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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 aged 20–34. Although the pathogenesis of TGCC is poorly understood, sub-optimal androgen levels or impaired androgen signaling may play a role. Some persistent organochlorine pesticides commonly found in human tissue possess anti-androgenic 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* (*AR*) gene. We conducted a population-based case-control study among 18–44 year-old male residents of three Washington State counties. Cases (n=246) were diagnosed during 1999–2003 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 *AR* CAG (<23 vs.23+ repeats) or GGN (<17 vs.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.

Keywords

testicular cancer; organochlorines; DDE; pesticides; androgen receptor

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INTRODUCTION

The incidence of testicular germ cell carcinoma (TGCC), the most common malignancy among men aged 20–34, has increased several-fold since the 1940's in many western countries (1–3). The reason for the rise 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 sub-optimal androgen levels or impaired androgen signaling as possibly playing a role (8). A number of persistent organochlorine pesticides commonly found in human tissue samples possess anti-androgenic 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 eleven 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 androgen receptor 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.

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 18–44 year-old male residents of King, Pierce, and Snohomish counties, Washington State. Eligible ATLAS cases are men 1) diagnosed with a first, invasive TGCC between January 1, 1999 and December 31, 2008; and 2) 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 (CSS), a part of the Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute, based on the following International Classification of Diseases – Oncology topography and histology codes: topography C62.0 – C62.9, histology 9060 – 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 prior to 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 (RDD) (29;30). The RDD 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 prior to 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 time period prior to 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 RDD. Information collected during the interview included demographic characteristics, medical history, physical characteristics, and other suspect TGCC risk factors. Prior to the in-person interview, each participant was asked to complete self-administered questionnaires eliciting information on family history of cancer and pre- and post- natal 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 impact 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 23.1 months 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 (NCEH), Centers for Disease Control and Prevention. Over the course of the study, serum samples were shipped frozen to the NCEH 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 1) randomly ordered case and control samples in an approximately 1:3 ratio, and 2) 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 non-participant 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 [β-HCH], γ-hexachlorocyclohexane [γ-HCH or lindane], dieldrin, hexachlorobenzene (HCB), heptachlor epoxide, mirex, p,p'-DDT, o,p-DDT, p,p'-DDE, oxychlordane, and trans-nonachlor), as well as 36 polychlorinated biphenyl (PCB) congeners were measured using accelerated solvent extraction (ASE) and a combined PCB/organochlorine pesticide gas chromatography-high-resolution mass spectrometry (GC-HRMS) analysis as previously described (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), two laboratory QC samples, two blanks, two recovery reference samples, and a replicate of one of

the 19 unknowns to assess within run variation. Staff conducting the analyses, and overseeing the calculation of the analyte concentrations from the GC-HRMS was blinded to the identity of the unknowns from subjects and QC aliquots. Mean limits of detection (LOD) corrected for extraction recovery were approximately 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 each target analyte and compared to 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 non-target compounds, IR ratios that are different from expected can occur when the measured analyte concentration is close to the LOD. 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 LOD (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, 8 QC) due to loss of the sample during analysis or insufficient recovery (n=36), or matrix effects (n=5). Organochlorine measurements were performed on 247 case, 630 control, and 70 quality control specimens.

AR microsatellite repeat polymorphisms

The number of CAG and GGN repeats in the *androgen receptor* gene was determined by a polymerase chain reaction-based fragment analysis as previously described (34). Briefly, the gene fragments containing these microsatellites were amplified in separate reactions, and fluorescently-labeled PCR products from the same individual were pooled, diluted, and a small aliquot was added to a mixture of formamide and GeneScan-500 LIZ size standard (Applied Biosystems Inc (ABI), Foster City, CA). The samples were then denatured and analyzed by capillary electrophoresis on an ABI 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 non-detectable 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 non-detects, conditional on the value being between zero and the LOD. 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 datasets to compute multiple-imputation estimates of regression parameters and valid

variance estimates (36). Simulations have shown that this imputation procedure generates unbiased estimates of the mean, and 95% confidence intervals close to the nominal level, for samples sizes of 200–400, even when the percentage of non-detects is as high as 60–70% (35).

To assess the reliability of serum organochlorine measurements, we calculated intraclass correlation coefficients (ICCs) for within-run replicates 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% confidence intervals (CI), conditioning on the assay run number. For the primary analysis, we categorized analyte concentrations *a priori* as low ($<50^{th}$ percentile), medium (50^{th}–85^{th} percentile), and high ($> 85^{th}$ 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.

In order to identify characteristics associated with organochlorine levels, we selected a single organochlorine (p,p'-DDE) and calculated least-square adjusted mean concentrations by categories of age at reference date, race, BMI, change in BMI, breastfeeding 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 Inc., Cary, NC) for the imputation procedure and Stata (Stata Statistical Software, version 9; StataCorp, College Station, TX) for all other analyses. All statistical tests were two-sided.

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

RESULTS

Compared to 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 breastfed as infants. Body mass index was similar in cases and controls.

Median concentrations, interquartile ranges, proportion of non-detects and IR-excluded measurements, and within- and between- run intraclass correlation coefficients 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 above 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 to white men, overweight and obese men compared to normal weight men, men who had been breastfed 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-HCH; a 10 pg/g increase in γ-HCH 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).

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-HCH 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 HCB, *trans*-nonachlordane, or *cis*-nonachlordane above the median.

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 TGCCs) (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 time period. Non-differential exposure measurement error of this type would attenuate relative risk estimates towards the null. Serum levels of DDE and HCB measured as much as 10 years apart are strongly correlated in adult men (r=0.92 and r=0.91, respectively) (41), and a moderate correlation (r=0.39) between umbilical cord serum DDE

concentrations at birth and serum DDE levels at 14 years old of age 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 (approximately 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 or not a participant reported having been breastfed as an infant. Because breastfeeding 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 fewer than 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 odds ratio and decrease our ability to detect even strong associations.

Our study was also limited by the fact that we used post-diagnostic 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 sub-analyses 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 non-differential 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.

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Table 1

Selected characteristics of ATLAS TGCC cases and controls.

Characteristic	Controls (n=630)		Cases (n=246)	
	N	%	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 1° 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
Breastfed as an infant				
No	237	37.6	106	43.1
Yes	318	50.5	111	45.1
Unknown†	75	11.9	29	11.8
Body mass index (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, 18 cases) and those that did not complete family history questionnaire (38 controls, 11 cases)

†includes participants that did not know breast feeding history (47 controls, 20 cases) and those that did not complete natal questionnaire (28 controls, 9 cases)

‡change in body mass index between reference date and blood draw date

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).

			ICC[‡]		Concentration, pg/g serum	Concentration, ng/g lipid
Pesticide	**%ND[*]**	**%IR[†]**	**Within run**	**Between run**	**Median (IQR)[§]**	**Median (IQR)[§]**
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	1089.10 (722.70 – 1770.92)	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)

[*] ND = non-detects

[†] IR = ion ratio out of tolerance indicating possible interference

[‡] ICC = intraclass correlation coefficient

[§] IQR = 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		
No. cases/No. controls	113/285	83/200	25/85		
OR* (95% CI)	1.00 (Ref.)	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		
No. cases/No. controls	130/312	73/217	38/93		
OR* (95% CI)	1.00 (Ref.)	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		
No. cases/No. controls	116/295	86/207	29/88		
OR* (95% CI)	1.00 (Ref.)	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		
No. cases/No. controls	139/315	71/220	35/94		
OR* (95% CI)	1.00 (Ref.)	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		
No. cases/No. controls	113/287	91/200	24/85		
OR* (95% CI)	1.00 (Ref.)	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		
No. cases/No. controls	125/311	89/221	28/93		
OR* (95% CI)	1.00 (Ref.)	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		
No. cases/No. controls	110/301	94/210	32/90		
OR* (95% CI)	1.00 (Ref.)	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		
No. cases/No. controls	104/290	83/200	37/83		
OR* (95% CI)	1.00 (Ref.)	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)	≤1101	>1101–2473	>2473		
No. cases/No. controls	130/311	94/218	21/93		
OR* (95% CI)	1.00 (Ref.)	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		
No. cases/No. controls	121/299	87/209	29/89		
OR* (95% CI)	1.00 (Ref.)	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		
No. cases/No. controls	124/315	91/221	31/94		
OR* (95% CI)	1.00 (Ref.)	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		
No. cases/No. controls	120/315	93/221	33/94		
OR* (95% CI)	1.00 (Ref.)	1.18 (0.81–1.71)	0.93 (0.51–1.68)	0.88	0.94 (0.79–1.12)

* adjusted for age, race, change in body mass index between reference date and blood draw, assay run number and serum lipids

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† trend test performed by assigning ordinal scores to pesticide categories and modeling as continuous term

‡ except p,p'-DDE which is per 100 pg/g

§ Sum of chlordane derived compounds including oxychlordane, trans-nonachlor, and heptachlor epoxide

Table 4

Joint association of serum p'p-DDE concentration and androgen receptor genotype with TGCC risk.

AR genotype	Adjusted odds ratio* (95% confidence interval) (No. cases/No. controls) p'p-DDE concentration (pg/g) ≤1,101	>1101–2473	>2,473	$P_{interaction}$
CAG repeat length				
≤23	1.0 (Referent) (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
GGC repeat length				
≤17	1.0 (Referent) (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 body mass index between reference date and blood draw, assay run number and serum lipids