, only Pearson correlations are reported for all variables, and Spearman correlations are also shown for variables that required a reciprocal transformation to be normalized. Bivariate analyses using regression models were conducted for the different reproductive outcomes, CAG, and questionnaire variables to determine the risk factors and to identify potential confounding factors. Afterward, linear and smoothed multivariate models were created for each dependent variable (transformed semen parameters); we accomplished this by initially including all of the independent variables that reached a level of statistical significance of *P* is less than 0.15 in the bivariate analyses. In sum, multivariate models were used to evaluate the effect of *p,p′*-DDE on different reproductive outcomes, accounting for CAG, age, smoking, duration of abstinence period, Quetelet Index, age of puberty onset, intercourse frequency, history of sexually transmitted diseases, and fever in the last 3 months.

Linear and logistic multivariate analyses were done introducing semen parameters as continuous or dichotomous (according to WHO [1999] reference values) response variables, respectively. Negative binomial regressions were used to analyze sperm head, neck, and tail defects as outcomes, since the distribution of these variables was discrete and the median was different to the variance.

A backward elimination procedure was applied in all multivariate models to discard, one by one, variables that did not reach the selected statistical cut-off point of *P* less than .05 or that did not significantly affect the rest of the coefficients in the model (did not produce a larger than 10% change in them).

Statistical analyses were conducted using Stata (Stata Statistical Software, Release 7.0; Stata Corporation, College Station, Tex) and S-Plus 6.1 packages.

Quality Control

For the semen analyses, a quality control program was followed in collaboration with the Andrology Unit at the University of Pretoria (South Africa), a collaborator in the European Society of Human Reproduction and Embryology network. Statistical analyses were performed in MedCalc, using the Kruskal-Wallis test. The ideal is less than 1% deviation from the average and less than 5% for the standard deviation (Mortimer, 1994).

Results

Seventeen of the 133 men who volunteered to participate did not meet the inclusion criteria as a result of medical criteria, so 116 men were retained. Of the 116 men recruited in the study, 92 provided 2 semen samples and 24 only 1 sample. Where applicable, the mean value for the 2 samples was used in the study.

Since there was no record of the number of men who attended informative meetings, a participation rate could not be calculated. Participants were men from a low socioeconomic status who had never been occupationally exposed to DDT, and most of them were farmers.

Exposure data and andrological information from the questionnaires are summarized in Table 1. The mean se-

Table 1. *Characteristics of participants*

Characteristics	N	%	Mean (SD)	Median	Ranges	P25–75*
Age of participants, y	116		27.08 (8.2)	25	18–49	20–34
Sexual abstinence, d	116		3.77 (0.97)	3.5	3–8	3–4.5
Sexual frequency, times/mo	116		6.08 (5.4)	4	1–32	2–8
Born in study area	63	54.8				
Duration of residence in study area, y	116		15.4 (11.6)	16.5	1–43	5–22.5
Smokers	39	33.6				
Houses sprayed with DDT, official	113	97.4				

* P25–75 represents the 25th and 75th percentiles.

rum *p,p′*-DDE concentration was 245 plus or minus 177 (mean ± SD) μg/L (median = 227 μg/L). When expressed on a lipid basis, the mean *p,p′*-DDE concentration was 45 plus or minus 31 μg/g lipids (median = 41 μg/g).

Table 2 shows the mean levels of seminal parameters and differences of men with 1 or 2 semen samples. Only 4 parameters proved significantly different ($.01 \leq P \leq .06$) between the men with 1 or 2 samples: percentage of head and tail defects, sperm concentration, and Aniline Blue staining (Grade 2). Separate results for all men and those with 2 samples are reported for these parameters. The mean lipid adjusted *p,p′*-DDE concentration was not statistically different between both groups.

The description of seminal parameters for all the participants is shown in Table 3. The mean semen volume was below the WHO (1999) reference value of 2 mL. Sperm counts were within the reference range ($\geq 40 \times 10^6$ sperm/ejaculate), but the percentage of progressively motile sperm was relatively low; WHO (1999) reference values are greater than 25% Grade a and greater than 50% Grade a + b motility. The WHO (1999) reference value is greater than or equal to 15% morphologically normal sperm, and the normal proportion observed was lower than previous values or indicators (WHO, 1992). The sperm IgG MAR test was positive (>10% sperm with adherent particles) in 8 participants (6.9% of cases), indicating the presence of antisperm autoantibodies. In only 2.6% of the cases (n = 3) did the immunological test have clinical significance (>50%) according to WHO (1999).

Correlations and crude linear regressions between the seminal parameters and blood plasma lipid–adjusted *p,p′*-DDE concentration are also given in Table 3.

The coefficients of regression on the 4 seminal param-

Table 2. *Comparison of seminal parameters and lipid-adjusted* p,p′*-DDE (μg/g) between participants with 1 (N = 24) or 2 (N = 92) semen samples*

Parameter	1 Sample Mean (SD)	2 Samples Mean (SD)	*P* Value of *t* Test
Volume, mL	1.86 (1.0)	1.78 (.96)	0.73
Total sperm count, $\times 10^6$ sperm/ejaculate	130 (111)	168 (164)	0.18
Sperm concentration, $\times 10^6$ sperm/mL	74.6 (55.6)	98.8 (11.9)	0.04
Viability	72.8 (11.0)	71.2 (15.8)	0.57
Rapid progressive motility, % Grade a	10.8 (7.4)	13.4 (16)	0.25
Progressive motility, % Grades a + b	52.6 (14.0)	52.3 (15.6)	0.92
Motility, % Grades a + b + c	60.7 (15.0)	59.2 (17.1)	0.67
Path velocity (VAP), μm/s	11.9 (2.3)	12.7 (4.6)	0.23
Progressive velocity (VSL), μm/s	7.53 (2.2)	8.02 (4.0)	0.43
Linear index (LIN), %	58.4 (5.9)	58.0 (5.6)	0.76
VSL/LIN*100 (VCL), μm/s	12.6 (2.6)	13.5 (4.7)	0.21
Morphology, % normal	8.69 (6.4)	7.33 (5.1)	0.34
Morphology, % abnormal heads	67.5 (8.0)	64.1 (7.4)	0.06
Morphology, % abnormal necks	20.0 (4.4)	21.4 (4.8)	0.18
Morphology, % abnormal tails	9.98 (4.8)	12.5 (5.9)	0.03
Morphology (TZI)	3.90 (10.9)	1.51 (.16)	0.29
α-Glucosidase, mU/ejaculate	17.5 (13.2)	21.7 (16.9)	0.20
Aniline Blue, % Grade 0	51.2 (24.5)	48.6 (26.9)	0.65
Aniline Blue, % Grade 1	28.8 (13.5)	28.2 (12.8)	0.84
Aniline Blue, % Grade 2	15.6 (11.0)	22.6 (14.7)	0.01
Aniline Blue, % Grade 3	5.86 (5.3)	6 (4.5)	0.90
Seminal pH	7.98 (0.12)	7.98 (.13)	1.00
Lipid adjusted *p,p′*-DDE, μg/g	35.7 (29.6)	47.2 (32.1)	0.12

Table 3. *Seminal parameters and their association with lipid adjusted* p,p′*-DDE (μg/g) concentration (n = 116)*

Parameter	Mean (SD)	Median	Pearson Correlation (*P*)	Crude Linear Regression (IC 95%)
Volume, mL*	1.84 (1.0)	1.62	−0.0248 (0.86)	−.003 (−.003, 0.002)
Total sperm count, $\times 10^6$ sperm/ejaculate*	137 (123)	109	−0.095 (0.32) −0.026 (0.81)‖	−15.38 (−45.99, 15.23) −11.70 (−33.31, 9.91)
Sperm concentration, $\times 10^6$ sperm/mL*	76.2 (60)	60.0	−0.102 (0.29)	−4.73 (−26.31, 16.85)†
Viability	72.53 (12.0)	75.75	−0.128 (0.18)	−0.0515 (−.123, .020)
Rapid progressive motility, % Grade a	10.82 (9.4)	8	−0.094 (0.37)	−0.0257 (−.083, .031)
Progressive motility, % Grades a + b	52.69 (14.3)	56	−0.201 (0.034)	−0.0908 (−.175, −.007)
Motility, % Grades a + b + c	60.51 (15.3)	64	−0.229 (0.02)	−.1003 (−.191, −.010)
Path velocity (VAP), μm/s*	12.10 (2.9)	11.5	0.165 (0.05)	.00001 (−1.73e−06, .00003)
Progressive velocity (VSL), μm/s§	7.65 (2.6)	7	0.152 (0.11) −0.19 (0.05)¶	.0002 (−.00004, .0004)
Linear index (LIN), %§	58.40 (5.8)	57.75	0.043 (0.66) −0.08 (0.40)¶	2.16e−06 (−7.42e−06, .00001)
VSL/LIN*100 (VCL), μm/s§	12.81 (3.1)	12.35	0.18 (0.05) −0.23 (0.02)¶	.0001 (−1.76e−06, 0.0002)
Morphology, % normal	8.43 (6.2)	7.5	−0.041 (0.67)	−0.0057 (−.044, .032)
Morphology, % abnormal heads	66.87 (8.0)	68	−0.100 (0.30) −0.142 (0.18)‖	−.0250 (−.072, .022) −.0358 (−.088, 0.17)‖
Morphology, % abnormal necks	20.29 (4.5)	20	0.057 (0.55)	.0079 (−0.18, 0.34)
Morphology, % abnormal tails	10.45 (5.1)	10	0.229 (0.02) 0.292 (0.005)‖	.0373 (.007, .067) .0435 (.013, .074)‖
Morphology (TZI)‡	3.45 (9.9)	1.43	−0.092 (0.34)	.0042 (−.056, .064)
α-Glucosidase, mU/ejaculate	18.14 (14.0)	14.58	0.023 (0.82)	.0012 (−.005, .007)
Aniline Blue, % Grade 0	50.77 (24.8)	49.2	−0.071 (0.46)	−.0547 (−.202, 0.93)
Aniline Blue, % Grade 1	28.72 (13.4)	28.75	0.123 (0.20)	.0518 (−.028, .132)
Aniline Blue, % Grade 2	16.82 (11.9)	17.25	−0.045 (0.64) 0.038 (0.72)‖	−.017 (−.086, .053) .0130 (−.058, .084)‖
Aniline Blue, % Grade 3	5.88 (5.1)	5	0.223 (0.04)	.0356 (.0010, .0703)
Seminal pH	7.9 (0.1)	8	0.057 (0.55)	.0002 (−.001, .001)

* Square-root transformed to normalize for correlations and regressions.
† Reciprocal transformed to normalize for correlations and regressions.
‡ Square-root transformed to normalize for correlations and regressions.
§ One subject with the highest *p,p′*-DDE level and only 1 semen sample was excluded since including it changed the direction of the association.
‖ Analysis done on 92 men with 2 semen samples.
¶ Spearman correlation in nontransformed variables.

eters with statistical differences between men with 1 or 2 samples showed little or no difference, except for sperm concentration, which was higher for the sum of all men. Sperm concentration was, however, not associated with *p,p′*-DDE. The association between *p,p′*-DDE and tail defects was the only one that was statistically significant among these previous 4 parameters. Because the *p,p′*-DDE and tail defects association was slightly more conservative among all 116 men than the among the 92 with both samples, the whole group was used in other tests with this variable and in reporting and interpreting of the results.

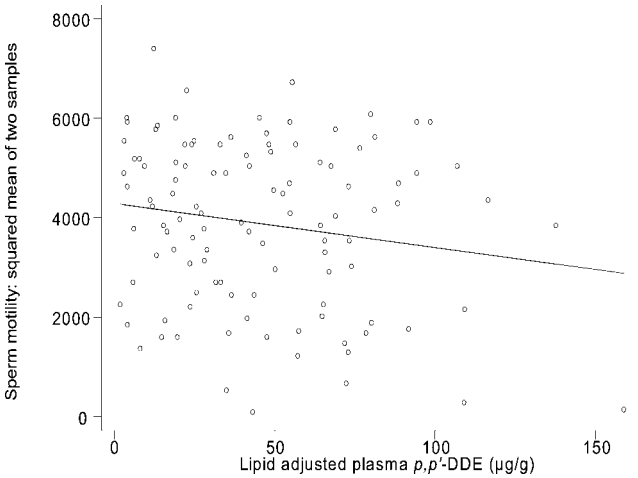

Figure 1. The negative association between lipid-adjusted plasma *p,p′*-DDE concentrations and the percentage of motile sperm (motility Grades a + b + c) from 116 men living in selected communities in Chiapas (Mexico). Sperm motility declines with increasing *p,p′*-DDE concentrations ($P = .074$).

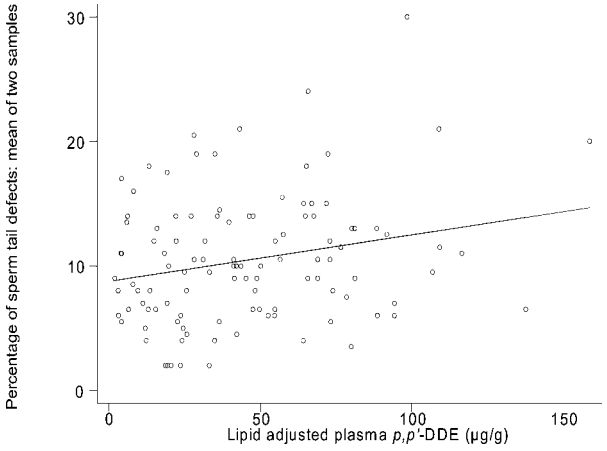

Figure 2. The positive association between lipid-adjusted plasma *p,p′*-DDE concentrations and the percentage of sperm with abnormal tail morphology from 116 men living in selected communities in Chiapas (Mexico). Flagellar abnormalities increase with increasing *p,p′*-DDE concentrations ($P = .017$).

The mean motility (% Grade a + b + c sperm) and the mean progressive motility (% Grade a + b sperm) were both weakly but significantly associated with *p,p′*-DDE levels: both of these categories of motility decreased nearly 0.1% per increase unit of lipid-adjusted *p,p′*-DDE. A weak positive correlation between mean path velocity and *p,p′*-DDE indicated slower sperm with increasing exposure. Results on the crude and adjusted (by age) linear regressions between squared motility and *p,p′*-DDE showed coefficients of −8.85 ($P = .074$) and −7.90 ($P = .102$), respectively (Figure 1).

Although negative binomial regression was considered the proper analysis for head, neck, and tail defects, linear regression results are shown in Table 3 for practical purposes. There was a positive and significant correlation between the percentage of sperm with tail defects and *p,p′*-DDE concentration. A negative binomial regression between tail defects and *p,p′*-DDE resulted in a crude coefficient of .003 ($P = .017$) (Figure 2).

Higher *p,p′*-DDE levels were associated with lower sperm count, semen volume, sperm density, viability, and higher percentage of sperm neck defects. However, none of these associations was statistically significant in correlations or crude or adjusted linear regressions. In the case of volume, a subject with the highest *p,p′*-DDE level and only one semen sample was eliminated, since its inclusion alone turned the direction of the association positive.

According to the Aniline Blue staining test, changes in nucleoprotein composition took place (>25% Grade 2 + 3) in 46.6% (54/116) of participants. The WHO (1999) reference value is less than 25% Grade 2 + 3 staining, indicating excessive improper sperm DNA condensation. Furthermore, the percentage of Grade 3–stained samples (the least condensed sperm) was positively correlated to *p,p′*-DDE concentration: there is an 0.04% increase in Grade 3 staining per unit of lipid adjusted *p,p′*-DDE (Table 3). The mean α-glucosidase concentration in the semen was lower than the reference value set by the WHO (1992, 1999), 20 mU/ejaculate (Table 3), although it was not affected by DDT exposure.

The correlation between each seminal parameter and the measured covariates is shown in Table 4. In general, age was the most important covariate, being negatively and significantly associated with several motility parameters and with the percentage of normal sperm. Polymorphism of the androgen receptor (CAG) was not correlated to any of the seminal parameters (Table 4), and it was also not a significant covariate when added to regression models of the association between these parameters and *p,p′*-DDE (data not shown). Since only 1 or 2 covariates were associated with some seminal parameters (Table 4) and their inclusion did not change the meaning of the findings, multivariate analysis is not shown.

Some participants had very poor semen characteristics compared to others that appeared normal. To test the hypothesis that the aberrant seminal characteristics are linked to DDT exposure, the participants were divided into groups according to abnormal sperm characteristics, as described by the WHO (1999). The results of separate group correlations with *p,p′*-DDE and logistic regression comparisons between normozoospermic samples and oligo-, astheno-, and teratozoospermia semen samples are summarized in Table 5. Total sperm counts in the oligozoospermic group correlated positively with *p,p′*-DDE.

Table 4. *Correlations between covariates and seminal parameters from the 116 men (probability values indicated in parentheses)*

Semen Parameter	CAG	Age, y	Sexual Abstinence, d	Smoking Cigarettes/d	Body Mass Index, kg/cm²	Age of Puberty Onset, y	Intercourse Frequency, Times/mo
Volume, mL*	.03 (.77)	.16 (.09)	.06 (.55)	.07 (.46)	.07 (.51)	−.11 (.25)	−.13 (.17)
Total sperm count, $\times 10^6$ sperm/ejaculate*	.07 (.47)	−.01 (.95)	.15 (.11)	.08 (.42)	−.08 (.47)	−.15 (.13)	−.13 (.17)
Sperm concentration, $\times 10^6$ sperm/mL*	.02 (.80)	−.10 (.28)	.25 (.01)	.02 (.81)	−.11 (.29)	.01 (.94)	−.05 (.58)
Viability		−.18 (.06)	.02 (.80)	−.01 (.94)	.05 (.62)	.03 (.78)	.07 (.46)
Rapid progressive motility, % Grade a	−.13 (.21)	−.18 (.09)	.08 (.46)	−.04 (.70)	−.03 (.80)	−.06 (.60)	−.10 (.32)
Progressive motility, % Grades a+b	.08 (.38)	−.31 (.001)	.02 (.86)	−.09 (.32)	.13 (.24)	−.07 (.51)	.03 (.76)
Motility, % Grades a+b+c	.08 (.39)	−.28 (.002)	.03 (.78)	−.11 (.23)	.11 (.31)	−.03 (.75)	.002 (.98)
Morphology, % normal	−.02 (.82)	−.18 (.05)	.07 (.48)	−.08 (.40)	−.13 (.24)	.04 (.72)	−.12 (.20)
Morphology, % abnormal heads	−.03 (.75)	−.05 (.60)	−.04 (.70)	−.10 (.30)	.11 (.33)	−.07 (.46)	.09 (.36)
Morphology, % abnormal necks	.08 (.39)	.16 (.08)	.01 (.89)	.12 (.22)	.03 (.79)	.24 (.02)	−.01 (.94)
Morphology, % abnormal tails	−.06 (.56)	.04 (.65)	.11 (.25)	.05 (.63)	−.17 (.11)	−.12 (.22)	−.09 (.37)
Morphology (.TZI)†	−.10 (.21)	.13 (.17)	−.07 (.45)	.003 (.97)	.08 (.45)	.19 (.05)	−.001 (.99)

* Square-root transformed to normalize.
† 1/Square-root transformed to normalize.

No correlation was found in the group with normal sperm counts. Sperm morphology in the teratozoospermic group and sperm motility in the asthenozoospermic group both correlated negatively with *p,p′*-DDE. No associations with *p,p′*-DDE were found in the groups with normal morphology and motility. There were also no significant associations indicating a higher risk of astheno- or tetatozoospermia with increasing *p,p′*-DDE levels in the logistic regressions done (not shown). Although the logistic regression analysis on volume indicates that there might be a weak relation, the risk of being oligospermic increased 1% per unit of lipid adjusted *p,p′*-DDE, with a borderline statistical significance of *P* equals .09.

Discussion

This study provides evidence that, as measured by plasma *p,p′*-DDE concentrations, nonoccupational exposure to the organochlorine insecticide DDT is associated with reduced seminal quality in a population of men in rural Mexico (Chiapas). The major findings in the semen analyses included poor sperm motility, abnormal sperm morphology, and inadequate sperm chromatin condensation in the presence of increasing levels of *p,p′*-DDE in serum lipids. *p,p′*-DDE concentration in plasma lipids is 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.

We also considered that polyglutamine polymorphism of the androgen receptor gene could be associated with semen parameters and/or any relationship among these parameters and *p,p′*-DDE. Indeed, the number of polyglutamines in the androgen receptor protein has been reported to influence individual risk of breast cancer in women via an increased sensitivity to androgens (Giguère et al, 2001). However, there was no association among the reproductive parameters, exposure to DDT, and polyglutamine polymorphism of the androgen receptor gene, indicating that this polymorphism is not implicated in the response to *p,p′*-DDE either in this population or at this level of exposure.

In this population, the mean *p,p′*-DDE concentration adjusted for total lipids was 45 plus or minus 32 μg/g lipid, about 100 times the value found in an apparently nonexposed group of women (Smith, 1999). A pilot study on a small group of men (n = 24) performed 2 years earlier in Chiapas reported a mean *p,p′*-DDE concentration of 77.9 mg/kg (range = 17.0–177.2; Ayotte et al, 2001). Although this difference could be interpreted as a positive outcome of the newly adopted DDT phase out, the impact of other factors should be considered for the apparent drop in *p,p′*-DDE levels. In the present study, different communities participated (compared to the pilot

Table 5. *Levels of dichotomized sperm parameters (according to WHO [1999] classifications) and their associations with lipid-adjusted p,p′-DDE (μg/g) in 116 men*

	Sperm Parameter	N	Mean (SD)	Median	Pearson Correlation (*P*)
Count	Oligozoospermia $<40 \times 10^6$ sperm/ejaculate	28	2.28×10^7 (1.3×10^7)	2.52×10^7	.335 (.08)
	Normozoospermia $\geq 40 \times 10^6$ sperm/ejaculate	88	1.77×10^8 (1.2×10^8)	1.45×10^8	.030 (.78)
Morphology	Teratozoospermia % normal <5%	28	2.73 (1.1)	3	−.408 (.03)
	Normozoospermia % normal ≥5%	88	138 (6.0)	9	−.038 (.74)
Motility	Asthenozoospermia Grade a+b <50%	38	36.15 (11.3)	39	−.387 (.02)
	Normal motility Grade a+b ≥50%	78	61.08 (5.7)	61.5	−.080 (.50)

study), and the use of DDT may not necessarily be similar. Regardless, the current levels of *p,p′*-DDE in human tissue and the environment remain high in Chiapas.

The results obtained from the complete semen analysis and motility parameters permitted evaluating the testicular function in the subjects. The parameters assessed reflect the integrity of the germinal line of the seminiferous tubules, which produce the male gametes, and thus provide information on the effectiveness of spermatogenesis. The production of functional male gametes is dependent of a series of complex modifications that cannot be completely analyzed by these tests (Schrader et al, 1992). However, other andrological tests, such as the seminal volume and pH and the α-glucosidase assay, were performed to evaluate the characteristics of the sperm and posttesticular male reproductive tract. A total of 33.6% of the participants from the exposed communities were smokers. As smoking is thought to affect sperm characteristics (Sofikitis et al, 1995; Kunzle et al, 2003), it was initially included in the statistical models so as not to bias the effects of DDT, even though the average number of cigarettes smoked was only 2.6 per day. However, smoking was not associated with seminal parameters.

There were negative associations between sperm motility (both percentages of total and progressive motility, or Grades a + b + c and a + b, respectively) and *p,p′*-DDE concentration, indicating the percentage of motile sperm decreases as *p,p′*-DDE increases. Thirty-eight cases (32.8%) presented asthenozoospermia (impaired sperm motility), and in this particular subgroup, good progressive motility correlated negatively and significantly with the *p,p′*-DDE concentration. The computer-assisted sperm analysis (CASA) results for sperm motility parameters confirmed the decreased percentage of progressively motile sperm observed on the wet preparations. CASA is being used increasingly in reproductive toxicology, as some CASA parameters are sensitive to reproductive toxicants (European Society of Human Reproduction and Embryology–Special Interest Group on Andrology, 1998). Nontransformed variables, according to Spearman's correlations, had a negative association with *p,p′*-DDE. A tendency toward weak correlation was found for the curvilinear velocity (VCL; $r = -.23$; $P = .02$) indicating the time average velocity of a sperm head along its actual curvilinear trajectory. Straight-line velocity (VSL) also was significantly negatively correlated with *p,p′*-DDE concentration (VSL; $r = -.19$; $P = .05$). These findings imply that DDT exposure decreases the velocity of the sperm head along its curvilinear and average paths, thereby affecting motility.

A major challenge of morphological assessment of sperm is the pleomorphism of human spermatozoa. A significant proportion of sperm in each sample may be morphologically abnormal (teratozoospermia), but if this proportion is above 5%, this may account for impaired fertility (Menkveld et al, 1990). In this study group, 8.4% plus or minus 6% of sperm were classified as morphologically normal sperm, which is well below the cut-off value of 30% (WHO, 1992). Below 15% normal forms might reduce fertilization rates in vitro (WHO, 1999). Although no association was established between sperm head and neck defects, the percentage of tail defects increased with increasing *p,p′*-DDE levels. In participants with severe teratozoospermia (<5% morphologically normal sperm; n = 28) the levels of *p,p′*-DDE values were higher and correlated negatively with the percentage normal sperm ($r = -0.387$; $P = 0.016$).

In a normal ejaculate, less than or equal to 25% of the sperm should be classified as Grades 2 and 3 according to Aniline Blue staining for chromatin condensation (Foresta et al, 1992). A high percentage of chromatin defects of sperm nuclei (>25% Grades 2 and 3) relate to the nucleoprotein content of DNA (incomplete or defective sperm DNA condensation) and, therefore, will have a negative effect on sperm function. The combined effect on Grades 2 and 3 staining was calculated to be positive in 46.6% of the cases (n = 54). In this study, the mean Grades 2 plus 3 (combined) was 23%, which was still

within normal limits for the test, but toward the cut-off limit of 25%. Furthermore, those sperm that would theoretically be most severely affected with insufficient condensation (Grade 3) correlated significantly positively with the *p,p′*-DDE concentrations. This might indicate a subtle effect, one that, if it persisted, would in time have a negative impact on fertility, as Aniline Blue staining provides an indication of male fertility potential (Foresta et al, 1992).

It is, therefore, possible that DDT exposure (reflected by *p,p′*-DDE levels in the blood) detrimentally affected the quality of sperm motility by several different mechanisms. Higher levels of *p,p′*-DDE were observed in cases with impaired sperm motility, and the high frequency of morphological tail defects in the sperm (positively associated to *p,p′*-DDE) might also explain part of the impaired motility. Furthermore, subchronic DDT exposure is associated with an increase in free radical generation by lipid peroxidation (Koner et al, 1998). It is well established that reactive oxygen species impair sperm motility (Aitken et al, 1998) and could represent another mechanism by which DDT exposure leads to reduced sperm motility.

Both DDT and *p,p′*-DDE are considered to be hormonally active, with DDT having estrogenic activity via binding and activation of the estrogen receptor and DDE being antiandrogenic (Kelce et al, 1995). Regardless 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 impeded androgen action (via DDE's effects on the androgen receptor)—the physiological consequence would be impaired Sertoli cell function (Parvinen, 1998). The primary role of these cells is to support spermatogenesis. Therefore, the abnormal sperm function and structure observed in the present study in the presence of increasing plasma *p,p′*-DDE could be because the affected Sertoli cells are unable to maintain normal spermatogenesis.

It appears that the DDE could be, at least in part, responsible for the low-normal sperm motility, impaired sperm morphology, and abnormal sperm condensation. Any antiandrogenic effect on spermatogenesis will possibly be more pronounced in the later stages of spermatogenesis or during the spermiogenic phase in which spermatids transform into spermatozoa structurally equipped to reach and fertilize the egg (Parvinen, 1998). During spermiogenesis, spermatids are transformed to sperm by processes including condensation and structural shaping of the cell nucleus, the formation of a flagellum, and the expulsion of a large part of cytoplasm. Any effect on this stage of development may become evident in impaired sperm condensation, motility, and morphology, as were seen in this study group.

Crude results rather than multivariate results were generally reported in this article because little or no extra information was added to the models by other covariates. This most likely reflects the small variance in participants' characteristics in combination with little possible influence on seminal parameters.

Since many outcomes were evaluated, the possibility of significant results due to chance (type I error) cannot be ruled out. However, our results are biologically plausible and in agreement with those of other studies (Guillette et al, 1995; Kelce, 1995; Toppari et al, 1996; Ayotte et al, 2002). On the other hand, the relatively small sample size might have provided insufficient power to detect truly significant associations (type II error). This could be the case with regard to the negative, but not significant, association between *p,p′*-DDE and seminal volume.

Conclusions

The present results provide sufficient evidence for concern that male reproductive health and fertility potential is influenced by nonoccupational, subchronic DDT exposure. The mean age of the participants was 27 years, and they have been living in the study area for 15 years. It is impossible to predict what long-term effect would be evident in, for example, another 10 years. However, in view of the apparent lack of control over individual use of DDT, male fertility potential could well be compromised. The duration of time to achieve pregnancy in this study group would provide additional information to elucidate the clinical impact of DDT on male reproduction.

The subtle but apparent effect on reproductive parameters of the participants living in a DDT-exposed area may have far-reaching implications for human health. The sample size of the present study is relatively small, which might have limited the statistical power of our multivariate regression analyses to detect all possible significant effects of DDT exposure. Despite this limitation, together with well-documented effects of DDT on animals (Toppari et al, 1996), this study provides sufficient evidence to be concerned about the impact of this pesticide and its major 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 tissues. Other effects may occur in the young, as they seem to be the most vulnerable (Longnecker et al, 2000). Indeed, it is not the amount of DDT to which a 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). Finally, these findings are not only applicable to populations in rural Mexico, but also to other populations in other de-

veloping countries that still depend mainly on DDT for malaria control. Clearly, even nonoccupational exposure to DDT has adverse consequences on male reproductive health, and thus the development of alternative methods of pest management should be encouraged.

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