Serum Organochlorine Pesticide Residues and Risk of Testicular Germ Cell Carcinoma: A Population-Based Case-Control Study

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Abstract

Testicular germ cell carcinoma (TGCC) is the most common malignancy among men ages 20 to 34 years. Although the pathogenesis of TGCC is poorly understood, suboptimal androgen levels or impaired androgen signaling may play a role. Some persistent organochlorine pesticides commonly found in human tissue possess antiandrogenic properties. We examined whether the risk of TGCC is associated with serum levels of 11 organochlorine pesticides, including *p,p′*-DDE, and whether the *p,p′*-DDE-TGCC association is modified by CAG or GGN repeat polymorphisms in the *androgen receptor* gene. We conducted a population-based case-control study among 18- to 44-year-old male residents of three Washington State counties. Cases (n = 246) were diagnosed during 1999 to 2003 with a first, primary TGCC. Controls (n = 630) were men of similar age with no history of TGCC from the same population identified through random-digit telephone dialing. Questionnaires elicited information on demographic, medical, and lifestyle factors. A blood specimen provided serum for gas chromatography-high-resolution mass spectrometry analysis of organochlorine pesticide residues and DNA for genotyping. We observed no clear patterns between TGCC risk and concentrations of any of the organochlorines measured, nor did we observe that the risk associated with *p,p′*-DDE was modified by *androgen receptor* CAG (<23 versus ≥23 repeats) or GGN (<17 versus ≥17 repeats) genotype. This study does not provide support for the hypothesis that adult exposure to organochlorine pesticides is associated with risk of TGCC. Due to uncertainty regarding how well organochlorine levels measured in adulthood reflect exposures during early life, further research is needed using exposure measurements collected *in utero* or during infancy. (Cancer Epidemiol Biomarkers Prev 2008;17(8):2012–8)

Introduction

The incidence of testicular germ cell carcinoma (TGCC), the most common malignancy among men ages 20 to 34 years, has increased several-fold since the 1940s in many western countries (1-3). The reason for the increase in incidence is unknown, but the rapid nature of the increase, and the apparent recent plateauing in some populations (4-7), suggests a role for a widespread, temporally varying, environmental exposure.

Although the pathogenesis of TGCC is poorly understood, risk factors such as undescended testes, reduced fertility and semen quality, and testicular atrophy, and the apparent absence of these cancers in men with congenital androgen insufficiency, point to suboptimal androgen levels or impaired androgen signaling as possibly playing a role (8). Several persistent organochlorine pesticides commonly found in human tissue samples possess antiandrogenic properties (9-14), with the strongest evidence for *p,p′*-DDE, which is a potent androgen receptor (AR) antagonist *in vitro* and *in vivo* (15-21). It has been hypothesized that exposure to certain environmental contaminants could increase the risk of TGCC by interfering with normal functioning of endocrine pathways (22), but to date, only a single small epidemiologic study has examined this hypothesis (23).

We conducted a population-based case-control study to determine whether serum organochlorine pesticide concentrations are associated with the risk of TGCC. We studied 11 of the most common persistent organochlorine pesticide residues in the U.S. population, including *p,p′*-DDE, which is detectable in the blood of nearly all individuals (24). Because of the strong evidence that *p,p′*-DDE acts as an AR antagonist, we also examined whether variation in the number of CAG and/or GGN repeats in the *AR* gene (25-27) modified the risk of TGCC associated with *p,p′*-DDE.

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Materials and Methods

Study Population. The Adult Testicular Lifestyle and Blood Specimen (ATLAS) Study is an ongoing molecular epidemiologic investigation of risk factors for TGCC among 18- to 44-year-old male residents of King, Pierce, and Snohomish counties, Washington State. Eligible ATLAS cases are men (*a*) diagnosed with a first, invasive TGCC between January 1, 1999 and December 31, 2008 and (*b*) who had a residential telephone at diagnosis (because controls are ascertained through random-digit telephone dialing). We identify potentially eligible cases from the files of the population-based Cancer Surveillance System, a part of the Surveillance, Epidemiology and End Results Program of the National Cancer Institute, based on the following *International Classification of Diseases for Oncology* topography and histology codes: topography C62.0 to C62.9 and histology 9060 to 9091 (28). The ancillary ATLAS Organochlorine Study included only cases diagnosed through December 31, 2003. During that period, 397 eligible cases were identified, of whom 272 (68.5%) were recruited into the study. The reasons that eligible cases did not participate were subject refusal (n = 48), subject could not be located (n = 38), subject had moved from the study region (n = 25), physician refused permission to contact subject (n = 10), and subject death before recruitment (n = 4).

Controls were men with no history of TGCC, frequency matched to the cases on 5-year age group and residing in the any of the same three counties as the cases during the case diagnosis period, who were identified using random-digit telephone dialing (29, 30). The random-digit telephone dialing household screening response was 84.2%, and of the 1,377 apparently eligible controls identified, 1,187 allowed us to send them a recruitment letter. Among these latter men, 726 (61.2%) were recruited into the study. The reasons that eligible controls did not participate were subject refusal (n = 372), subject could not be located (n = 57), subject had moved from the study region (n = 31), and subject death before recruitment (n = 1).

Interviews. After providing signed informed consent, cases and controls were interviewed in-person using a structured questionnaire. All questions referred to the period before each man's reference date. For each case, the reference date was the month and year of his TGCC diagnosis. Each control was assigned a reference date selected at random from among all possible dates given the distribution of diagnosis years of cases identified as of the time of selection of the control via random-digit telephone dialing. Information collected during the interview included demographic characteristics, medical history, physical characteristics, and other suspect TGCC risk factors. Before the in-person interview, each participant was asked to complete self-administered questionnaires eliciting information on family history of cancer and prenatal and postnatal history, including information on breast-feeding during infancy. At the time of blood specimen collection, interviewers administered to each participant a brief in-person questionnaire that elicited information on his current health status and other characteristics that were known or suspected of influencing organochlorine concentrations [current weight, frequency of consumption of dark-fleshed fish (such as salmon and tuna) since reference date, and receipt of chemotherapy treatment (yes/no and date of most recent treatment)] or otherwise might affect the quality or composition of the specimen.

Blood Collection. At the time of interview, each case and control was asked to provide a venous blood sample that was processed into serum, plasma, and peripheral leukocytes. Of the 272 cases and 726 controls who were enrolled in the ATLAS Study through December 2003, 259 and 659, respectively, provided blood specimens. Blood samples were drawn a median of 23.1 and 14.8 months following reference date for controls and cases, respectively. All cases had completed the first course of cancer treatment at the time of the specimen collection. We drew one extra blood sample from a random subset of participants to serve as quality-control (QC) replicate samples. All blood aliquots were stored at −70°C.

Organochlorine Analysis. Serum samples were analyzed for organochlorine residues at the National Center for Environmental Health, Centers for Disease Control and Prevention. Over the course of the study, serum samples were shipped frozen to the National Center for Environmental Health at regular intervals and analyzed as samples were received. In each shipment, serum aliquots (4 mL) were arranged in analytic sets of 19 samples (''unknowns'') from ATLAS subjects consisting of (*a*) randomly ordered case and control samples in ~1:3 ratio and (*b*) between one and three QC replicate aliquots. In addition to replicate serum aliquots from participants that were inserted into different analytic sets to assess between-run variation, we also included two aliquots of pooled QC serum (created from nonparticipant volunteers) in each shipment (consisting of between 9 and 12 analytic sets) to monitor analytic drift over the course of the study.

Concentrations of pesticides [β-hexachlorocyclohexane, γ-hexachlorocyclohexane (lindane), dieldrin, hexachlorobenzene, heptachlor epoxide, mirex, *p,p*′-DDT, *o,p*-DDT, *p,p*′-DDE, oxychlordane, and *trans*-nonachlor] as well as 36 polychlorinated biphenyl congeners were measured using accelerated solvent extraction and a combined polychlorinated biphenyl/organochlorine pesticide gas chromatography-high-resolution mass spectrometry analysis as described previously (31). We report the results of the organochlorine pesticide analyses here. Each analytic run consisted of 26 samples: 19 unknowns (samples from cases and controls, QC replicates, or pooled QC replicates), 2 laboratory QC samples, 2 blanks, 2 recovery reference samples, and a replicate of 1 of the 19 unknowns to assess within-run variation. Staff conducting the analyses and overseeing the calculation of the analyte concentrations from the gas chromatography-high-resolution mass spectrometry was blinded to the identity of the unknowns from subjects and QC aliquots. Mean limits of detection corrected for extraction recovery were ~20 pg/g for dieldrin, oxychlordane, and heptachlor epoxide and 14 pg/g for the other pesticides. To identify samples that may have had errors in organochlorine quantification due to interfering compounds, ion ratios (IR) reflecting the level of chlorination were calculated for

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each target analyte and compared with the expected IR for that analyte. Observed IRs that were outside ±20% of the expected IR were flagged as being out of tolerance. In addition to indicating possible interference with nontarget compounds, IRs that are different from expected can occur when the measured analyte concentration is close to the limit of detection. We therefore excluded from the analysis those organochlorine measurements that were flagged as having an IR that was out of tolerance if the measured concentration was well above the limits of detection (defined as >50 pg/g for dieldrin, oxychlordane, and heptachlor epoxide and >40 pg/g for the other pesticides).

Total cholesterol, free cholesterol, triglycerides, and phospholipids were determined for each serum aliquot using standard enzymatic methods (32) and used to calculate an estimate of total serum lipids (33).

Eight participant serum specimens were not sent for analysis either because they were collected after the final laboratory shipment or because they had insufficient volume for organochlorine analysis. Of the 988 total specimens shipped to the laboratory, organochlorine levels were not reported for 41 (9 cases, 24 controls, and 8 QC specimens) due to loss of the sample during analysis or insufficient recovery ($n = 36$) or matrix effects ($n = 5$). Organochlorine measurements were done on 247 cases, 630 control, and 70 QC specimens.

AR Microsatellite Repeat Polymorphisms. The number of CAG and GGN repeats in the *AR* gene was determined by a PCR-based fragment analysis as described previously (34). Briefly, the gene fragments containing these microsatellites were amplified in separate reactions, fluorescently labeled PCR products from the same individual were pooled and diluted, and a small aliquot was added to a mixture of formamide and GeneScan-500 LIZ size standard (Applied Biosystems). The samples were then denatured and analyzed by capillary electrophoresis on an Applied Biosystems Genetic Analyzer 3100. The peaks from cases and controls were compared with peaks of known GeneScan-500 size standards and against cloned and sequenced standards. Every set of 96 injections included three clone standards along with a negative control at the beginning and end of each run to control for plate-to-plate variation of allele calling. GeneMapper 4.0 software was used to assign repeat number based on size bins relative to the clone standards. Laboratory personnel were blind to the characteristics of the study samples, including which corresponded to cases, controls, or QC replicates. These three types of samples were interspersed throughout the reaction plates.

Statistical Analyses. To allow the use of continuous variables in statistical modeling, we imputed values for samples with nondetectable analytes using an unbiased multiple-imputation procedure (35). In brief, the imputation method is applied by fitting a parametric distribution to the known data for an analyte then using the fitted distribution to draw a random value for each of the nondetects, conditional on the value being between zero and the limit of detection. Preliminary analyses, including evaluation of histograms and *q-q* plots of log-transformed concentrations, indicated that the observed organochlorine concentrations were consistent with a log-normal distribution. We created five complete data sets consisting of the observed and imputed values and used the five data sets to compute multiple-imputation estimates of regression variables and valid variance estimates (36). Simulations have shown that this imputation procedure generates unbiased estimates of the mean, and 95% confidence intervals (95% CI) close to the nominal level, for samples sizes of 200 to 400, even when the percentage of nondetects is as high as 60% to 70% (35).

To assess the reliability of serum organochlorine measurements, we calculated intraclass correlation coefficients (ICC) for within-run and between-run replicates. Because the between-run ICCs were quite low for many of the pesticides, indicating poor between-run reliability, we adjusted for run number in multivariable analyses.

We computed a summary measure of chlordane-derived compounds by summing concentrations of oxychlordane, *trans*-nonachlor, and heptachlor epoxide. We estimated the relative risk of TGCC associated with analyte levels using conditional logistic regression to calculate odds ratios (OR) and 95% CI, conditioning on the assay run number. For the primary analysis, we categorized analyte concentrations *a priori* as low (<50th percentile), medium (50-85th percentile), and high (>85th percentile), with the percentiles based on the distribution of analytes among the controls, based on the hypothesis that associations between analytes and TGCC risk would be limited to men with the highest exposures. However, because categorization of a continuous exposure results in the loss of information, we also modeled organochlorine levels as a continuous variable in secondary analyses.

To identify characteristics associated with organochlorine levels, we selected a single organochlorine (*p,p′*-DDE) and calculated least-squares adjusted mean concentrations by categories of age at reference date, race, body mass index (BMI), change in BMI, breast-feeding during infancy, birthplace, and frequency of consumption of dark-flesh fish.

Multivariate models fit to estimate the relative risk of TGCC associated with analyte levels were adjusted for age at reference date (18-24, 25-29, 30-34, 35-39, 40-44 years), race (White, non-White), change in BMI (kg/m^2) between reference date and blood draw date (<-2, -2 to +2, >+2), and total serum lipids. Because weight change between reference date and the blood draw was strongly associated with pesticide levels among ATLAS participants and differed according to case-control status, we repeated the analyses in the subset of participants that were relatively weight stable (change in BMI of not more than 2 kg/m^2). We also repeated the analyses excluding men treated with chemotherapy, which may increase or decrease serum organochlorine levels (37, 38).

To evaluate whether the risk of TGCC associated with *p,p′*-DDE was modified by *AR* genotype, we fit conditional logistic regression models containing multiplicative interaction terms. To test the statistical significance of the analyte-genotype interaction, we used the likelihood ratio test to compare models with and without the interaction term.

We used SAS (SAS System for Windows version 8.2; SAS Institute) for the imputation procedure and Stata (Stata Statistical Software version 9; StataCorp) for all other analyses. All statistical tests were two-sided.

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Table 1. Selected characteristics of ATLAS TGCC cases and controls

Characteristics	Controls (n = 630), n (%)	Cases (n = 246), n (%)
Age		
18-24	74 (11.8)	33 (13.4)
25-29	80 (12.7)	44 (17.9)
30-34	151 (24.0)	54 (22.0)
35-39	158 (25.1)	57 (23.2)
40-44	167 (26.5)	58 (23.6)
Race		
White	576 (91.4)	241 (98.0)
African American	13 (2.1)	0 (0)
Asian	24 (3.8)	4 (1.6)
Other	17 (2.7)	1 (0.4)
History of undescended testes		
No	614 (97.5)	221 (91.0)
Yes	16 (2.5)	22 (9.1)
Family history TGCC in first-degree relative		
No	548 (87.0)	211 (85.8)
Yes	3 (0.5)	6 (2.4)
Unknown*	79 (12.5)	29 (11.8)
Education		
High school or less	151 (24.0)	70 (28.5)
College/trade school	385 (61.1)	151 (61.4)
Graduate school	94 (14.9)	25 (10.2)
Birthplace		
North America	576 (91.4)	234 (95.1)
Outside North America	54 (8.6)	12 (4.9)
Breast-fed as an infant		
No	237 (37.6)	106 (43.1)
Yes	318 (50.5)	111 (45.1)
Unknown†	75 (11.9)	29 (11.8)
BMI (kg/m^2)		
18-24	239 (38.0)	96 (39.0)
25-29	281 (44.6)	108 (43.9)
≥30	110 (17.5)	42 (17.1)
BMI change‡ (kg/m^2)		
>-2	23 (3.7)	17 (7.0)
-2 to +2	466 (74.3)	182 (74.9)
>+2	138 (22.0)	44 (18.1)

*Includes participants that did not know family history (41 controls and 18 cases) and those that did not complete family history questionnaire (38 controls and 11 cases).
† Includes participants that did not know breast-feeding history (47 controls and 20 cases) and those that did not complete natal questionnaire (28 controls and 9 cases).
‡ Change in BMI between reference date and blood draw date.

The ATLAS Study protocol was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

Results

Compared with controls, TGCC cases were more likely to be White, to have a history of undescended testes, to have a family history of TGCC, and to have lost weight between their reference date and the date of the study blood draw (Table 1). Cases were somewhat less likely to be educated beyond high school, to have been born outside North America, and to have been breast-fed as infants. BMI was similar in cases and controls.

Median concentrations, interquartile ranges, proportion of nondetects and IR-excluded measurements, and within-run and between-run ICCs from QC specimens for each of the measured pesticide residues are shown in Table 2. Lipid-standardized values are also provided for comparison with other studies. Within-run ICCs were >0.90 for the majority of pesticides measured, but between-run ICCs were substantially lower. Concentrations of *p,p′*-DDE among controls increased with increasing age and were appreciably higher in African American and Asian men compared with White men, overweight and obese men compared with normal weight men, men who had been breast-fed as infants, and men born outside North America (results not shown).

The risk of TGCC was similar across categories of serum pesticide concentrations with no clear evidence of a trend with increasing serum pesticide levels (Table 3). OR estimates based on continuous measures also showed no meaningful associations between TGCC risk and serum pesticide levels, with the exception of γ-hexachlorocyclohexane; a 10 pg/g increase in γ-hexachlorocyclohexane was associated with an OR for TGCC of 5.54 (95% CI, 1.65-18.56). Results were similar when the analysis was restricted to weight-stable men, to men not treated with chemotherapy, to White men, or to men born within North America (results not shown).

There was no evidence that differences in number of *AR* CAG or GGN repeats modified the association between *p,p′*-DDE and risk of TGCC (Table 4).

Table 2. Serum concentrations of organochlorine pesticide residues in ATLAS Study participants (n = 876) expressed as whole weight (pg/g serum) and lipid-standardized values (ng/g lipid)

Pesticide	% Nondetects	%IR*	ICC		Median (IQR)†	
			Within run	Between run	Concentration, pg/g serum	Concentration, ng/g lipid
β-hexachlorocyclohexane	5.0	9.8	0.90	0.66	29.47 (19.64-50.66)	4.32 (2.89-6.52)
γ-hexachlorocyclohexane	23.9	1.5	0.58	0.42	9.58 (6.71-15.11)	1.37 (0.89-2.23)
Dieldrin	19.8	6.5	0.42	0.16	51.07 (26.94-76.01)	7.33 (4.13-10.38)
Hexachlorobenzene	0.0	0.2	0.90	0.33	186.34 (114.81-348.04)	25.46 (15.95-48.86)
Heptachlor epoxide	10.0	8.6	0.93	0.48	26.74 (17.38-43.74)	3.96 (2.64-5.75)
Mirex	4.3	1.0	0.92	0.62	11.65 (6.58-20.73)	1.62 (0.93-2.77)
p,p′-DDT	6.7	4.4	0.91	0.25	27.27 (18.62-38.27)	3.78 (2.72-5.28)
o,p-DDT	42.3	9.0	0.68	0.57	5.72 (2.65-10.45)	0.79 (0.37-1.49)
p,p′-DDE	0.0	0.9	0.97	0.66	1,089.10 (722.70-1,770.92)	153.28 (108.16-230.35)
Oxychlordane	13.1	4.6	0.91	0.52	42.86 (26.32-71.13)	6.15 (3.95-9.43)
trans-nonachlor	0.1	0.0	0.93	0.66	72.84 (52.43-112.74)	10.50 (7.74-15.26)

*IR out of tolerance indicating possible interference.
† Interquartile range.

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Table 3. Association between serum organochlorine pesticide concentrations and risk of TGCC

	Low	Medium	High	P_{trend}*	per 10 pg/g†
β-hexachlorocyclohexane					
Concentration (pg/g)	≤29	>29-65	>65		
Cases/controls (*n*)	113/285	83/200	25/85		
OR‡ (95% CI)	1.00 (reference)	1.26 (0.86-1.85)	0.92 (0.51-1.64)	0.83	0.97 (0.82-1.13)
γ-hexachlorocyclohexane					
Concentration (pg/g)	≤9	>9-20	>20		
Cases/controls (*n*)	130/312	73/217	38/93		
OR‡ (95% CI)	1.00 (reference)	0.80 (0.53-1.20)	1.36 (0.75-2.46)	0.69	5.54 (1.65-18.56)
Dieldrin					
Concentration (pg/g)	≤51	>51-98	>98		
Cases/controls (*n*)	116/295	86/207	29/88		
OR‡ (95% CI)	1.00 (reference)	1.00 (0.68-1.47)	0.79 (0.44-1.41)	0.53	0.78 (0.53-1.17)
Hexachlorobenzene					
Concentration (pg/g)	≤190	>190-473	>473		
Cases/controls (*n*)	139/315	71/220	35/94		
OR‡ (95% CI)	1.00 (reference)	0.79 (0.47-1.32)	0.85 (0.37-1.96)	0.56	1.05 (0.95-1.15)
Heptachlor epoxide					
Concentration (pg/g)	≤26	>26-59	>59		
Cases/controls (*n*)	113/287	91/200	24/85		
OR‡ (95% CI)	1.00 (reference)	1.25 (0.85-1.83)	0.67 (0.35-1.29)	0.69	1.04 (0.48-2.25)
Mirex					
Concentration (pg/g)	≤11	>11-28	>28		
Cases/controls (*n*)	125/311	89/221	28/93		
OR‡ (95% CI)	1.00 (reference)	1.27 (0.86-1.86)	0.87 (0.50-1.53)	0.98	1.38 (0.66-2.88)
p,p′-DDT					
Concentration (pg/g)	≤27	>27-47	>47		
Cases/controls (*n*)	110/301	94/210	32/90		
OR‡ (95% CI)	1.00 (reference)	1.39 (0.96-2.02)	1.17 (0.68-2.00)	0.30	1.14 (0.56-2.32)
o,p-DDT					
Concentration (pg/g)	≤5	>5-13	>13		
Cases/controls (*n*)	104/290	83/200	37/83		
OR‡ (95% CI)	1.00 (reference)	1.26 (0.83-1.91)	1.30 (0.67-2.53)	0.28	0.86 (0.33-2.26)
p,p′-DDE					
Concentration (pg/g)	≤1,101	>1,101-2,473	>2,473		
Cases/controls (*n*)	130/311	94/218	21/93		
OR‡ (95% CI)	1.00 (reference)	1.14 (0.78-1.67)	0.61 (0.32-1.14)	0.36	0.99 (0.97-1.01)
Oxychlordane					
Concentration (pg/g)	≤43	>43-97	>97		
Cases/controls (*n*)	121/299	87/209	29/89		
OR‡ (95% CI)	1.00 (reference)	1.18 (0.80-1.73)	0.93 (0.50-1.73)	0.89	0.97 (0.58-1.63)
trans-nonachlor					
Concentration (pg/g)	≤72	>72-146	>146		
Cases/controls (*n*)	124/315	91/221	31/94		
OR‡ (95% CI)	1.00 (reference)	1.16 (0.80-1.69)	0.89 (0.49-1.61)	0.99	0.87 (0.63-1.19)
Total chlordanes§					
Concentration (pg/g)	≤144	>144-283	>283		
Cases/controls (*n*)	120/315	93/221	33/94		
OR‡ (95% CI)	1.00 (reference)	1.18 (0.81-1.71)	0.93 (0.51-1.68)	0.88	0.94 (0.79-1.12)

*Trend test done by assigning ordinal scores to pesticide categories and modeling as continuous term.
† Except *p,p*′-DDE, which is per 100 pg/g.
‡ Adjusted for age, race, change in BMI between reference date and blood draw, assay run number, and serum lipids.
§ Sum of chlordane-derived compounds including oxychlordane, *trans*-nonachlor, and heptachlor epoxide.

Discussion

We found little evidence to support the hypothesis that serum levels of 11 organochlorine pesticide residues measured in adulthood are associated with risk of TGCC. The observed positive association between the continuous measure of γ-hexachlorocyclohexane and risk of TGGC was not confirmed by the categorical analysis and may have been a chance finding resulting from the numerous comparisons made. We also found no evidence that the risk of TGCC associated with serum DDE was modified by the number of *AR* CAG or GGN repeats.

Our results are generally consistent with those of a smaller case-control study of 58 Swedish TGCC cases and 61 age-matched controls that found no statistically significant associations between many of the same pesticides and risk of TGCC (23). It is noteworthy that concentrations of the pesticides measured in common between the two studies were very similar. We did not measure concentrations of *cis*-nonachlordane, for which plasma levels above the median was associated with a 2.6-fold increased risk of TGCC in the Swedish study. In addition, the Swedish study measured maternal organochlorine levels for cases and controls, finding a statistically significant increased risk of TGCC in men whose mothers had concentrations of hexachlorobenzene, *trans*-nonachlordane, or *cis*-nonachlordane above the median.

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Table 4. Joint association of serum *p,p′*-DDE concentration and *AR* genotype with TGCC risk

AR genotype	Adjusted OR* (95% CI), cases/controls (*n*), *p,p′*-DDE concentration (pg/g)			$P_{interaction}$
	≤1,101	>1,101-2,473	>2,473	
CAG repeat length				
≤23	1.0 (reference), 87/189	1.3 (0.8-2.0), 69/138	0.6 (0.3-1.2), 15/67	
>23	0.9 (0.5-1.4), 35/94	0.9 (0.5-1.6), 24/67	0.6 (0.2-2.0), 5/21	0.8
GGN repeat length				
≤17	1.0 (reference), 74/177	0.9 (0.6-1.6), 47/125	0.5 (0.2-1.2), 12/57	
>17	0.9 (0.6-1.4), 48/106	1.4 (0.9-2.4), 46/80	0.7 (0.3-1.7), 8/31	0.3

*Adjusted for age, race, change in BMI between reference date and blood draw, assay run number, and serum lipids.

Although our results indicate that adult levels of organochlorines are unlikely to be strong risk factors for TGCC, there are several limitations that should be considered when interpreting these findings. The early age at onset of TGCC, the strong association with undescended testes (39), plus robust evidence for a fetal origin of carcinoma *in situ* (the precursor to all adult TGCC; ref. 40), strongly suggests that the *in utero* environment is important in establishing risk. To the extent that it is exposure to organochlorines during early life that influences TGCC risk, the concentrations that we measured in adults may not accurately reflect exposure in the etiologically relevant period. Nondifferential exposure measurement error of this type would attenuate relative risk estimates toward the null. Serum levels of DDE and hexachlorobenzene measured as much as 10 years apart are strongly correlated in adult men (r = 0.92 and 0.91, respectively; ref. 41), and a moderate correlation (r = 0.39) between umbilical cord serum DDE concentrations at birth and serum DDE levels at age 14 years has been reported (42), but there are no published data on the correlation between childhood and adult organochlorine concentrations. Besides the long time-span between birth and the blood sample collection among ATLAS Study participants (~40 years, on average), children are known to metabolize and clear xenobiotics at a different rate than adults (43). Taken together, this limited evidence suggests that organochlorine levels measured in adulthood may correlate poorly with those present during infancy. However, we did observe an appreciable difference in current serum *p,p′*-DDE levels according to whether a participant reported having been breast-fed as an infant. Because breast-feeding is an important determinant of organochlorine levels in children (42, 44, 45), the presence of this association in our study suggests that levels measured in these adult men do reflect, at least to some extent, levels in early life.

Another important limitation of this study was the poor between-run reliability of the analytic method for several of the analytes. Although we adjusted for run number, because each run had <20 case and control samples, and many runs had little, or no, variation among exposure categories (some runs contained samples in only 1 or 2 exposure categories), it is uncertain how adequate the statistical adjustment was. Poor reliability would attenuate the OR and decrease our ability to detect even strong associations.

Our study was also limited by the fact that we used postdiagnostic blood samples and thus cannot exclude the possibility that some aspect of the disease or treatment affected organochlorine levels in the TGCC cases. Both chemotherapy (37, 38) and changes in body weight (41, 46) are reported to influence blood organochlorine levels. Results from subanalyses, which excluded men treated with chemotherapy or who experienced notable weight change, were similar to those from the main analysis, but we cannot rule out other unmeasured effects of either treatment or disease that could result in either differential or nondifferential exposure misclassification.

We interviewed 68.5% and 51.5% of eligible cases and controls, respectively. Nonresponse could have biased the results if the association between organochlorine pesticides and risk of TGCC was different among cases and controls that did not participate. However, the results of our analyses of known risk factors for TGCC (age, race, undescended testes, and family history of TGCC), and of relationships between demographic and lifestyle characteristics and organochlorine levels, are consistent with those found in many previous studies and provide a reassuring measure of external validity of our case and control groups.

Within the limitations described above, this study provides some reassurance that contemporary adult concentrations of the organochlorine pesticide residues measured in this study are not associated with an increased risk of TGCC. Because of the uncertainty regarding how well organochlorine levels measured in adulthood reflect exposures during early life, further research is needed using exposure measurements collected *in utero* and/or during infancy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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