

Maternal Serum Level of 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene and Risk of Cryptorchidism, Hypospadias, and Polythelia among Male Offspring

Matthew P. Longnecker,¹ Mark A. Klebanoff,² John W. Brock,³ Haibo Zhou,^{1,4} Kimberly A. Gray,⁵ Larry L. Needham,³ and Allen J. Wilcox¹

1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE) is a metabolite of the insecticide 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) and is a ubiquitous environmental contaminant. Nearly everyone in the United States has a detectable serum level of DDE. DDE was recently found to inhibit binding of androgen to its receptor and to block androgen action in rodents. Normal development of male genitalia in mammals depends on androgen action. The authors used stored serum samples to examine the relation between maternal DDE levels during pregnancy and adjusted odds of cryptorchidism ($n = 219$), hypospadias ($n = 199$), and polythelia (extra nipples) ($n = 167$) among male offspring, using a nested case-control design with one control group ($n = 552$). Subjects were selected from the Collaborative Perinatal Project, a US birth cohort study begun in 1959–1966, when DDE levels were much higher than they are at present. Compared with boys whose mother's recovery-adjusted serum DDE level was less than 21.4 $\mu\text{g/liter}$, boys with maternal levels greater than or equal to 85.6 $\mu\text{g/liter}$ had adjusted odds ratios of 1.3 (95% confidence interval (CI): 0.7, 2.4) for cryptorchidism, 1.2 (95% CI: 0.6, 2.4) for hypospadias, and 1.9 (95% CI: 0.9, 4.0) for polythelia. For cryptorchidism and polythelia, the results were consistent with a modest-to-moderate association, but in no instance was the estimate very precise. The results were inconclusive. *Am J Epidemiol* 2002;155:313–22.

abnormalities; androgen antagonists; androgens; cryptorchidism; DDE; hypospadias; nipples

In 1995, Kelce et al. (1) reported that the ubiquitous environmental contaminant 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE; hereafter called DDE) inhibited androgen binding to the androgen receptor. DDE is a metabolite of 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT), an insecticide that was once used worldwide. While the use of DDT has been restricted in the United States since 1972, it is still used in some countries for disease-vector control. DDE is resistant to degradation; it bioaccumulates in the food chain, and it persists in measurable amounts in the serum of nearly everyone in the United States (2).

Received for publication November 14, 2000, and accepted for publication August 29, 2001.

Abbreviations: CI, confidence interval; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane.

¹Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

²Division of Epidemiology, Statistics, and Prevention Research, National Institute of Child Health and Human Development, Rockville, MD.

³National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA.

⁴Department of Biostatistics, University of North Carolina, Chapel Hill, NC.

⁵Epidemiologic Research and Information Center, Veterans Affairs Medical Center, Durham, NC.

Correspondence to Dr. Matthew P. Longnecker, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709 (e-mail: longnecker@niehs.nih.gov).

Normal development of male external genitalia depends on androgen action (3, 4). For example, genetic defects that interfere with androgen action in humans can result in cryptorchidism (failure of one or both testicle(s) to descend into the scrotum) or hypospadias (a urethral opening on the underside of the penis or on the perineum) (5). In addition, in male rodents, the destruction of mammary rudiments requires androgen (6, 7), and treatment with DDE in utero leads to retained nipples (1, 8). In humans, extra nipples are called supernumerary nipples or polythelia. We hypothesized that in-utero exposure to the androgen antagonist DDE could be related to the frequency of cryptorchidism, hypospadias, and polythelia among boys (9). To test this hypothesis, we studied a population with relatively high serum DDE levels: persons born in the United States in the early 1960s.

MATERIALS AND METHODS

Study subjects

The subjects were enrolled in the Collaborative Perinatal Project, a prospective study of neurologic disorders and other conditions in children (10). Pregnant women were recruited between 1959 and 1966 from 12 US study centers (Baltimore, Maryland; Boston, Massachusetts; Buffalo, New York; Memphis, Tennessee; Minneapolis, Minnesota; New Orleans, Louisiana; New York, New York (two centers); Philadelphia, Pennsylvania; Portland, Oregon; Providence, Rhode Island;

and Richmond, Virginia). In 11 study centers, patients were recruited from the participating university hospital's prenatal care clinic, and in one study center (Buffalo), patients were recruited from 13 participating private medical practices. The method of subject selection varied across study centers. For example, at Columbia-Presbyterian Medical Center, every sixth woman who was potentially eligible was invited to participate; at Charity Hospital in New Orleans, potentially eligible subjects were selected if their patient identification number ended in zero; and in Boston, all potentially eligible women were invited to participate. Women were ineligible if they were incarcerated, if they were planning to leave the area or give up the child up for adoption, or if they gave birth on the day they were recruited into the study. Records of the number of women who refused to participate at baseline were not kept, but participation rates were assumed to be high (e.g., the rate was >99 percent at the Johns Hopkins center in Baltimore (Janet Hardy, Johns Hopkins University, personal communication, 2001)).

The characteristics of women in the sample at registration were essentially the same as those in the sampling frame (10). Four percent of subjects who enrolled were lost to follow-up before delivery. Once the women were enrolled, nonfasting blood samples were collected from them approximately every 8 weeks, as well as at delivery and 6 weeks postpartum. Serum samples were stored in glass at -20°C , with no recorded thaws. Approximately 42,000 women were enrolled in the study, and 55,000 children were born into the study. The children were systematically assessed for the presence of birth defects and other outcomes through the age of 7 years. Follow-up to age 7 years was completed for approximately 75 percent of subjects born into the study.

We used a nested case-control design to examine the association between maternal serum DDE level and risk of cryptorchidism, hypospadias, and polythelia among sons. Eligibility criteria were that the infant had to be 1) male, 2) liveborn, and 3) a singleton and that 4) a 3-ml aliquot of third-trimester maternal serum had to be available. We considered subjects to have cryptorchidism if they were classified as having an undescended testicle(s) at any time during the first year of life. Subjects first observed to have an undescended testicle(s) after the first year of life were not considered cryptorchid, because they might have had retractile testes. For hypospadias and polythelia, diagnosis at any time up to age 7 years was accepted. Among the 28,444 boys in the Collaborative Perinatal Project, 267 were not liveborn and 441 were not singletons; for 5,389, no maternal blood sample was available.

Among the eligible 22,347 boys, there were 241 cases of cryptorchidism, 214 cases of hypospadias, and 185 cases of polythelia. The eight subjects with more than one of these defects were included in each defect group that applied. From the eligible subjects, we selected 599 controls at random, so that the control:case ratio would be more than 2:1 for each defect under study. Other than restriction of the study to males, there were no other matching criteria. A laboratory result for DDE was not obtained for 100 of the 1,239 potentially eligible subjects (8 percent), mainly because the measured value did not meet quality control criteria for

acceptance (11). Two additional subjects were excluded because no laboratory results for serum lipid were available. Thus, in the final analysis, there were 219 boys with cryptorchidism, 199 with hypospadias, and 167 with polythelia and 552 controls. The proportion of subjects without a DDE measure was similar across these groups (cryptorchidism, 8 percent; hypospadias, 9 percent; polythelia, 7 percent; controls, 9 percent).

Among boys diagnosed as cryptorchid during the first year of life, the study records indicated that the testicles were descended at birth in 103. Because the cremasteric reflex (which causes retraction of the testicles) is not well developed in the first year of life (3), we assumed that these 103 subjects had been misclassified as normal on their birth examination. To evaluate this assumption, we considered these subjects separately in a sensitivity analysis (described below). Among boys with at least one undescended testicle at birth ($n = 138$), all but one also had a subsequent observation of the abnormality in at least one of the three subsequent physical examinations (ages 4 months, 1 year, and 7 years) or orchidopexy. Regarding hypospadias, the Collaborative Perinatal Project records did not note the degree. For polythelia, the presence of mammary tissue or areola or the number of supernumerary nipples was not recorded. Of the eight subjects who had more than one of the defects, five subjects had cryptorchidism and hypospadias, two had cryptorchidism and polythelia, and one had hypospadias and polythelia.

The socioeconomic index calculated for subjects in the Collaborative Perinatal Project was the mean of three percentile scores (for education, occupation, and family income), where education was that of the head of the household, occupation was that of the head of the household or the chief wage-earner, and the score used to calculate the percentile for an occupation was based on the percentiles of education and income among persons with the same occupation (12).

Laboratory assays

Serum levels of p,p' -DDT and p,p' -DDE were measured in 1997–1999 after solid-phase extraction, cleanup, and dual-column gas chromatography using electron capture detection (11). The proportion of DDE in the samples recovered by extraction averaged 70 percent (range, 57–85 percent), and the between-batch coefficient of variation in recovery was 8.2 percent ($n = 312$). The within-batch variation in recovery was not estimated; without this, it was not possible to know whether adjustment of DDE levels for proportion of material recovered gave estimates that were more accurate and precise than the unadjusted values. Thus, results are presented with and without recovery adjustment. All subjects had serum DDE values above the method detection limit (0.61 $\mu\text{g/liter}$). Among the analytical batches that included a subject with a given type of birth defect, 91 percent or more included at least one male control from the same center, and 87 percent of batches contained an aliquot from a single large pool, used to calculate the between-assay coefficient of variation. The between-assay coefficient of variation was 19 percent at 29 $\mu\text{g DDE/liter}$ ($n = 291$). The

order of specimens within-batch was determined by a random process. The laboratory personnel were masked with respect to the type of sample. Serum levels of cholesterol and triglycerides were measured using standard enzymatic methods (13). Serum sodium was measured by inductively coupled plasma-atomic emission spectroscopy (14), to assess desiccation in the samples.

We determined DDE levels in serum from the third trimester, because these samples were the most complete. In 67 women selected at random from the Collaborative Perinatal Project, the Pearson's correlation coefficient between lipid-adjusted DDE levels measured in the first and third trimesters was 0.86 (15). DDE crosses the placenta, and in a US study conducted around 1980, levels in the mother's serum at delivery and the child's umbilical cord serum were correlated at $r = 0.79$ (16).

Statistical methods

To divide subjects into categories based on DDE exposure, we identified a set of four equally spaced cutpoints that yielded at least 20 controls per group and a large contrast in exposure between those in the lowest and highest categories. We estimated the odds of having a birth defect in relation to DDE level using conditional logistic regression, conditional on study center (12 strata). Serum levels of DDE are proportional to those of lipid; thus, serum triglycerides and cholesterol were included as continuous variables in models wherein DDE was expressed per unit volume of serum. In one set of models, we expressed DDE on a per-unit-serum-lipid basis and did not include the lipid levels as independent variables. To express DDE on a per-unit-serum-lipid basis, we estimated the lipid content of serum using the formula given by Phillips et al. (17) and the level of total cholesterol and triglycerides in each sample. Furthermore, race was related to the occurrence of polythelia and was associated with DDE levels (Blacks had more polythelia and higher DDE levels than Whites), so all models were adjusted for race (White, Black, other).

Confounding was evaluated by comparing the coefficient for DDE from models including lipids and race with the coefficients in a model with an additional factor. DDE was modeled as a continuous variable and as a categorical variable, and results from both types of models were used to evaluate the change in estimate. If the odds ratio per 15 $\mu\text{g/liter}$ of DDE changed by 15 percent or more or the odds ratio for the contrast between the highest exposure categories and the lowest changed by 15 percent or more, the factor was considered a confounder. The factors considered as potentially confounding variables were season of birth, mother's age, parity, socioeconomic index, body mass index (weight (kg)/height (m)²) before pregnancy, weight gain during pregnancy, smoking during pregnancy, hyperemesis gravidarum, hypertension, age at menarche, history of infertility, menstrual cycle irregularity, estrogen use during pregnancy, progesterone use during pregnancy, method of delivery, and serum sodium level. We similarly considered the effect of adjustment for preterm birth, birth weight, smallness for gestational age, and gestational age, even though these were

potentially intermediate variables. Using a similar approach but with cross-product terms, we evaluated effect modification by race, birth weight, gestational age, hormone use, and study center. Evaluation of effect modification by categorical variables with more than two categories was supplemented by comparing the model-fit statistics for models with and without the cross-product terms. For effect modification, the p values associated with the interaction terms or likelihood ratio tests all had values greater than 0.10, and thus the degree of potential effect modification was not considered further. All statistical analyses were conducted using the SAS statistical software package, version 8.0 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Based on the distribution among controls (table 1), the study population was nearly half White and nearly half Black, with the remainder being of other races (primarily Hispanic and Asian). Based on median levels in controls, birth weight and gestational age were typical of healthy births; the mothers were relatively young (about one third were primiparas); and the socioeconomic index of the subjects was just under that for the United States as a whole. The skewed distribution of DDE values (a long tail to the right) was evident from the category counts among controls. The median level of recovery-adjusted DDE among controls was roughly five times greater than it is at present in the United States (see Discussion). The median sodium level among controls was 130 mmol/liter, which is in the range that would be expected for pregnant women if fresh specimens were used.

In comparison with White controls, Blacks were more likely to have polythelia, and boys of "other" races were less likely to have it (table 1). Birth weight was slightly lower among boys with hypospadias. The median age of mothers of boys with cryptorchidism and hypospadias was slightly greater than that for controls. The median socioeconomic index was lower among mothers of boys with hypospadias and polythelia than among control mothers. The median levels of cholesterol and triglycerides were lowest among the mothers of boys with polythelia. The median level of DDE was elevated among mothers of boys with polythelia as compared with the other subject groups.

For cryptorchidism and hypospadias, the adjusted odds ratio among boys in the highest category of serum DDE compared with boys in the lowest was modestly elevated, but the confidence intervals were wide (table 2). For polythelia, the adjusted odds ratio for the same exposure contrast was moderately increased, though again the confidence interval was wide. For all three birth defects, when DDE was modeled as a continuous variable (scaled to odds ratio per 15- $\mu\text{g/liter}$ increase), all odds ratios were greater than 1 but were small and imprecise. The addition of a quadratic term for DDE to models that included DDE as a continuous variable did not improve the fit. The crude and adjusted results were essentially the same, although for polythelia the crude odds ratio was increased in the highest exposure category because of confounding by race. Further

TABLE 1. Characteristics of mothers and male offspring in a study of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene and male birth defects, according to the child subject's case/control status, Collaborative Perinatal Project, 1959–1966*

Characteristic	Cases			Controls (<i>n</i> = 552)
	Cryptorchidism (<i>n</i> = 219)	Hypospadias (<i>n</i> = 199)	Polythelia (<i>n</i> = 167)	
<i>Child subjects</i>				
Race (%)				
White	57	51	17	47
Black	42	44	82	47
Other	1	5	1	6
Season of birth (%)				
January–March	25	17	30	24
April–June	28	25	25	24
July–September	25	30	24	26
October–December	23	28	21	26
Method of delivery (%)				
Spontaneous vertex	89	89	96	92
Spontaneous breech	3	4	1	2
Cesarean	8	7	3	6
Median gestational age (weeks)	40 (38, 41)†	39 (38, 41)	40 (38, 41)	40 (38, 41)
Preterm birth (%)				
Yes	15	19	15	14
No	85	81	85	86
Median birth weight (g)	3,260 (2,863, 3,629)	3,147 (2,665, 3,487)	3,317 (2,977, 3,572)	3,260 (2,948, 3,600)
Small for gestational age (%)				
Yes	9	18	5	5
No	91	82	95	95
<i>Mothers</i>				
Median age (years)	24 (20, 30)	23 (20, 29)	22 (19, 27)	22 (20, 28)
Parity‡ (%)				
0	26	31	34	31
1	23	22	17	23
≥2	51	47	49	46
Median socioeconomic index§	47 (33, 63)	45 (30, 63)	37 (23, 50)	47 (33, 60)
Median prepregnancy body mass index¶	22.2 (20.4, 25.0)	21.8 (19.6, 24.1)	22.0 (20.1, 24.4)	22.1 (19.7, 24.9)
Median rate of pregnancy weight gain (average g/week)	249 (177, 341)	256 (195, 344)	262 (214, 351)	268 (198, 344)
Median age at menarche (years)	13 (12, 14)	12 (12, 13)	13 (12, 14)	13 (12, 13)
Smoking (%)				
Yes	49	48	41	46
No	51	52	59	54
Gestational hypertension (%)				
Yes	6	6	5	7
No	94	94	95	93
History of infertility (%)				
Yes	0	1	1	1
No	100	99	99	99

Table continues

TABLE 1. Continued

Characteristic	Cases			Controls (n = 552)
	Cryptorchidism (n = 219)	Hypospadias (n = 199)	Polythelia (n = 167)	
History of regular menstrual cycles (%)				
No	6	6	4	7
Yes	94	94	94	93
Hyperemesis gravidarum (%)				
Yes	1	2	1	0
No	99	98	99	100
Estrogen use during pregnancy (%)				
Yes	0	1	0	1
No	100	99	100	99
Progestin use during pregnancy (%)				
Yes	4	1	2	3
No	96	99	98	97
Median total cholesterol level (mg/dl)	232 (190, 273)	232 (192, 278)	223 (189, 261)	233 (195, 279)
Median triglyceride level (mg/dl)	202 (161, 252)	188 (153, 256)	173 (136, 222)	205 (159, 259)
Median sodium level (mmol/liter)	128 (123, 138)	129 (124, 136)	128 (125, 134)	130 (125, 141)
Study center (%)				
Boston, Massachusetts	33	27	8	24
Buffalo, New York	7	4	4	4
New Orleans, Louisiana	7	4	9	4
New York, New York (Columbia- Presbyterian Medical Center)	2	4	4	3
Baltimore, Maryland	6	6	3	8
Richmond, Virginia	7	6	12	6
Minneapolis, Minnesota	4	4	3	6
New York, New York (New York Medical College)	1	5	1	8
Portland, Oregon	4	6	7	7
Philadelphia, Pennsylvania	15	24	17	18
Providence, Rhode Island	11	8	2	5
Memphis, Tennessee	3	2	30	7
Median DDE# level ($\mu\text{g/liter}$)	23.6 (15.9, 35.3)	23.9 (16.6, 34.4)	31.9 (20.6, 47.7)	24.4 (16.6, 37.1)
Median recovery-adjusted DDE level ($\mu\text{g/liter}$)	34.1 (22.8, 51.4)	34.1 (24.0, 49.3)	45.2 (29.6, 69.3)	34.3 (23.9, 52.7)
Median lipid-basis recovery-adjusted DDE level ($\mu\text{g/g}$)	4.31 (2.85, 6.52)	4.18 (2.88, 5.85)	6.33 (4.02, 10.19)	4.24 (2.95, 6.62)

* Numbers of subjects with missing data: smallness for gestational age, 94; parity, 1; socioeconomic index, 11; prepregnancy body mass index, 88; rate of pregnancy weight gain, 38; age at menarche, 13; smoking status, 7; gestational hypertension, 105; infertility, 7; regularity of menses, 42.

† Numbers in parentheses, quartile cutpoints.

‡ Number of previous livebirths.

§ Percentile relative to contemporaneous US distribution. (See Materials and Methods section for details).

¶ Weight (kg)/height (m)².

DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene.

adjustment for the other factors considered as potentially confounding variables had no substantial effect on results (the largest change in the odds ratio due to the inclusion of any factor was 11 percent); as mentioned above, notable effect modification was not observed.

Use of recovery-adjusted DDE levels gave results (table 3) that were nearly the same as those found when no recovery adjustment was done (table 2), although for polythelia the odds ratio for the highest category of DDE compared with the lowest was slightly increased. When recovery-

TABLE 2. Crude and adjusted odds ratios for specified birth defects among male offspring in relation to concentration of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene in the mother's serum, Collaborative Perinatal Project, 1959–1966*

Birth defect and DDE† exposure (μg/liter)	No. of cases	No. of controls	Crude odds ratio	Adjusted‡ odds ratio	95% confidence interval
Cryptorchidism					
<15.0	45	104	1§	1§	
15.0–29.9	104	239	1.0	1.1	0.8, 1.6
30.0–44.9	41	121	0.9	1.0	0.6, 1.5
45.0–59.9	14	58	0.7	0.8	0.4, 1.5
≥60	15	30	1.3	1.4	0.8, 2.6
Total no.	219	552			
Per 15.0 μg/liter			1.041	1.060	0.953, 1.178
Hypospadias					
<15.0	39	104	1	1	
15.0–29.9	95	239	1.1	1.1	0.8, 1.7
30.0–44.9	35	121	0.9	0.9	0.6, 1.5
45.0–59.9	18	58	0.9	1.1	0.6, 1.9
≥60	12	30	1.1	1.3	0.6, 2.5
Total no.	199	552			
Per 15.0 μg/liter			0.995	1.019	0.903, 1.150
Polythelia					
<15.0	16	104	1	1	
15.0–29.9	64	239	1.4	1.3	0.7, 2.2
30.0–44.9	42	121	1.4	1.2	0.6, 2.2
45.0–59.9	21	58	1.4	1.1	0.5, 2.2
≥60	24	30	2.1	1.7	0.8, 3.3
Total no.	167	552			
Per 15.0 μg/liter			1.079	1.051	0.955, 1.156

* Results were obtained from conditional logistic regression models (conditional on study center).

† DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene.

‡ Adjusted for race (White, Black, other), triglyceride level, and cholesterol level.

§ Referent.

adjusted DDE levels were expressed on a per-unit-serum-lipid basis (table 4), again the results were similar, but for hypospadias the odds ratio for the highest category of DDE compared with the lowest was 1.0.

We also evaluated the results for cryptorchidism after including only cases who had at least one undescended testicle at birth; the results were essentially the same as those shown in table 2. We attempted to examine results according to whether the cryptorchidism was unilateral ($n = 153$) or bilateral ($n = 66$), but only three cases with bilateral cryptorchidism were in the top two exposure categories—too few to allow a meaningful comparison. For each birth defect, we evaluated associations separately according to whether there were other birth defects present (defects of any type—for example, cardiac defects and oral clefts). This stratification resulted in case cell counts in the ≥ 60 -μg DDE/liter exposure category of ≤ 5 ; thus, results were imprecise (data not shown), and differences between multiple and single defects were difficult to interpret. For example, the odds ratios from the ≥ 60 -μg/liter versus < 15 -μg/liter comparisons were as follows: for hypospadias—single defect, 1.0 (95 percent confidence interval (CI): 0.4, 2.4); multiple defects, 1.8 (95 percent CI: 0.6, 5.1); for polythelia—single

defect, 2.6 (95 percent CI: 1.0, 6.5); multiple defects, 1.0 (95 percent CI: 0.3, 3.4). In addition, when the eight subjects with more than one of the male birth defects were excluded, the results were essentially unchanged (data not shown). The relation of serum DDT level to the odds of defects was also evaluated, and associations were weaker than those in table 2 (data not shown).

DISCUSSION

For boys in the highest category of DDE exposure compared with those in the lowest, the odds ratios were consistent with an association of modest (cryptorchidism) or moderate (polythelia) size, but in no instance was the estimate precise. Although some findings suggested a modest association of DDE with hypospadias, this depended on how exposure was represented in the models. Overall, our results were inconclusive.

Among mothers of controls in the present study, representing 12 US cities in 1959–1966, the median serum recovery-adjusted DDE level was 34.3 μg/liter. More recently reported serum DDE levels in the United States have been lower (e.g., 1.3 μg/liter (18) and 7.1 μg/liter (19)), which is

TABLE 3. Adjusted odds ratios for specified birth defects among male offspring in relation to recovery-adjusted concentration of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene in the mother's serum, Collaborative Perinatal Project, 1959–1966*

Birth defect and DDE† exposure ($\mu\text{g/liter}$)	No. of cases	No. of controls	Adjusted‡ odds ratio	95% confidence interval
Cryptorchidism				
<21.4	48	97	1§	
21.4–42.7	99	247	0.9	0.7, 1.3
42.8–64.1	43	119	0.9	0.6, 1.5
64.2–85.5	13	59	0.7	0.4, 1.3
≥ 85.6	16	30	1.3	0.7, 2.4
Total no.	219	552		
Per 21.4 $\mu\text{g/liter}$			1.060	0.952, 1.180
Hypospadias				
<21.4	41	97	1	
21.4–42.7	91	247	1.0	0.7, 1.4
42.8–64.1	42	119	1.0	0.6, 1.6
64.2–85.5	11	59	0.6	0.3, 1.2
≥ 85.6	14	30	1.2	0.6, 2.4
Total no.	199	552		
Per 21.4 $\mu\text{g/liter}$			1.014	0.897, 1.146
Polythelia				
<21.4	13	97	1	
21.4–42.7	63	247	1.4	0.7, 2.6
42.8–64.1	43	119	1.3	0.7, 2.6
64.2–85.5	23	59	1.3	0.6, 2.6
≥ 85.6	25	30	1.9	0.9, 4.0
Total no.	167	552		
Per 21.4 $\mu\text{g/liter}$			1.053	0.958, 1.157

* Results were obtained from conditional logistic regression models (conditional on study center).

† DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene.

‡ Adjusted for race (White, Black, other), triglyceride level, and cholesterol level.

§ Referent.

consistent with the known decrease that has taken place since about 1970 (20). In approximate terms, then, the median exposure level in the Collaborative Perinatal Project was more than five times greater than that which exists at present. By comparison, in a South African population whose homes are sprayed annually with DDT for mosquito control, the mean serum DDE level is 103 $\mu\text{g/liter}$ (21).

Steady increases in rates of cryptorchidism and hypospadias have been reported in the United States since 1970, when data first became available (22). In the United States, at the same time that rates of cryptorchidism and hypospadias increased, background-level exposure to DDE was decreasing (20). These trends, being in opposite directions, suggest that background-level DDE exposure had little effect on risks of cryptorchidism and hypospadias. Nonetheless, if a maternal serum recovery-adjusted DDE level of ≥ 85.6 $\mu\text{g/liter}$ (the highest category shown in table 3) did slightly increase risk, the contribution to the number of US cases would have been small, given the relatively small proportion of subjects (about 5 percent) exposed at that level. Thus, an effect of DDE on trends in cryptorchidism or hypospadias, if any, could easily have been lost among the effects of other determinants of trend that either

affected a greater proportion of the population or had larger effects.

In animals, antiandrogenic agents clearly can cause cryptorchidism, hypospadias, and retained nipples (23). The first report on the antiandrogenic effect of DDE (1) showed that a large dose of DDE given to pregnant rats caused male offspring to have reduced anogenital distance and retained nipples. In a subsequent study (8), DDE effects were found to vary across rat species, and, in sensitive species, dose-response analyses revealed that DDE was a relatively weak antiandrogen. In rodents, the outcome most sensitive to DDE is retained nipples, and these were seen in the offspring of rat dams whose mean serum DDE level was 110 $\mu\text{g/liter}$ while pregnant, 2 days after their last dose (8). Because our results were more suggestive of an association for polythelia than for the other defects, and because our results for polythelia were most suggestive in the group with recovery-corrected DDE levels of ≥ 85.6 $\mu\text{g/liter}$ (the highest exposure category in table 3), the animal and human data show interesting parallels.

In recent studies of risk factors for cryptorchidism and hypospadias (24–26), both defects were directly linked to preterm birth and smallness for gestational age and were

TABLE 4. Adjusted odds ratios for specified birth defects among male offspring in relation to recovery-adjusted lipid-basis concentration of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene in the mother's serum, Collaborative Perinatal Project, 1959–1966*

Birth defect and DDE† exposure (μg/g)	No. of cases	No. of controls	Adjusted‡ odds ratio	95% confidence interval
Cryptorchidism				
<2.67	48	102	1§	
2.67–5.34	92	256	0.9	0.6, 1.2
5.35–8.01	45	97	1.1	0.7, 1.7
8.02–10.69	14	54	0.8	0.4, 1.4
≥10.70	20	43	1.3	0.7, 2.2
Total no.	219	552		
Per 2.67 μg/g			1.072	0.972, 1.183
Hypospadias				
<2.67	42	102	1	
2.67–5.34	96	256	0.9	0.6, 1.4
5.35–8.01	33	97	0.9	0.6, 1.5
8.02–10.69	12	54	0.7	0.3, 1.3
≥10.70	16	43	1.0	0.6, 1.9
Total no.	199	552		
Per 2.67 μg/g			1.013	0.902, 1.137
Polythelia				
<2.67	13	102	1	
2.67–5.34	59	256	1.1	0.6, 2.1
5.35–8.01	38	97	1.2	0.6, 2.4
8.02–10.69	21	54	1.0	0.5, 2.2
≥10.70	36	43	1.7	0.8, 3.4
Total no.	167	552		
Per 2.67 μg/g			1.058	0.969, 1.155

* Results were obtained from conditional logistic regression models (conditional on study center).

† DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene.

‡ Adjusted for race (White, Black, other).

§ Referent.

inversely associated with parity. This suggests that the etiology of these two male defects is partly shared (24). In multivariate analyses of our data, preterm birth and smallness for gestational age were also associated with an increased risk for these conditions, but the study was relatively small and most effect estimates were imprecise (data not shown). Risk factors for polythelia have been less well studied, although race and urogenital malformations have been consistently related to risk (27, 28).

The greater risk of polythelia among Blacks could be due in part to DDE exposure (Blacks have higher DDE levels (2)). All studies of polythelia among Blacks were US studies, beginning in the 1960s. In our data, Black race remained an important predictor of polythelia when DDE was included in the model. The odds ratio for polythelia among Blacks as compared with Whites (in a model adjusted for triglycerides, cholesterol, and "other" race) was 3.5 (95 percent CI: 1.9, 6.5) prior to adjustment for DDE (five categories) and 3.3 (95 percent CI: 1.7, 6.2) after adjustment. The Pearson correlation coefficient between race (Black vs. other) and log_e DDE was 0.33—that is, not high enough that keeping both in the model would obscure an effect of DDE.

In the Collaborative Perinatal Project, birth defects were ascertained through the age of 7 years. A greater proportion of cases than of controls were followed to age 7 (82 percent vs. 75 percent). The greater loss to follow-up among controls could have led to a relative underdiagnosis of birth defects. However, such defects were sufficiently rare that if they had been undiagnosed, the resulting misclassification would not have affected our results much. Furthermore, at least 85 percent of the known defects were diagnosed during the first year of life. Median DDE levels among all cases and controls followed to age 7 years were 25.0 mg/liter and 23.8 μg/liter, respectively; and among boys not followed to age 7 years, median DDE levels among all cases and controls were 27.4 mg/liter and 26.9 μg/liter, respectively. These figures, and the combination of the relatively complete follow-up and the predominance of early diagnosis of birth defects, suggest that bias from cases' being misclassified as controls because of loss to follow-up would be positive but negligible in magnitude. The rates of cryptorchidism and hypospadias reported in the Collaborative Perinatal Project (29) were higher than those in other US reports (22). In the Collaborative Perinatal Project, cases were identified in a series of systematic examinations, whereas data in birth defect registries are typically

based on routine reports and records only (29). We excluded as cases boys for whom cryptorchidism was initially diagnosed after the first year of life, to reduce the possibility of mixing boys with retractile testes into our case group. For polythelia, the systematic examination used in the Collaborative Perinatal Project was not especially effective in detecting cases. When newborns have been examined specifically for polythelia (30, 31), the rate has been higher than that seen in the Collaborative Perinatal Project. In any event, the diagnoses of the three defects studied were made by personnel who had no information about the subject's DDE exposure, so any bias due to misclassification of cases probably caused the odds ratios to be closer to the null than they would have been if such error had been absent (32). Similarly, if an association was present, nondifferential error in exposure measurement probably caused the odds ratio per unit of change in serum DDE to appear smaller than it actually was (33).

Expression of serum organochlorine levels on a per-unit-serum-lipid basis has been recommended (34), and we did so here to provide results in a format familiar to some readers. The usual method underlying the lipid-basis metric is estimation of total serum lipids from total cholesterol and triglyceride levels using a formula (17). The origins of the formula, however, have never been clearly described. If one applies the formula in the population used to derive it (35), using the mean total cholesterol and triglyceride levels in that population, total serum lipid levels are not especially well predicted. Furthermore, whether the formula works well enough to estimate total lipids in pregnant women (or any other population, for that matter) is unclear. The goal here was to examine associations with organochlorine levels after accounting for variation in organochlorine levels due to the concentration of lipids, and that goal was better achieved by letting our own data determine the best coefficients for lipid adjustment. Moreover, because a substantial portion of organochlorine in serum is not associated with lipid, expressing concentration on a per-unit-serum-lipid basis is misleading (36). The attenuation of the odds ratios for the high:low exposure category comparison in table 4 as compared with table 3 may be entirely due to misclassification resulting from expression of exposure levels on a per-unit-serum-lipid basis. Similarly, for the reasons stated above (see "Laboratory assays"), we have little faith that recovery adjustment of our exposure values improved DDE measures in these data. Our adjusted measures were useful, however, when comparing our DDE levels with those reported by other investigators.

In summary, these data alone provide no clear evidence of an effect of DDE on male development, but given the experimental and emerging human evidence (37) of DDE as an androgen antagonist, there remains the suspicion that high maternal levels of DDE may affect the development of male offspring.

REFERENCES

1. Kelce WR, Stone CR, Laws SC, et al. Persistent DDT metabolite *p,p'*-DDE is a potent androgen receptor antagonist. *Nature* 1995;375:581-5.

2. Stehr-Green PA. Demographic and seasonal influences on human serum pesticide residue levels. *J Toxicol Environ Health* 1989;27:405-21.
3. Hutson JM, Hasthorpe S, Heys CF. Anatomical and functional aspects of testicular descent and cryptorchidism. *Endocr Rev* 1997;18:259-80.
4. Aaronson IA, Cakmak MA, Key LL. Defects of the testosterone biosynthetic pathway in boys with hypospadias. *J Urol* 1997;157:1884-8.
5. Ahmed SF, Cheng A, Dovey L, et al. Phenotypic features, androgen receptor binding, and mutational analysis in 278 clinical cases reported as androgen insensitivity syndrome. *J Clin Endocrinol Metab* 2000;85:658-65.
6. Imperato-McGinley J, Sanchez RS, Spencer JR, et al. Comparison of the effects of the 5 alpha-reductase inhibitor finasteride and the antiandrogen flutamide on prostate and genital differentiation: dose-response studies. *Endocrinology* 1992;131:1149-56.
7. Durnberger H, Kratochwil K. Specificity of tissue interaction and origin of mesenchymal cells in the androgen response of the embryonic mammary gland. *Cell* 1980;19:465-71.
8. You L, Casanova M, Archibeque-Engle S, et al. Impaired male sexual development in perinatal Sprague-Dawley and Long-Evans hooded rats exposed in utero and lactationally to *p,p'*-DDE. *Toxicol Sci* 1998;45:162-73.
9. Dolk H. Rise in prevalence of hypospadias. *Lancet* 1998;351:770.
10. Niswander KR, Gordon M. The women and their pregnancies. (Publication no. (NIH) 73-379). Washington, DC: US Department of Health, Education, and Welfare, 1972.
11. Brock JW, Burse VW, Ashley DL, et al. An improved analysis for chlorinated pesticides and polychlorinated biphenyls (PCBs) in human and bovine sera using solid-phase extraction. *J Anal Toxicol* 1996;20:528-36.
12. Myrianthopoulos NC, French KS. An application of the U.S. Bureau of the Census socioeconomic index to a large, diversified patient population. *Soc Sci Med* 1968;2:283-99.
13. McNamara JR, Schaefer EJ. Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. *Clin Chim Acta* 1987;166:1-8.
14. Mermet JM, Hubert J. Analysis of biological materials using plasma atomic emission spectroscopy. *Prog Analyt Atom Spectrosc* 1982;5:1-32.
15. Longnecker MP, Klebanoff MA, Gladen BC, et al. Serial levels of serum organochlorines during pregnancy and postpartum. *Arch Environ Health* 1999;54:110-14.
16. Rogan WJ, Gladen BC, McKinney JD, et al. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethane (DDE) in human milk: effects of maternal factors and previous lactation. *Am J Public Health* 1986;76:172-7.
17. Phillips DL, Pirkle JL, Burse VW, et al. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch Environ Contam Toxicol* 1989;18:495-500.
18. Berkowitz GS, Lapinski RH, Wolff MS. The role of DDE and polychlorinated biphenyl levels in preterm birth. *Arch Environ Contam Toxicol* 1996;30:139-41.
19. Laden F, Neas LM, Spiegelman D, et al. Predictors of plasma concentrations of DDE and PCBs in a group of U.S. women. *Environ Health Perspect* 1999;107:75-81.
20. Kutz FW, Wood PH, Bottimore DP. Organochlorine pesticides and polychlorinated biphenyls in human adipose tissue. *Rev Environ Contam Toxicol* 1991;120:1-82.
21. Bouwman H, Cooppan RM, Becker PJ, et al. Malaria control and levels of DDT in serum of two populations in KwaZulu. *J Toxicol Environ Health* 1991;33:141-55.
22. Paulozzi LJ. International trends in rates of hypospadias and cryptorchidism. *Environ Health Perspect* 1999;107:297-302.
23. Gray LE Jr, Ostby JS, Kelce WR. Developmental effects of an environmental antiandrogen: the fungicide vinclozolin alters sex differentiation of the male rat. *Toxicol Appl Pharmacol* 1994;129:46-52.
24. Akre O, Lipworth L, Cnattingius S, et al. Risk factor patterns

- for cryptorchidism and hypospadias. *Epidemiology* 1999;10:364–9.
25. Weidner IS, Moller H, Jensen TK, et al. Risk factors for cryptorchidism and hypospadias. *J Urol* 1999;161:1606–9.
 26. Berkowitz GS, Lapinski RH, Godbold JH, et al. Maternal and neonatal risk factors for cryptorchidism. *Epidemiology* 1995;6:127–31.
 27. Gilmore HT, Milroy M, Mello BJ. Supernumerary nipples and accessory breast tissue. *S D J Med* 1996;49:149–51.
 28. Velanovich V. Ectopic breast tissue, supernumerary breasts, and supernumerary nipples. *South Med J* 1995;88:903–6.
 29. Myriantopoulos NC, Chung CS. Congenital malformations in singletons: epidemiologic survey. Report from the Collaborative Perinatal Project. *Birth Defects* 1974;10:1–58.
 30. Mimouni F, Merlob P, Reisner SH. Occurrence of supernumerary nipples in newborns. *Am J Dis Child* 1983;137:952–3.
 31. Kenney RD, Flippo JL, Black EB. Supernumerary nipples and renal anomalies in neonates. *Am J Dis Child* 1987;141:987–8.
 32. Weinberg CR, Umbach DM, Greenland S. Re: “When will nondifferential misclassification of an exposure preserve the direction of a trend?” Weinberg et al. reply. (Letter). *Am J Epidemiol* 1995;142:784.
 33. Carrol RJ. Measurement error in epidemiologic studies. In: Armitage P, Colton T, eds. *Encyclopedia of biostatistics*, vol 3. New York, NY: John Wiley and Sons, Inc, 1998:2491–519.
 34. Woodruff T, Wolff MS, Davis DL, et al. Organochlorine exposure estimation in the study of cancer etiology. *Environ Res* 1994;65:132–44.
 35. Patterson DG Jr, Needham LL, Pirkle JL, et al. Correlation between serum and adipose tissue levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in 50 persons from Missouri. *Arch Environ Contam Toxicol* 1988;17:139–43.
 36. Noren K, Weistrand C, Karpe F. Distribution of PCB congeners, DDE, hexachlorobenzene, and methylsulfonyl metabolites of PCB and DDE among various fractions of human blood plasma. *Arch Environ Contam Toxicol* 1999;37:408–14.
 37. Ayotte P, Giroux S, Dewailly E, et al. DDT spraying for malaria control and reproductive function in Mexican men. (Letter). *Epidemiology* 2001;12:366–7.