Persistent Organochlorine Pesticides and Risk of Testicular Germ Cell Tumors

Katherine A. McGlynn, Sabah M. Quraishi, Barry I. Graubard, Jean-Philippe Weber, Mark V. Rubertone, Ralph L. Erickson

Background Exposure to endocrine-disrupting chemicals, such as persistent organochlorine pesticides, has been suggested to increase the risk of testicular germ cell tumors (TGCTs).

Methods To study the relationship of POP exposure to TGCT risk, prediagnostic serum samples from 754 case subjects and 928 control subjects enrolled in the Servicemen's Testicular Tumor Environmental and Endocrine Determinants Study were analyzed for *cis*-nonachlor, *trans*-nonachlor, oxychlordane, total chlordanes, β-hexachlorocyclohexane, mirex, *p*,*p*′-dichlorodiphenyldichloroethylene (*p*,*p*′-DDE), and *p*,*p*′-dichlorodiphenyltrichloroethane. Adjusted odds ratios (ORs) and their associated 95% confidence intervals (CIs) for the risk of TGCT overall and for the histological subgroups, seminoma and nonseminoma, were estimated using multivariable logistic regression. All statistical tests were two-sided.

Results TGCT risk was statistically significantly associated with higher plasma levels of *p*,*p*′-DDE (for highest quartile [Q4] vs lowest quartile [Q1], OR = 1.71, 95% CI = 1.23 to 2.38, P_{trend} = .0002) and of two chlordane components, *cis*-nonachlor (Q4 vs Q1, OR = 1.56, 95% CI = 1.11 to 2.18, P_{trend} = .009) and *trans*-nonachlor (Q4 vs Q1, OR = 1.46, 95% CI = 1.07 to 2.00, P_{trend} = .026). Seminoma risk was statistically significantly associated with *p*,*p*′-DDE (Q4 vs Q1, OR = 1.91, 95% CI = 1.22 to 2.99, P_{trend} = .0008), *cis*-nonachlor (Q4 vs Q1, OR = 1.93, 95% CI = 1.27 to 2.93, P_{trend} = .0045), *trans*-nonachlor (Q4 vs Q1, OR = 1.72, 95% CI = 1.11 to 2.67, P_{trend} = .033), and a chlordane metabolite, oxychlordane (Q4 vs Q1, OR = 1.64, 95% CI = 1.04 to 2.60, P_{trend} = .048), whereas nonseminoma risk showed a statistically significant association with *p*,*p*′-DDE only (Q4 vs Q1, OR = 1.63, 95% CI = 1.10 to 2.42, P_{trend} = .0044).

Conclusions Increased exposure to *p*,*p*′-DDE may be associated with the risk of both seminomatous and nonseminomatous TGCTs, whereas exposure to chlordane compounds and metabolites may be associated with the risk of seminoma. Because evidence suggests that TGCT is initiated in very early life, it is possible that exposure to these persistent organic pesticides during fetal life or via breast feeding may increase the risk of TGCT in young men.

J Natl Cancer Inst 2008;100:663–671

Testicular germ cell tumors (TGCTs) are the most common cancer among US men between the ages of 15 and 44 years, and their incidence has been increasing for at least three decades (1). Caucasian ethnicity, cryptorchism, prior history of TGCT, family history of TGCT, and, perhaps, increased adult stature are the only well-documented risk factors (2). The association of TGCT with cryptorchism, as well as the similarity of testicular carcinoma in situ to primordial germ cells, suggests that TGCT risk may be determined very early in life, possibly even in utero (3–6). The in utero factors that increase the risk of TGCT are unknown, although the speculation of Henderson et al. (7,8) that maternal estrogen levels could be a major risk factor for TGCT has received much attention. The ability of synthetic hormonal drugs, phytoestrogens, and persistent organochlorine pesticides (POPs) to bind to estrogen receptors has led to speculation that exposures to these endocrine-dirupting chemicals might also be related to the development of TGCT and of the other male reproductive disorders that are speculated to be part of the testicular dysgenesis syndrome: cryptorchism, hypospadias, and impaired spermatogenesis (9,10).

Affiliations of authors: Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD (KAM, SMQ, BIG); Toxicology Centre, Institut National de Sante Publique du Quebec, Quebec, QC (JPW); Army Medical Surveillance Activity, U.S. Army Center for Health Promotion and Preventive Medicine, Washington, DC (MVR); GEIS, Division of Preventive Medicine, Walter Reed Army Institute of Research, Silver Spring, MD (RLE).

Correspondence to: Katherine A. McGlynn, PhD, Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, EPS—Ste 550, 6120 Executive Blvd, Rockville, MD 20852-7234 (e-mail: mcglynnk@mail.nih.gov).

See "Funding" and "Notes" following "References."

DOI: 10.1093/jnci/djn101

Published by Oxford University Press 2008.

CONTEXT AND CAVEATS

Prior knowledge

Previous work had suggested that exposure to organochlorine pesticides was associated with increased risk of testicular germ cell tumors (TGCTs).

Study design

A case–control design was used to test the association of serum levels of several organochlorines and the risk of TGCTs in young men.

Contribution

The pesticide dichlorodiphenyldichloroethylene was statistically significantly associated with the risk of TGCTs, and the data suggested that chlordane isomers may be associated with the risk of seminoma.

Implications

Further examination of the association of organochlorine pesticides with TGCTs in other populations is needed, particularly given that more widespread use is being considered in the developing world.

Limitations

Because assessment of the association of several chemicals with TGCT risk entailed multiple comparisons, some of the results should be interpreted with caution.

To our knowledge, only one prior study of the association of POP levels and TGCT has been reported (11). Examining blood samples from patients with TGCT and control subjects, the investigators found that the men with TGCT had statistically significantly increased levels of a congener of the pesticide chlordane, *cis*-nonachlor. Mothers of the case subjects had statistically significantly increased levels of the chlordane congeners *trans*-nonachlor, *cis*-nonachlor, and total chlordane. To examine the association of prediagnostic serum levels of chlordane and other POPs with the risk of TGCT, we have conducted a case–control study.

Materials and Methods

Subjects and Eligibility

The US Servicemen's Testicular Tumor Environmental and Endocrine Determinants (STEED) Study enrolled participants between April 4, 2002, and January 17, 2005. To be eligible for the study, case subjects had to be 45 years or younger at the time of diagnosis and to have donated at least one serum sample between January 1, 1987, and December 31, 2002, to the Department of Defense Serum Repository (DoDSR). At the DoDSR, specimens are stored in precisely documented locations in walk-in freezers at −30°C. Using a person-specific identification number, the specimens in the DoDSR computerized database were linked to the Defense Medical Surveillance System (DMSS) and to other military medical databases to determine which military personnel had developed TGCT. Men with a serum sample in the DoDSR who had not developed TGCT were eligible to participate as control subjects.

Diagnoses of TGCT were limited to classic seminoma or nonseminoma (embryonal carcinoma, yolk sac carcinoma, choriocarcinoma, teratoma, or mixed germ cell tumor). Spermatocytic seminoma was not included because it is thought to have an etiology distinct from other adult-onset TGCTs. The diagnoses were based on the original pathology reports or, in the case of 6.5% of the case participants, review of the pathology slides.

Study Design

The study was designed as a pair-matched case–control study, although additional control subjects were initially identified due to the transient nature of the military population. Each case subject was matched to all available potential control subjects on birth year (within 1 year), race/ethnicity (white, black, other), and date of available serum sample (within 30 days) using the computerized DMSS database. From the list of possible control subjects, four men were chosen at random as the control set. The first man on the list was designated as the primary control subject. Attempts were made to enroll the primary control subject for 30 days via electronic tracing attempts, letters, and telephone calls. If the primary control subject was not completely enrolled in the study within 30 days, similar attempts to enroll the next possible control in the set were initiated. In some cases, this scheme resulted in more than one control subject being enrolled for a particular case subject.

The database linkage identified 961 men diagnosed with TGCT between 1988 and 2003 who appeared to meet the study criteria. Further review found that 76 men could not be traced, 27 had died, 3 were known to be deployed to a combat zone, and 2 were ineligible, leaving 853 possible participants. Of these men, 22 were in the process of being contacted when the study closed. Of the 831 men contacted, 754 (91%) agreed to participate. Among potential control subjects, 2579 were evaluated for inclusion. Of these, 385 could not be traced, 18 had died, 64 were known to be deployed to a combat zone, and 2 were ineligible. In addition, 928 could not be enrolled within 30 days. Of the remaining 1182 men, 32 were in the process of being contacted when the study closed. Thus, of the 1150 potential control subjects contacted, 928 (81%) agreed to participate. Among the 754 case subjects and 928 control subjects enrolled, 720 were matched case–control pairs.

To participate, each man agreed to complete a study questionnaire, donated a buccal cell sample collected in mouthwash, granted permission to use his DoDSR serum specimen, and signed an informed consent document. The study was approved by institutional review boards of the National Cancer Institute, Bethesda, MD, and the Walter Reed Army Institute for Research, Silver Spring, MD.

Questionnaire Data

Each participant was administered a computer-assisted telephone interview composed of nine modules. Case subjects were asked questions in reference to a date 1 year before their TGCT diagnosis (referred to as the reference date). Control subjects were assigned the same reference date as their matched case subject. For the current analysis, participants were asked to report their height and weight as of the reference date and to report whether they had a personal history of cryptorchism and/or a family history of testicular cancer in first- or second-degree relatives.

Laboratory Methods

Eleven organochlorine compounds were analyzed at the Human Toxicology Laboratory of the Institut National de Santé Publique du Québec. Each case sample was analyzed in the same batch as its matched control, and laboratory personnel were blinded to the case or control status of all samples. Two quality control samples consisting of pooled serum were randomly inserted in each batch. The compounds analyzed were α-chlordane, γ-chlordane, oxychlordane, *trans*-nonachlor, *cis*-nonachlor, aldrin, mirex, β-hexachlorocyclohexane, hexachlorobenzene, *p*,*p*′-dichlorodiphenyltrichloroethane (*p*,*p*′-DDT), and *p*,*p*′-dichlorodiphenyldichloroethylene (*p*,*p*′-DDE). The plasma samples, enriched with isotopically labeled internal standards, were denatured using formic acid. Analytes were then automatically extracted from the matrix by solid-phase extraction. Extracts were automatically cleaned on a florisil column and analyzed by gas chromatography–mass spectrometry. Detection of ions generated from negative chemical isolation was accomplished in the selected ion monitoring mode. Evaluation of concentrations was done by considering recoveries of the labeled internal standards. A linear calibration extended up to 10 μg/L for most analytes. The amounts of analytes present in higher concentrations were determined after appropriate dilutions. The mean detection limit of all analytes was approximately 0.005 μg/L. Average within-day variability ranged from 2% to 5%, and average recovery was 80%. For each compound, the limit of detection, median levels, and coefficients of variation, which incorporate both within- and between-batch variability, are available online as supplementary materials. The laboratory's accuracy in detection of organochlorine pesticide levels has been validated through successful participation in two external quality assessment schemes, the German External Quality Assurance Scheme (Erlangen University) and the Arctic Monitoring and Assessment Program Ring Test (Quebec).

To permit adjustment of the POP measurements for lipid levels, serum samples were analyzed for triglycerides, free and total cholesterol, and phospholipids. Each POP level was then divided by the total lipid level. The lipid measurements were made with enzyme bioassays using reagents produced by Randox Laboratories (Antrim, UK). Measurements of POPs and lipid levels were obtained for 739 case subjects and 915 control subjects.

Statistical Analysis

To conduct logistic regression analyses, lipid-adjusted POP levels were categorized into quartiles based on the levels in those control subjects that were above the limit of detection. Organochlorine levels equal to or below the limit of detection were imputed as the midpoint of the limit and were included in the first quartile for regression analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the association of each POP quartile with the risk of TGCT overall and with risk of seminoma or nonseminoma separately. One case subject was excluded from the histology-specific analyses because his tumor histology was unknown. Given the matched case–control design, risk estimates adjusting for confounders were first generated using conditional logistic regression, restricting the analysis to only the matched sets. Modeling using unconditional logistic regression was subsequently performed using the data from all participants. Because the latter involved breaking the match, risk estimates derived from the unconditional logistic regression models were adjusted for the three matching factors: age at reference date, race, and date of serum sample collection. Both logistic regression models were adjusted for the known TGCT risk factors: personal history of cryptorchism (yes, no), family history of testicular cancer (yes, no), and height, as well as for age at serum draw and for body mass index (BMI; kg/m^2) because BMI is associated with plasma organochlorine levels (11). Tests for trend in risk were computed using scored variables for plasma levels, based on the median levels of each quartile, to evaluate possible dose–response relationships. A measure of total chlordane exposure was evaluated in risk analysis by adding together the standardized levels [(level − mean level)/SD] of lipid-adjusted *cis*-nonachlor, *trans*-nonachlor, and oxychlordane. Because results using conditional and unconditional logistic regression were similar, only those using the latter approach are presented. Attributable risk calculation was done as described (12). Statistical analyses were conducted using SAS Release 9.1 (SAS Institute Inc, Cary, NC). All tests were two-sided, with *P* less than .05 defined as the level of statistical significance.

Results

We included 739 case subjects (313 with seminoma and 425 with nonseminoma) and 915 control subjects in the analysis (Table 1). The set of serum samples included 685 matched pairs (300 seminoma and 385 nonseminoma). Case and control subjects were matched on birth year and ethnicity, and thus, there were no appreciable differences in the distributions of these variables. Overall, the seminoma case subjects were somewhat older than the control subjects ($P < .001$), and the nonseminoma case subjects were somewhat younger ($P < .0001$). The case subjects ($P < .001$), particularly the nonseminoma case subjects ($P < .0001$), were statistically significantly more likely than the control subjects to have a history of cryptorchism. The case subjects were also statistically significantly more likely to have a family history of testicular cancer ($P < .001$) and were taller ($P < .001$) than the control subjects. No difference in BMI was evident between the case subjects and control subjects ($P = .86$).

The serum analyses of POP levels were conducted in 2006. Among all participants, the mean storage time (from serum donation until laboratory analysis) was 14.2 years (14.1 and 14.3 years in case and control subjects, respectively). The median time was 15.5 years for both case subjects and control subjects. The minimum storage time for any sample was 3.8 years, and the maximum was 18.9 years. There were no statistically significant differences in storage time between the samples of the case and control subjects ($P = .12$, data not shown). There was also no statistically significant difference in the average length of time between sample donation and reference date among case and control subjects (case subjects = 3.94 years, control subjects = 3.96 years, $P = .89$, data not shown). Because the samples were analyzed in batches, an analysis that examined the effect of batch was conducted for each compound. No statistically significant effects of batch were identified (data not shown).

Of the 11 POPs analyzed, four (aldrin, α-chlordane, γ-chlordane, and hexachlorobenzene) were excluded from data analysis because fewer than 35% of the study samples had values above the limit of detection (see Supplementary Material, available online, for limit

Table 1. Selected characteristics of case subjects and control subjects in Servicemen's Testicular Tumor Environmental and Endocrine Determinants Study*

	Control subjects (n = 915)	Case subjects					
		All TGCT (n = 739)		Seminoma (n = 313)		Nonseminoma (n = 425)	
Characteristic	No. (%)	No. (%)	*P* value†	No. (%)	*P* value†	No. (%)	*P* value†
Reference age (y)			.94		<.001		<.001
18–20	67 (7.3)	64 (8.7)		12 (3.8)		52 (12.2)	
21–25	306 (33.4)	240 (32.5)		57 (18.2)		182 (42.8)	
26–30	261 (28.5)	206 (27.9)		106 (33.9)		100 (23.5)	
31–35	159 (17.4)	132 (17.9)		74 (23.6)		58 (13.6)	
36–40	96 (10.5)	75 (10.2)		49 (15.7)		26 (6.1)	
41–45	26 (2.8)	22 (3.0)		15 (4.8)		7 (1.6)	
Race/ethnicity			.47		.10		.37
White	781 (85.4)	625 (84.6)		253 (80.8)		372 (87.5)	
Black	34 (3.7)	22 (3.0)		12 (3.8)		10 (2.4)	
Other	100 (10.9)	92 (12.4)		48 (15.3)		43 (10.1)	
History of cryptorchism			<.001		.067		<.0001
No	899 (98.3)	700 (94.7)		302 (96.5)		397 (93.4)	
Yes	16 (1.7)	39 (5.3)		11 (3.5)		28 (6.6)	
Family history of testicular cancer‡			<.001		<.001		.02
No	901 (98.5)	708 (95.8)		297 (94.9)		410 (96.5)	
Yes	14 (1.5)	31 (4.2)		16 (15.1)		15 (3.5)	
Body mass index§			.86		.24		.93
<18.5	11 (1.2)	9 (1.2)		4 (1.3)		4 (0.9)	
18.5–24.9	405 (44.3)	325 (44.0)		136 (43.5)		189 (44.4)	
25.0–29.9	452 (49.4)	360 (48.7)		147 (47.0)		213 (50.1)	
≥30.0	47 (5.1)	45 (6.1)		26 (8.3)		19 (4.5)	
Adult height (inches)							
<68	257 (28.1)	151 (20.4)	<.001	60 (19.2)	.01	91 (21.4)	.016
68–70	253 (27.6)	217 (29.4)		96 (30.7)		121 (28.5)	
71–72	237 (25.9)	193 (26.1)		84 (26.8)		109 (25.6)	
≥73	168 (18.4)	178 (24.1)		73 (23.3)		104 (24.5)	

* Tumor histology was unknown for one case subject. TGCT = testicular germ cell tumor.

† *P* value of χ^2 test.

‡ Family history in first- and second-degree relatives.

§ Body mass index = weight in kg divided by square of the height in m.

of detection of each compound). A fifth compound, *p,p′*-DDT, was detectable in only 20% of the samples but was retained in the analysis because it is the parent compound of *p,p′*-DDE.

After adjustment for age at blood donation, ethnicity, date of serum draw, age at reference date, personal history of cryptorchism, family history of TGCT, height, and BMI, there were statistically significant associations between TGCT risk and serum levels of *cis*-nonachlor (for highest quartile [Q4] vs lowest quartile [Q1], OR = 1.56, 95% CI = 1.11 to 2.18, P_{trend} = .009), *trans*-nonachlor (Q4 vs Q1, OR = 1.46, 95% CI = 1.07 to 2.00, P_{trend} = .026), and total chlordanes (ie, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane) (Q4 vs Q1, OR = 1.51, 95% CI = 1.09 to 2.10, P_{trend} = .027) (Table 2). There was a statistically significant association between *p,p′*-DDE (Q4 vs Q1, OR = 1.71, 95% CI = 1.23 to 2.38, P_{trend} = .0002) and risk of TGCT. There were no statistically significant associations between serum levels of oxychlordane, *p,p′*-DDT, β-HCH, or Mirex and the risk of TGCT.

We performed similar analyses to test the associations of organochlorines and seminoma (Table 3). *Cis*-nonachlor (Q4 vs Q1, OR = 1.93, 95% CI = 1.27 to 2.93, P_{trend} = .0045), *trans*-nonachlor (Q4 vs Q1, OR = 1.72, 95% CI = 1.11 to 2.67, P_{trend} = .033), oxychlordane (Q4 vs Q1, OR = 1.64, 95% CI = 1.04 to 2.60, P_{trend} = .048), total chlordanes (Q4 vs Q1, OR = 1.90, 95% CI = 1.20 to 3.00, P_{trend} = .025), and *p,p′*-DDE (Q4 vs Q1, OR = 1.91, 95% CI = 1.22 to 2.99, P_{trend} = .0008) were statistically significantly associated with increased risk of seminoma. By contrast, analyses of associations with nonseminoma showed that, among the organochlorine pesticides, only *p,p′*-DDE was statistically significantly related to increased risk of nonseminoma (Q4 vs Q1, OR = 1.63, 95% CI = 1.10 to 2.42, P_{trend} = .0044, Table 4).

Because the study was based on a matched case–control design, all analyses presented in Tables 2–4 were conducted both as conditional and unconditional analyses. There were no statistically significant differences in the results of the analyses (data not shown); thus, only the unconditional results are displayed.

Discussion

In 1993, Sharpe and Skakkebaek (8) first stated the estrogen hypothesis, which postulates that the increasing incidence of reproductive abnormalities in the human male may be related to increased estrogen exposure in utero. In the same year, Colborn et al. (13) suggested that adverse developmental effects are associated with in utero exposures to endocrine-disrupting chemicals.

Table 2. Adjusted relative risk of testicular germ cell tumors by quartile of lipid-adjusted serum levels of persistent organochlorine pesticides in the Servicemen's Testicular Tumor Environmental and Endocrine Determinants Study*

Quartile characteristic	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Cis-nonachlor					
μg/g lipid	≤0.00218	0.00219–0.00280	0.00281–0.00386	>0.00386	
Case subjects (n)	511	72	66	87	
Control subjects (n)	670	81	82	80	
Relative risk (95% CI)	1.00 (referent)	1.30 (0.91 to 1.85)	1.12 (0.78 to 1.61)	1.56 (1.11 to 2.18)	.009
Trans-nonachlor					
μg/g lipid	≤0.0105	0.0105–0.0165	0.0166–0.0245	>0.0245	
Case subjects (n)	193	186	147	210	
Control subjects (n)	252	220	221	220	
Relative risk (95% CI)	1.00 (referent)	1.25 (0.93 to 1.68)	0.98 (0.71 to 1.34)	1.46 (1.07 to 2.00)	.026
Oxychlordane					
μg/g lipid	≤0.00805	0.00806–0.0116	0.0117–0.0171	>0.0171	
Case subjects (n)	187	170	188	191	
Control subjects (n)	238	225	225	225	
Relative risk (95% CI)	1.00 (referent)	1.10 (0.81 to 1.50)	1.21 (0.88 to 1.66)	1.27 (0.92 to 1.76)	.14
p,p′-DDE					
μg/g lipid	≤0.157	0.158–0.250	0.251–0.390	>0.390	
Case subjects (n)	186	167	146	236	
Control subjects (n)	238	230	220	224	
Relative risk (95% CI)	1.00 (referent)	1.01 (0.75 to 1.36)	1.00 (0.73 to 1.38)	1.71 (1.23 to 2.38)	.0002
p,p′-DDT					
μg/g lipid	≤0.0209	0.0210–0.259	0.260–0.397	>0.397	
Case subjects (n)	630	27	40	37	
Control subjects (n)	784	43	43	42	
Relative risk (95% CI)	1.00 (referent)	0.81 (0.49 to 1.35)	1.27 (0.81 to 2.01)	1.13 (0.71 to 1.82)	.50
β-hexachlorocyclohexane					
μg/g lipid	≤0.00582	0.00583–0.00804	0.00805–0.0115	>0.0115	
Case subjects (n)	306	160	125	143	
Control subjects (n)	355	185	188	185	
Relative risk (95% CI)	1.00 (referent)	1.05 (0.80 to 1.40)	0.82 (0.60 to 1.11)	0.90 (0.65 to 1.24)	.40
Mirex					
μg/g lipid	≤0.00474	0.00475–0.00640	0.00641–0.00952	>0.00952	
Case subjects (n)	518	70	63	83	
Control subjects (n)	642	91	89	90	
Relative risk (95% CI)	1.00 (referent)	1.07 (0.76 to 1.51)	0.89 (0.62 to 1.27)	1.24 (0.90 to 1.74)	.32
Total chlordanes†					
Case subjects (n)	170	196	156	214	
Control subjects (n)	229	227	228	229	
Relative risk (95% CI)	1.00 (referent)	1.31 (0.97 to 1.77)	1.05 (0.76 to 1.46)	1.51 (1.09 to 2.10)	.027

* Adjusted for age at blood donation, ethnicity, date of serum draw, age at reference date, cryptorchism, family history, height, and body mass index. Relative risk assumed to be equivalent to the odds ratio based on the rare disease assumption. CI = confidence interval; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; *p,p′*-DDT = *p,p′*-dichlorodiphenyltrichloroethane.

† Total chlordanes = *cis*-nonachlor + *trans*-nonachlor + oxychlordane.

Together, these papers focused attention on the possible link between endocrine-disrupting chemicals and the group of disorders (testicular cancer, cryptorchism, hypospadias, and impaired spermatogenesis) that came to be known as the testicular dysgenesis syndrome (10). Only recently have studies testing the endocrine-disrupting chemicals hypothesis begun to emerge. The conclusions of this study, that *p,p′*-DDE and chlordane-related compounds (*cis*-nanachlor, *trans*-nonachlor, oxychlordane, total chlordanes), are associated with risk of TGCT, support the endocrine-disrupting chemicals hypothesis and suggest that some of the increase in TGCT incidence in recent decades could be related to accumulation of these chemicals in the environment.

Among the endocrine-disrupting chemicals, the organochlorine pesticides (POPs) have been a focus of particular interest because they include the first modern synthetic pesticide, DDT. DDT, now banned in the United States, was in general commercial use in the United States between 1945 and 1973 (14). As a consequence of the ban, measured serum levels of DDT and its main persistent metabolite, dichlorodiphenyldichloroethylene (*p,p′*-DDE), have declined in the US population in the past few decades (15). DDT mimics the actions of estrogen by binding to estrogen receptors, whereas *p,p′*-DDE has been demonstrated to be antiandrogenic (16). The association between *p,p′*-DDE and TGCT in this study suggests that the risks of both seminoma and nonseminoma may be more related to antiandrogenic activity than to estrogenic activity.

This study cannot determine how and when the study participants were exposed to *p,p′*-DDT and/or *p,p′*-DDE. Because DDT can cross the placenta and is present in breast milk, at least some of the exposure may have occurred in utero and/or via breast feeding

Table 3. Adjusted relative risk of seminoma by quartile of lipid-adjusted levels of serum organochlorines in the Servicemen's Testicular Tumor Environmental and Endocrine Determinants Study*

Quartile characteristic	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Cis-nonachlor					
μg/g lipid	≤0.00218	0.00219–0.00280	0.00281–0.00386	>0.00386	
Case subjects (n)	200	33	31	49	
Control subjects (n)	670	81	82	80	
Relative risk (95% CI)	1.00 (referent)	1.26 (0.80 to 2.00)	1.00 (0.62 to 1.61)	1.93 (1.27 to 2.93)	.0045
Trans-nonachlor					
μg/g lipid	≤0.0105	0.0105–0.0165	0.0166–0.0245	>0.0245	
Case subjects (n)	59	77	75	102	
Control subjects (n)	252	220	221	220	
Relative risk (95% CI)	1.00 (referent)	1.44 (0.94 to 2.21)	1.28 (0.82 to 2.00)	1.72 (1.11 to 2.67)	.033
Oxychlordane					
μg/g lipid	≤0.00805	0.00806–0.0116	0.0117–0.0171	>0.0171	
Case subjects (n)	58	70	89	96	
Control subjects (n)	238	225	225	225	
Relative risk (95% CI)	1.00 (referent)	1.33 (0.86 to 2.06)	1.62 (1.04 to 2.53)	1.64 (1.04 to 2.60)	.048
p,p′-DDE					
μg/g lipid	≤0.157	0.158–0.250	0.251–0.390	>0.390	
Case subjects (n)	59	68	57	128	
Control subjects (n)	238	230	220	224	
Relative risk (95% CI)	1.00 (referent)	1.17 (0.76 to 1.78)	0.98 (0.62 to 1.54)	1.91 (1.22 to 2.99)	.0008
p,p′-DDT					
μg/g lipid	≤0.0209	0.0210–0.259	0.260–0.397	>0.397	
Case subjects (n)	260	11	19	22	
Control subjects (n)	784	43	43	42	
Relative risk (95% CI)	1.00 (referent)	0.59 (0.29 to 1.19)	1.20 (0.67 to 2.14)	1.30 (0.73 to 2.30)	.40
β-hexachlorocyclohexane					
μg/g lipid	≤0.00582	0.00583–0.00804	0.00805–0.0115	>0.0115	
Case subjects (n)	104	64	66	78	
Control subjects (n)	355	185	188	185	
Relative risk (95% CI)	1.00 (referent)	1.07 (0.72 to 1.58)	1.03 (0.68 to 1.55)	0.97 (0.63 to 1.49)	.83
Mirex					
μg/g lipid	≤0.00474	0.00475–0.00640	0.00641–0.00952	>0.00952	
Case subjects (n)	208	30	34	40	
Control subjects (n)	642	91	89	90	
Relative risk (95% CI)	1.00 (referent)	0.84 (0.53 to 1.34)	0.86 (0.55 to 1.36)	1.15 (0.75 to 1.77)	.74
Total chlordanes†					
Case subjects (n)	52	80	73	108	
Control subjects (n)	229	227	228	229	
Relative risk (95% CI)	1.00 (referent)	1.64 (1.06 to 2.54)	1.33 (0.84 to 2.10)	1.90 (1.20 to 3.00)	.025

* Adjusted for age at blood donation, ethnicity, date of serum draw, age at reference date, cryptorchism, family history, height, and body mass index. Relative risk assumed to be equivalent to the odds ratio based on the rare disease assumption. CI = confidence interval; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; *p,p′*-DDT = *p,p′*-dichlorodiphenyltrichloroethane.

† Total chlordanes = *cis*-nonachlor + *trans*-nonachlor + oxychlordane.

(17,18). If substantial exposure occurred in utero, it is conceivable that *p,p′*-DDT and/or *p,p′*-DDE could also be related to the risk of the congenital malformations of the testicular dysgenesis syndrome.

Several studies in humans have examined the relationship between serum DDT or DDE and cryptorchism (19–23), and none have reported a statistically significant association. Similarly, at least three studies of hypospadias and DDT/DDE have been reported (19,20,24), and all yielded null results. In addition, a proxy measure of fetal androgen action, anogenital distance, was not associated in humans with in utero exposure to DDE (25). Taken together, these results do not support a strong association between DDT/DDE exposure and the congenital anomalies that comprise the testicular dysgenesis syndrome.

Of the two testicular dysgenesis syndrome disorders that arise in adulthood, impaired spermatogenesis and TGCT, there is more evidence of a link between DDT and the former condition. Studies of semen quality (26–29) have reported that decreased sperm concentration and motility and/or abnormal morphology may be associated with DDT/DDE exposure, although not all study results have been consistent (30). As for TGCT, only one prior study of DDE has been reported (31). Examining a Swedish population and using a case–control design, Hardell et al. (31) found no association between serum levels of DDE and TGCT in the study participants or in mothers of the participants. The study was small, however (58 case subjects and 61 control subjects), so it may not have had adequate power to detect an association. Furthermore, in the study of Hardell et al. (31), the blood samples were drawn between 1997 and 2000, in contrast with this study, for which the blood samples were drawn as early as 1987. Because DDT/DDE levels in the populations of developed countries have been

Table 4. Adjusted relative risk of nonseminoma by quartile of lipid-adjusted levels of serum organochlorines in the Servicemen's Testicular Tumor Environmental and Endocrine Determinants Study*

Quartile characteristic	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Cis-nonachlor					
μg/g lipid	≤0.00218	0.00219–0.00280	0.00281–0.00386	>0.00386	
Case subjects (n)	310	39	35	38	
Control subjects (n)	670	81	82	80	
Relative risk (95% CI)	1.00 (referent)	1.32 (0.86 to 2.03)	1.22 (0.78 to 1.90)	1.32 (0.86 to 2.03)	.12
Trans-nonachlor					
μg/g lipid	≤0.0105	0.0105–0.0165	0.0166–0.0245	>0.0245	
Case subjects (n)	134	109	72	107	
Control subjects (n)	252	220	221	220	
Relative risk (95% CI)	1.00 (referent)	1.16 (0.82 to 1.64)	0.83 (0.56 to 1.21)	1.39 (0.96 to 2.00)	.10
Oxychlordane					
μg/g lipid	≤0.00805	0.00806–0.0116	0.0117–0.0171	>0.0171	
Case subjects (n)	129	100	99	94	
Control subjects (n)	238	225	225	225	
Relative risk (95% CI)	1.00 (referent)	1.02 (0.71 to 1.45)	1.03 (0.71 to 1.50)	1.11 (0.75 to 1.63)	.58
p,p′-DDE					
μg/g lipid	≤0.157	0.158–0.250	0.251–0.390	>0.390	
Case subjects (n)	127	98	89	108	
Control subjects (n)	238	230	220	224	
Relative risk (95%CI)	1.00 (referent)	0.98 (0.69 to 1.39)	1.10 (0.76 to 1.59)	1.63 (1.10 to 2.42)	.0044
p,p′-DDT					
μg/g lipid	≤0.0209	0.0210–0.259	0.260–0.397	>0.397	
Case subjects (n)	369	16	21	15	
Control subjects (n)	784	43	43	42	
Relative risk (95% CI)	1.00 (referent)	1.02 (0.55 to 1.90)	1.39 (0.79 to 2.42)	0.94 (0.50 to 1.77)	.86
β-hexachlorocyclohexane					
μg/g lipid	≤0.00582	0.00583–0.00804	0.00805–0.0115	>0.0115	
Case subjects (n)	202	96	58	65	
Control subjects (n)	355	185	188	185	
Relative risk (95% CI)	1.00 (referent)	1.05 (0.75 to 1.45)	0.66 (0.45 to 0.97)	0.85 (0.57 to 1.26)	.24
Mirex					
μg/g lipid	≤0.00474	0.00475–0.00640	0.00641–0.00952	>0.00952	
Case subjects (n)	310	40	29	42	
Control subjects (n)	642	91	89	90	
Relative risk (95% CI)	1.00 (referent)	1.28 (0.85 to 1.95)	0.90 (0.57 to 1.43)	1.24 (0.82 to 1.88)	.36
Total chlordanes†					
Case subjects (n)	118	116	83	105	
Control subjects (n)	229	227	228	229	
Relative risk (95% CI)	1.00 (referent)	1.17 (0.82 to 1.67)	0.91 (0.62 to 1.34)	1.37 (0.93 to 2.02)	.13

* Adjusted for age at blood donation, ethnicity, date of serum draw, age at reference date, cryptorchism, family history, height, and body mass index. Relative risk assumed to be equivalent to the odds ratio based on the rare disease assumption. CI = confidence interval; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; *p,p′*-DDT = *p,p′*-dichlorodiphenyltrichloroethane.

† Total chlordanes = *cis*-nonachlor + *trans*-nonachlor + oxychlordane.

declining over time, it is possible that lower levels in the period between 1997 and 2000 would make an association difficult to detect (32). Finally, evidence from studies of DDT/DDE in breast milk suggests that DDT/DDE exposures in Sweden were lower, in general, than those in the United States (33). For example, the serum DDE levels found in this study were approximately 250% higher than the levels reported in the Swedish study. If the relative risks calculated in this study are accurate, the population-attributable risk of DDE (ie, the proportion of disease in the study population that is attributable to DDE exposure) would be approximately 15% for all TGCT (95% CI = 0% to 31%) and approximately 29% for seminoma (95% CI = 0% to 46%).

If DDE exposure is associated with the adult testicular dysgenesis syndrome disorders and not with the congenital malformations, it may indicate that male reproductive disorders do not become manifest until a higher body burden of DDE is attained. As noted by several studies (11,32), DDE levels increase with age. Alternatively, the etiologies of the perinatal and adult reproductive disorders may differ with respect to DDT exposure.

Chlordane, a cyclodiene insecticide that is metabolized to oxychlordane, consists of more than 140 isomers, including *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and a closely related cyclodiene insecticide, heptachlor (34). Chlordane was used in the United States between 1947 and 1988 on agricultural crops and as a termiticide. The association of the chlordane isomers with the testicular dysgenesis syndrome disorders has not been studied as extensively as that of DDT/DDE. Two studies have examined whether higher chlordane levels are associated with cryptorchism. One of the studies (21) examined adipose levels of chlordane in boys and found no association, whereas the other

study (22) examined breast milk chlordane levels in mothers of affected boys and found a statistically significant association. Studies of the association between cryptorchism and serum heptachlor have also resulted in inconsistent findings (20,22,35). Collectively, these studies suggest that the chlordane isomers might be associated with risk of cryptorchism.

An association between TGCT and serum levels of chlordane isomers was examined by Hardell et al. (31) in the same Swedish population in which serum DDE levels were examined. Consistent with the association detected in this study, serum *cis*-nonachlor levels were statistically significantly higher in men with TGCT, particularly in men with seminoma (OR = 4.8, 95% CI = 1.4 to 16.0, $P = .04$). In addition, the mothers of case subjects in the Swedish study had statistically significantly higher levels of *cis*-nonachlor ($P = .02$), *trans*-nonachlor ($P = .008$), and total chlordanes ($P = .04$) than mothers of control subjects. In contrast to the men, however, the levels of DDE were statistically significantly elevated only among the mothers of nonseminoma case subjects, although the odds ratios were somewhat elevated among the mothers of the seminoma case subjects as well. However, given the small number of tumors, particularly after stratification by histology, it is unclear whether any discrepancy between the sons' and mothers' findings exists.

The underlying mechanism that would explain the association of chlordane with risk of TGCT is not clear. Chlordane does not appear to be estrogenic, as indicated by its inability to stimulate proliferation in the estrogen-dependent breast tumor cell line MCF-7 (36,37). There is some evidence from rodent models, however, that chlordane affects the hepatic metabolism of estrogens (38). Furthermore, chlordane may act as an androgenic agent by inhibiting the actions of aromatase, and, by acting as an antagonist of the estrogen-related receptor α-1, it may have a negative regulatory effect on aromatase expression (39). Based on studies in rodents of its carcinogenic properties, the International Agency for Research on Cancer classified chlordane as a possible human carcinogen (34). Whatever the underlying mechanism, an association of serum chlordane with seminoma, but not nonseminoma, is consistent with trends in TGCT incidence in the United States (1): rates of seminoma have continued to increase, whereas rates of nonseminoma appear to have plateaued. If DDE is associated with both seminoma and nonseminoma and DDE levels are falling in the population, the trend may indicate that DDE's contribution to TGCT incidence has peaked. Chlordane, however, was used for at least 15 years after DDT was banned, so levels may be proportionally higher than DDE levels in the population. The specific association of chlordane with the risk of seminoma might explain why seminoma rates are continuing to increase. Further research, however, will be required to determine whether the findings of this study are replicated in other populations. Based on the risks seen in this study, the population-attributable risks of the chlordane compounds for seminoma would be 28% (95% CI = 0% to 50%) for oxychlordane, 26% (95% CI = 0% to 48%) for *trans*-nonachlor, and 9% (95% CI = 0% to 12%) for *cis*-nonachlor.

A major advantage of this study was that prediagnostic serum samples were analyzed. Other advantages were that participants were drawn from a well-defined population, the response rate was high, the classification of the tumors was histologically confirmed, and the participants were likely to be representative of a wide spectrum of the underlying population. Our study also had several limitations. Some potential participants could not be contacted due to deployment, which could present a bias if the deployed men were systematically different than the nondeployed men. Because most young men in the military are healthy and fit, however, it is unlikely that deployment status introduced a substantial bias. Another limitation is that the analysis adjusted for self-reported body size rather than measured body size. The study, also, could not determine when and how the participants were exposed to POPs and thus could not determine the critical window of exposure. Finally, our analyses included multiple comparisons; thus, some caution should be exercised when interpreting the results. It is worth noting, however, that the relationship between *p,p′*-DDE and TGCT would remain statistically significant even after adjusting the *P* value using a Bonferroni correction and that the chlordane relationship with TGCT had been previously reported.

In conclusion, this study suggests that DDE is associated with the risk of all TGCTs in young men and that chlordane isomers are associated with the risk of seminoma. The results argue for further examination of the association of POPs and TGCT in other populations as chlordane exposure continues to occur and more widespread use of DDT is being considered in the developing world. Because greater use of DDT in any location may contribute to higher body burdens of DDT and DDE around the world, any relationship with deleterious outcomes should be investigated carefully.

References

1. McGlynn KA, Devesa SS, Sigurdson AJ, Brown LM, Tsao L, Tarone RE. Trends in the incidence of testicular germ cell tumors in the United States. *Cancer.* 2003;97(1):63–70.
2. McGlynn KA. Environmental and host factors in testicular germ cell tumors. *Cancer Invest.* 2001;19(8):842–853.
3. Dieckmann KP, Pichlmeier U. Clinical epidemiology of testicular germ cell tumors. *World J Urol.* 2004;22(1):2–14.
4. Skakkebaek NE, Berthelsen JG, Giwercman A, Muller J. Carcinoma-in-situ of the testis: possible origin from gonocytes and precursor of all types of germ cell tumours except spermatocytoma. *Int J Androl.* 1987;10(1):19–28.
5. Toppari J, Kaleva M. Maldescendus testis. *Horm Res.* 1999;51(6): 261–269.
6. Rajpert-De Meyts E, Bartkova J, Samson M, et al. The emerging phenotype of the testicular carcinoma in situ germ cell. *APMIS.* 2003;111(1): 267–278.
7. Henderson BE, Benton B, Jing J, Yu MC, Pike MC. Risk factors for cancer of the testis in young men. *Int J Cancer.* 1979;23(5):598–602.
8. Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet.* 1993;341(8857): 1392–1395.
9. Toppari J, Larsen JC, Christiansen P, et al. Male reproductive health and environmental xenoestrogens. *Environ Health Perspect.* 1996;104(suppl 4):741–803.
10. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod.* 2001;16(5):972–978.
11. Wolff MS, Britton JA, Teitelbaum SL, et al. Improving organochlorine biomarker models for cancer research. *Cancer Epidemiol Biomarkers Prev.* 2005;14(9):2224–2236.
12. Graubard BI, Fears TR. Standard errors for attributable risk for simple and complex sample designs. *Biometrics.* 2005;61(3):847–855.
13. Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect.* 1993;101(5):378–384.

14. Beard J. DDT and human health. *Sci Total Environ.* 2006;355(1–3): 78–89.
15. Longnecker MP. Invited commentary: why DDT matters now. *Am J Epidemiol.* 2005;162(8):726–728.
16. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. *Nature.* 1995;375(6532):581–585.
17. Lopez-Carrillo L, Torres-Sanchez L, Moline J, Ireland K, Wolff MS. Breast-feeding and serum p,p'DDT levels among Mexican women of childbearing age: a pilot study. *Environ Res.* 2001;87(3):131–135.
18. Sagiv SK, Tolbert PE, Altshul LM, Korrick SA. Organochlorine exposures during pregnancy and infant size at birth. *Epidemiology.* 2007;18(1): 120–129.
19. Longnecker MP, Klebanoff MA, Brock JW, et al. Maternal serum level of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene and risk of cryptorchidism, hypospadias, and polythelia among male offspring. *Am J Epidemiol.* 2002;155(4):313–322.
20. Bhatia R, Shiau R, Petreas M, Weintraub JM, Farhang L, Eskenazi B. Organochlorine pesticides and male genital anomalies in the child health and development studies. *Environ Health Perspect.* 2005;113(2):220–224.
21. Hosie S, Loff S, Witt K, Niessen K, Waag KL. Is there a correlation between organochlorine compounds and undescended testes? *Eur J Pediatr Surg.* 2000;10(5):304–309.
22. Damgaard IN, Skakkebaek NE, Toppari J, et al. Persistent pesticides in human breast milk and cryptorchidism. *Environ Health Perspect.* 2006; 114(7):1133–1138.
23. Waliszewski SM, Infanzon RM, Arroyo SG, et al. Persistent organochlorine pesticides levels in blood serum lipids in women bearing babies with undescended testis. *Bull Environ Contam Toxicol.* 2005;75(5):952–959.
24. Flores-Luevano S, Farias P, Hernandez M, et al. DDT/DDE concentrations and risk of hypospadias. Pilot case-control study [in Spanish]. *Salud Publica Mex.* 2003;45(6):431–438.
25. Longnecker MP, Gladen BC, Cupul-Uicab LA, et al. In utero exposure to the antiandrogen 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) in relation to anogenital distance in male newborns from Chiapas, Mexico. *Am J Epidemiol.* 2007;165(9):1015–1022.
26. Hauser R, Chen Z, Pothier L, Ryan L, Altshul L. The relationship between human semen parameters and environmental exposure to polychlorinated biphenyls and p,p'-DDE. *Environ Health Perspect.* 2003;111(12): 1505–1511.
27. Ayotte P, Giroux S, Dewailly E, et al. DDT spraying for malaria control and reproductive function in Mexican men. *Epidemiology.* 2001;12(3): 366–367.
28. De Jager C, Farias P, Barraza-Villarreal A, et al. Reduced seminal parameters associated with environmental DDT exposure and p,p'-DDE concentrations in men in Chiapas, Mexico: a cross-sectional study. *J Androl.* 2006;27(1):16–27.
29. Aneck-Hahn NH, Schulenburg GW, Bornman MS, Farias P, de Jager C. Impaired semen quality associated with environmental DDT exposure in young men living in a malaria area in the Limpopo Province, South Africa. *J Androl.* 2007;28(3):423–434.
30. Martin SA Jr, Harlow SD, Sowers MF, et al. DDT metabolite and androgens in African-American farmers. *Epidemiology.* 2002;13(4):454–458.
31. Hardell L, van Bavel B, Lindstrom G, et al. Increased concentrations of polychlorinated biphenyls, hexachlorobenzene, and chlordanes in mothers of men with testicular cancer. *Environ Health Perspect.* 2003;111(7): 930–934.
32. CDC. *Third National Report on Human Exposure to Environmental Chemicals.*Altanta, GA: National Center for Environmental Health, Centers for Disease Control and Prevention; 2005. NCEH publication 05-0570.
33. National Resources Defense Council. Healthy Milk, Healthy Baby. 2007. www.nrdc.org/breastmilk. Accessed January 16, 2008.
34. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, International Agency for Research on Cancer, World Health Organization. *Some Thyrotropic Agents.* Lyon, France: IARC Press; 2001.
35. Pierik FH, Klebanoff MA, Brock JW, Longnecker MP. Maternal pregnancy serum level of heptachlor epoxide, hexachlorobenzene, and beta-hexachlorocyclohexane and risk of cryptorchidism in offspring. *Environ Res.* 2007;105(3):364–369.
36. Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. *Environ Health Perspect.* 1995; 103(suppl 7):113–122.
37. Verma SP, Salamone E, Goldin B. Curcumin and genistein, plant natural products, show synergistic inhibitory effects on the growth of human breast cancer MCF-7 cells induced by estrogenic pesticides. *Biochem Biophys Res Commun.* 1997;233(3):692–696.
38. Welch RM, Levin W, Kuntzman R, Jacobson M, Conney AH. Effect of halogenated hydrocarbon insecticides on the metabolism and uterotropic action of estrogens in rats and mice. *Toxicol Appl Pharmacol.* 1971;19(2): 234–246.
39. Yang C, Chen S. Two organochlorine pesticides, toxaphene and chlordane, are antagonists for estrogen-related receptor alpha-1 orphan receptor. *Cancer Res.* 1999;59(18):4519–4524.

Funding

Funding for this research was provided by the intramural research program of the National Cancer Institute, National Institutes of Health.

Notes

The authors wish to thank Emily Steplowski and Leslie Carroll of Information Management Services for their contributions in data management and analysis. The authors are also greatly indebted to the STEED participants, without whom there would have been no study. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

The authors take full responsibility for the study design, data collection, analysis and interpretation of the data, the decision to submit the manuscript for publication, and the writing of the manuscript.

Manuscript received October 4, 2007; revised February 17, 2008; accepted March 7, 2008.