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Wise, Lauren A.

National Institute of Environmental Health Sciences


http://hdl.handle.net/2144/2811

Boston University
Secondary Sex Ratio among Women Exposed to Diethylstilbestrol in Utero

Lauren A. Wise,1 Julie R. Palmer,1 Elizabeth E. Hatch,2 Rebecca Troisi,3,4 Linda Titus-Ernstoff,3 Arthur L. Herbst,5 Raymond Kaufman,6 Kenneth L. Noller,7 and Robert N. Hoover4

1Slone Epidemiology Center, Boston University, Boston, Massachusetts, USA; 2Department of Epidemiology, Boston University School of Public Health, Boston, Massachusetts, USA; 3Norris Cotton Cancer Center, Dartmouth Hitchcock Medical Center, Lebanon, New Hampshire, USA; 4Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA; 5Department of Obstetrics and Gynecology, University of Chicago, Chicago, Illinois, USA; 6Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas, USA; 7Department of Obstetrics and Gynecology, New England Medical Center, Boston, Massachusetts, USA

Diethylstilbestrol (DES) is a synthetic estrogen that was prescribed to > 2 million pregnant women during the mid-1900s. In later years, it was discovered to be associated with the occurrence of vaginal clear cell adenocarcinoma (Herbst et al. 1971), anatomic abnormalities of the reproductive tract (Stillman 1982), and poor reproductive outcomes in daughters (Beral and Colwell 1981; Kaufman et al. 2000; Palmer et al. 2001). DES exposure in utero may also exert long-term effects on female endocrine function, possibly leading to permanent dysregulation of the hypothalamic–pituitary–ovarian axis and alterations in hormone biosynthesis in adult women (Assies 1991; Peress et al. 1982; Wu et al. 1980). Although the effect of DES on plasma sex hormones has not been well studied, elevated levels of serum testosterone (Wu et al. 1980), but not luteinizing hormone (LH) (Peress et al. 1982; Wu et al. 1980), progesterone (Wu et al. 1980), or estrogens (Peress et al. 1982; Wu et al. 1980), have been documented in DES daughters. Levels of follicle-stimulating hormone (FSH) were elevated in DES-exposed women in one study, but no differences were found in LH or the ratio of FSH to LH (Peress et al. 1982); another study found no difference in FSH levels (Wu et al. 1980). Animal data show that in vitro production of testosterone, total estrogen, and progesterone by ovarian tissue was significantly greater in female mice exposed prenatally to DES, at all ages studied (Haney et al. 1984).

Secondary sex ratio (proportion of male births)—a prevalence measure that reflects both sex programming at the time of conception and survival until birth—may be influenced by exposure to endocrine disruptors such as DES. In humans, several studies have examined the relation between preconceptual exposure to endocrine-disrupting compounds and secondary sex ratio, but most associations were observed in men and were mixed in direction (James 2006). With respect to maternal exposure, a significant decrease in sex ratio was found in studies of polychlorinated biphenyls (PCBs) (Weisskopf et al. 2003) and mercury (Sakamoto et al. 2001), but the majority of studies have been null, including those that examined maternal exposure to dioxin (Mocarelli et al. 2000; Rogan et al. 1999; Ryan et al. 2002; Yoshimura et al. 2001). PCBs (del rio Gomez et al. 2002; Karmaus et al. 2002; Taylor et al. 2006; Taylor et al. 1989), lead (Jarrell et al. 2006), and dichlorodiphenyltrichloroethane (DDT) (Cocco et al. 2006; Karmaus et al. 2002).

Moreover, dose and timing of exposure have been incompletely characterized in many studies, and little is known about the relation of these chemicals to the maternal endogenous hormonal milieu. For instance, PCBs have estrogenic, antiestrogenic, and androgenic properties (Bonefeld-Jorgensen et al. 2001), making the direction and magnitude of their effects difficult to predict. In a small study of preconception maternal PCB concentrations that stratified by hormonal activity of the PCB, Taylor et al. (2006) found that the odds of a male birth were elevated among women exposed to estrogenic but not antiestrogenic PCBs. Although not statistically significant, these results suggest that PCBs have different biologic effects depending on their underlying hormonal activity.

A prevailing hypothesis is that endocrine disruptors such as DES may affect secondary sex ratio through changes in hormonal concentrations around the time of conception (James 1987). In women, high levels of gonadotropins (FSH and LH) and progesterone are hypothesized to change the ratio toward more girls, whereas high testosterone and estrogen levels change the ratio toward more boys (James 1987). Another hypothesis, the “over-ripeness ovopathy” theory (Jongbloet 2004), postulates that sex ratio is influenced by both oocyte maturation and the quality of cervical mucus, with nonoptimal hormonal modulation favoring male-biased progeny. Nonoptimal liquefaction of cervical mucus may facilitate differential migration of sperm, with increased fertilization by Y-bearing sperm because the head, length, perimeter, and area are significantly smaller and the neck and tail are shorter in Y-bearing sperm than in X-bearing sperm (Cui 1997). Because concurrence of both oocyte maturation and cervical mucus liquefaction is modulated by estrogens.
before the midcycle, any perturbations to the endogenous estrogenic milieu caused by endocrine disruptors may theoretically affect sex ratio (Jongbloet 2004).

To our knowledge, there are no studies of secondary sex ratio in women exposed to DES, either prenatally or preconceptually, and most animal studies of this association are null. Specifically, studies in female mice (Honma et al. 2002; Suzuki et al. 2002), rats (Odum et al. 2002), and Chinese rare minnows (Zhong et al. 2005) have found no association between prenatal DES exposure and secondary sex ratio, whereas studies in rats exposed preconceptionally to DES had an increased proportion of male offspring (Sharpe et al. 1995).

We evaluated the association between in utero DES exposure in women and the secondary sex ratio of their offspring in a large collaborative study of participants with and without documented exposure to DES. Based on previous studies of sex steroid hormone levels in women exposed in utero to DES and the possible influence of these hormones on sex ratio (James 1987), we hypothesized that DES-exposed women would have a higher proportion of male offspring than unexposed women.

Materials and Methods

Study population. The National Cancer Institute (NCI) DES Combined Cohort Study began in 1992 and includes four individual cohorts of DES-exposed and unexposed women that combine participants from several field centers. The methods of the original studies from which these cohorts were assembled have been described elsewhere (Bibbo et al. 1977; Colton et al. 1993; Dieckmann et al. 1953; Horne and Kundsinn 1985; Labarthe et al. 1978). Briefly, participants from three cohorts originally identified for study during the 1950s–1970s—the Diethylstilbestrol Adenosis Project (DESAD); Labarthe et al. 1978), Dieckmann (Bibbo et al. 1977; Dieckmann et al. 1953), and Horne (Horne and Kundsinn 1985)—were traced and contacted for follow-up by the NCI. The Dieckmann cohort study was a clinical trial designed to test the efficacy of DES in preventing adverse pregnancy outcomes among women receiving routine prenatal care at the University of Chicago (Chicago, IL). The DESAD and Horne cohorts were derived from clinic populations. A fourth cohort, the Women’s Health Study (WHS), included the female offspring of women who had participated in a 1970s health study of DES-exposed and unexposed mothers. The study protocol was approved by the human subjects committees at the field centers and by the NCI. Women provided informed consent by completing and returning the mailed questionnaires, or by participating in a telephone interview.

From the four cohorts, 7,439 daughters were identified in 1992 as eligible for follow-up. Of these, 84 were deceased, and 804 had refused further contact during the original cohort studies or were untraceable. In 1994, 6,551 study participants (88% of the original surviving cohort) were contacted by mail and were sent a baseline questionnaire eliciting information on reproductive and contraceptive history, lifestyle and behavioral factors, medical conditions, medication use, and health care utilization. Participants were called for a telephone interview if they did not respond to two mailed questionnaires. In 1997, a follow-up questionnaire was sent to update reproductive and medical information. A total of 5,707 (87%) participants responded to the 1994 questionnaire (3,546 exposed and 1,761 unexposed), and 5,579 (85%) responded to the 1997 questionnaire (3,853 exposed and 1,686 unexposed). For the present study, we included women who completed at least the 1994 questionnaire. We then excluded nulliparous women [1,433 (41%) exposed and 481 (27%) unexposed], parous women who did not give information on offspring sex [16 (1%) exposed and 5 (1%) unexposed], and women who reported multifetal births but no singletons [1 (1%) exposed and 0 (0%) unexposed].

Assessment of exposure, outcome, and covariates. DES exposure status was verified in all cohorts by medical record. The completeness of data on DES dose and gestational timing of first exposure varied across the four cohorts. Data on DES dose and timing were carefully documented in the Dieckmann cohort, with participants being exposed to high cumulative doses of DES (median cumulative dose, ~12 g) in adherence to the regimen of Smith and Smith (1949). Women in the Horne cohort were generally given high doses of DES, and records of dose and timing were available on almost all women. Exposure in the DESAD cohort was difficult to estimate because of incomplete information from medical records. In this cohort, estimates ranged from a median cumulative dose of approximately 1.5–2.5 g at Baylor College of Medicine (Houston, TX) and the Mayo Clinic (Rochester, MN) to 8.5 g at the Boston Lying In Hospital (Boston, MA) (Labarthe et al. 1978). Data on dose and timing were unavailable in the WHS cohort.

Information on DES dose was available for 36% of exposed women. We used 5 g as a cutpoint for “low” versus “high” dose because the distribution was bimodal, with peaks at about 2 g and 12 g. The cohorts included women from several regions of the United States. As regional DES prescribing practices were similar, we conducted a secondary analysis in which women with missing data on dose were assigned an imputed value based on the median dose for their specific field center. Assigned doses were as follows: Dieckmann, 12.4 g; Horne, 10.4 g; Boston/DESAD, 8.5 g; California/DESAD, 7.9 g; Baylor/DESAD, 2.6 g; Wisconsin/DESAD, 3.2 g; and Mayo/DESAD, 1.5 g.

Gestational age at first DES exposure (in weeks), available for 74% of exposed women, was evaluated to assess whether biological susceptibility was related to timing of exposure. Although gestational age at first exposure ranged considerably within the cohorts, mean values were generally lower (i.e., earlier) in the Horne cohort. In cross-tabulations of dose and timing, the data were dichotomized at 13 weeks gestational age because the first trimester represents a period of heightened susceptibility for the developing fetal reproductive system (Sadler 2004).

Information on DES dose and timing was available for 33% of exposed women: 100% of Dieckmann participants, 30% of DESAD, 70% of Horne, and 0% of WHS. Women with complete data on dose and timing were more likely than those without complete data to be younger (year of birth 1960 or later: 18.2% vs. 11.5%), report a history of infertility (41.3% vs. 33.3%), and be primiparous (34.5% vs. 27.9%). No significant differences were found with respect to maternal age at first birth, age at menarche, education, body mass index (BMI), or smoking status.

On both the 1994 and 1997 questionnaires, women reported the outcome of each pregnancy (singleton vs. multiple live birth, stillbirth, spontaneous abortion, induced abortion, and other), the sex of each birth, and the date of the child’s birth. Infertility history was elicited on the 1994 questionnaire using two commonly employed definitions: whether the participant ever tried to become pregnant for ≥12 months without success, and whether the participant ever sought medical assistance for infertility from a health care provider. Women were also asked if they had ever used fertility drugs.

Statistical methods. Participants were allowed to contribute more than one birth to the analysis. Analyses were restricted to 7,732 singleton live births with complete information on offspring sex and birth date (4,968 exposed and 2,764 unexposed). We estimated odds ratios (ORs) and 95% confidence intervals (CIs) for the association of prenatal DES exposure with secondary sex ratio using generalized estimating equations (GEE) to account for nonindependence (Li and Zeger 1993). ORs overestimate prevalence ratios when the outcome is common, as was the case in our study. Nevertheless, we present ORs for comparability with other studies of secondary sex ratio.

We considered maternal age, cigarette smoking, calendar year of child’s birth, parity
(i.e., birth order), and the use of fertility drugs as potential confounders. Factors associated with DES exposure that changed the OR by > 2% were included in the final regression models. Based on these criteria, multivariable models controlled for maternal age at conception (< 25, 25–29, 30–34, 35–39, ≥ 40 years), parity (1, 2, ≥ 3), and calendar year of child’s birth (before 1980, 1980–1984, 1985–1989, 1990 or later). Models were further adjusted for cohort (DESAD, Dieckmann, Horne, WHS) because of cohort-related differences in study methodology and participant recruitment. Tests for trend by dose or timing were performed by adding to the regression model a single ordinal term coded as 0, 1, and 2 for no DES, low dose, and high dose, or 0, 1, 2, and 3 for no DES, first exposure at 13 weeks or later, 9–12 weeks, and before 9 weeks, respectively (Breslow and Day 1987). Because plasma levels of endogenous estrogens have been shown to decrease with increasing age (Dorgan et al. 1995) and parity (Bernstein et al. 1986), we evaluated whether maternal age (< 30 vs. ≥ 30 years) and parity (1 vs. ≥ 2 live births) modified the association between DES and sex ratio. We also examined interaction by cohort. Likelihood ratio tests were used to evaluate statistical interaction by comparing models with and without cross-product terms between DES exposure and covariates of interest.

Finally, it was well established that in utero DES exposure is associated with decreased fertility (Kaufman et al. 2000; Palmer et al. 2001). Although GEE analyses that include multiple births per mother provide additional statistical power, the use of all births could conceivably bias results toward the null if the influence of DES varies among exposed women. For example, if there are women who are less sensitive to the effects of DES, and the effect of DES on parity is related to its effect on sex ratio, then the GEE approach may overrepresent DES-exposed women who are less sensitive to its effects (e.g., multiparous women). To address this concern, we performed a separate logistic regression analysis among first-borns only (i.e., one birth per mother), which may represent a less biased sample. Analyses were carried out using SAS statistical software (SAS Institute Inc. 2004).

### Results

Women exposed to DES in utero were slightly younger, more educated, more likely to report a history of infertility and use fertility drugs, and less likely to have ever smoked cigarettes than unexposed women (Table 1). Exposed and unexposed women were similar with respect to BMI at 20 years of age, ever use of oral contraceptives, sexual history, and alcohol consumption. Over 96% of women were white (data not shown).

Table 1 shows the association between prenatal DES exposure and secondary sex ratio, overall and according to DES dose and gestational age at first exposure. DES-exposed women gave birth to 2,607 boys and 2,361 girls (sex ratio = 0.525), and unexposed women gave birth to 1,406 boys and 1,358 girls (sex ratio = 0.509), resulting in an unadjusted OR for having a male birth of 1.07 (95% CI, 0.97–1.17). After adjustment for maternal age, calendar year of child’s birth, parity, and study cohort, the OR for having a male infant comparing exposed to unexposed women was 1.05 (95% CI, 0.95–1.17).

The odds of conceiving a male birth increased slightly with decreasing gestational age at first DES exposure, but there was no statistical evidence of a trend (p-trend = 0.186). Among women with complete information on timing of DES exposure, the fully adjusted ORs for first exposure at ≥ 13, 9–12, and < 9 weeks of gestation relative to no exposure were 1.03 (95% CI, 0.89–1.18), 1.06 (95% CI, 0.92–1.23), and 1.08 (95% CI, 0.93–1.24), respectively.

Women first exposed to DES earlier in gestation and at higher doses had the highest odds of having a male birth (Table 2). Among women with complete information on DES dose and timing (33%), the fully adjusted ORs for having a male birth were 0.91 (95% CI, 0.65–1.27) for first exposure

<table>
<thead>
<tr>
<th>Prevalence of parous women with and without prenatal DES exposure (94%)</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>(n = 2,496)</td>
<td>(n = 1,275)</td>
</tr>
<tr>
<td>Cohort</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DESAD</td>
<td>2,090 (83.7)</td>
<td>607 (47.6)</td>
</tr>
<tr>
<td>Dieckmann</td>
<td>137 (5.5)</td>
<td>156 (12.2)</td>
</tr>
<tr>
<td>Horne</td>
<td>70 (2.8)</td>
<td>46 (3.6)</td>
</tr>
<tr>
<td>WHS</td>
<td>199 (8.0)</td>
<td>466 (36.6)</td>
</tr>
<tr>
<td>New</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Born before 1950</td>
<td>463 (18.5)</td>
<td>374 (29.3)</td>
</tr>
<tr>
<td>1950–1954</td>
<td>1,063 (42.6)</td>
<td>537 (41.2)</td>
</tr>
<tr>
<td>1955–1959</td>
<td>629 (25.2)</td>
<td>300 (23.6)</td>
</tr>
<tr>
<td>1960 or later</td>
<td>341 (13.7)</td>
<td>64 (5.1)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school or less</td>
<td>393 (15.8)</td>
<td>292 (22.9)</td>
</tr>
<tr>
<td>Some college</td>
<td>644 (25.9)</td>
<td>353 (27.7)</td>
</tr>
<tr>
<td>College</td>
<td>840 (33.7)</td>
<td>374 (29.4)</td>
</tr>
<tr>
<td>Graduate school</td>
<td>612 (24.6)</td>
<td>254 (20.0)</td>
</tr>
<tr>
<td>BMI at 20 years of age (kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>1,051 (43.4)</td>
<td>522 (42.3)</td>
</tr>
<tr>
<td>20–24</td>
<td>1,200 (49.6)</td>
<td>626 (50.7)</td>
</tr>
<tr>
<td>≥ 25</td>
<td>169 (7.0)</td>
<td>86 (7.0)</td>
</tr>
<tr>
<td>Use of fertility drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>2,108 (84.7)</td>
<td>1,185 (93.0)</td>
</tr>
<tr>
<td>Ever</td>
<td>382 (15.3)</td>
<td>89 (7.0)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1,442 (58.0)</td>
<td>640 (50.7)</td>
</tr>
<tr>
<td>Ever</td>
<td>1,046 (42.0)</td>
<td>632 (49.3)</td>
</tr>
<tr>
<td>No. of live births</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1,583 (64.0)</td>
<td>1,030 (81.5)</td>
</tr>
<tr>
<td>Yes</td>
<td>889 (36.0)</td>
<td>234 (18.5)</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of parous women with and without prenatal DES exposure (94%).

<table>
<thead>
<tr>
<th>Number of children</th>
<th>Male</th>
<th>Female</th>
<th>Proportion</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
<th>P-value, test for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexposed</td>
<td>1,406</td>
<td>1,358</td>
<td>0.509</td>
<td>1.00</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>Exposed</td>
<td>2,607</td>
<td>2,361</td>
<td>0.525</td>
<td>1.07 (0.97–1.17)</td>
<td>1.05 (0.95–1.17)</td>
<td>—</td>
</tr>
<tr>
<td>DES dose (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5</td>
<td>444</td>
<td>378</td>
<td>0.540</td>
<td>1.13 (0.96–1.33)</td>
<td>1.12 (0.95–1.33)</td>
<td>0.038</td>
</tr>
<tr>
<td>≥ 5</td>
<td>494</td>
<td>405</td>
<td>0.550</td>
<td>1.18 (1.01–1.37)</td>
<td>1.16 (0.98–1.36)</td>
<td>—</td>
</tr>
<tr>
<td>Gestational age at first exposure (weeks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 13</td>
<td>683</td>
<td>632</td>
<td>0.519</td>
<td>1.05 (0.91–1.19)</td>
<td>1.03 (0.89–1.18)</td>
<td>0.186</td>
</tr>
<tr>
<td>≥ 13</td>
<td>584</td>
<td>516</td>
<td>0.531</td>
<td>1.09 (0.95–1.26)</td>
<td>1.06 (0.92–1.23)</td>
<td>—</td>
</tr>
<tr>
<td>&lt; 9</td>
<td>687</td>
<td>609</td>
<td>0.530</td>
<td>1.03 (0.95–1.25)</td>
<td>1.08 (0.93–1.24)</td>
<td>—</td>
</tr>
<tr>
<td>≥ 9</td>
<td>584</td>
<td>516</td>
<td>0.531</td>
<td>1.09 (0.95–1.26)</td>
<td>1.06 (0.92–1.23)</td>
<td>—</td>
</tr>
</tbody>
</table>

Data on dose, timing, and both dose and timing were available for 36%, 74%, and 33% of women, respectively.

*Adjusted for maternal age at conception, year of child’s birth, parity, and cohort. Reference group for all column comparisons.
at \( \geq 13 \) weeks to \(<5 \) g, 0.95 (95% CI, 0.71–1.27) for first exposure at \( \geq 13 \) weeks to \( \geq 5 \) g, 1.16 (95% CI, 0.96–1.41) for first exposure at \( <13 \) weeks to \(<5 \) g, and 1.24 (95% CI, 0.88–1.83) for first exposure at \( <13 \) weeks to \( \geq 5 \) g, compared with no exposure.

Although the association between DES and sex ratio appeared to be stronger for participants in the Horne cohort (Table 3), the association was based on small numbers and was not statistically different from the other cohorts. Dose and timing results among the DESAD cohort were consistent with those found in the overall sample. Dose and timing results could not be assessed in the WHS, Dieckmann, and Horne cohorts either because of lack of data (i.e., WHS cohort) or because of limited variation in dose (i.e., all of the Dieckmann participants received >5 g) or timing (i.e., all Horne participants were first exposed to DES before 9 weeks of gestation).

The adjusted OR for the association between DES and sex ratio was similar among women with (OR = 1.03; 95% CI, 0.83–1.29) and without (OR = 1.03; 95% CI, 0.92–1.17) a history of infertility, defined as women who tried for \( \geq 12 \) months to conceive without success or sought medical assistance for infertility (\( p \)-interaction = 0.975). Within the subgroup of women with a history of infertility, the OR was 1.00 among women who had used fertility drugs (95% CI, 0.68–1.47) and 1.05 among women who had not (95% CI, 0.80–1.37; \( p \)-interaction = 0.744). Likewise, the association between DES and sex ratio was similar among women <30 years of age at the time of delivery (OR = 1.05; 95% CI, 0.91–1.20) compared with those \( \geq 30 \) years of age (OR = 1.06; 95% CI, 0.91–1.24; \( p \)-interaction = 0.952). With respect to parity status at the time of birth, the adjusted OR was not significantly different among primiparous women (OR = 1.10; 95% CI, 0.95–1.28) compared with multiparous women (OR = 1.02; 95% CI, 0.88–1.17; \( p \)-interaction = 0.317). OR estimates for dose and timing were similar across these subgroups and showed no evidence of statistical interaction (data not shown).

In analyses restricted to first births only, overall and dose-specific results were generally stronger than results among all births (Table 4). The fully adjusted OR for having a male birth was 1.37 (95% CI, 1.06–1.77) for first exposure at \( <13 \) weeks to \( \geq 5 \) g compared with no exposure.

### Discussion

The present findings are based on the largest study to date of U.S. women with documented intrauterine exposure to DES. Although we found no overall association between \( \text{in utero} \) DES exposure and secondary sex ratio, DES-exposed women who were first exposed earlier in gestation and to higher doses gave birth to a significantly higher proportion of males. If the developing female reproductive system is more susceptible to endocrine disruptors in the first trimester, the stronger association observed for women exposed to higher DES doses earlier in gestation is biologically plausible (Sadler 2004). These findings are the first to suggest a link between \( \text{in utero} \) DES exposure among women and the sex ratio of their offspring.

Previous research on maternal exposure to endocrine disruptors and secondary sex ratio has focused on exposure at times other than the prenatal period. Although our finding of an increased sex ratio among DES-exposed women is not consistent with two positive studies that found a significant decrease in sex ratio after maternal exposure to polychlorinated biphenyls (PCBs) (Weisskopf et al. 2003) and mercury (Sakamoto et al. 2001), it is consistent with a small study of preconception maternal PCB concentrations (Taylor et al. 2006). In the latter study, the odds of a male birth were elevated among women in the second (OR = 1.29) and third (OR = 1.48) tertiles of estrogenic PCBs, whereas odds were reduced among women in the highest tertile (OR = 0.70) of antioestrogenic PCBs (Taylor et al. 2006). Although the sample size was small (\( n = 99 \)) and the results were not statistically significant, the Taylor et al. (2006) study suggests that maternal exposure to chemicals with estrogenic properties might increase the likelihood of a male birth.

An important limitation of the present study is the high proportion of missing data on dose and timing of DES exposure (67% of exposed). A significant finding was observed only among those exposed earlier in gestation and to higher doses, but it is unclear whether or how our results would have changed had we acquired complete data on dose and timing. Among the exposed participants, the male birth proportions for women who did

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### Table 3. Offspring sex ratio in relation to prenatal DES exposure by study cohort.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>No. of children</th>
<th>Proportion male</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)(^a)</th>
<th>( p )-Value, test for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>DESAD</td>
<td>Unexposed</td>
<td>671</td>
<td>639</td>
<td>0.512</td>
<td>1.00(^b)</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>2,204</td>
<td>1,990</td>
<td>0.526</td>
<td>1.05 (0.93–1.19)</td>
</tr>
<tr>
<td>Dieckmann</td>
<td>Unexposed</td>
<td>179</td>
<td>162</td>
<td>0.525</td>
<td>1.00(^b)</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>146</td>
<td>121</td>
<td>0.547</td>
<td>1.10 (0.80–1.53)</td>
</tr>
<tr>
<td>Horne</td>
<td>Unexposed</td>
<td>34</td>
<td>48</td>
<td>0.415</td>
<td>1.04 (0.80–1.36)</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>57</td>
<td>48</td>
<td>0.543</td>
<td>1.67 (0.94–2.99)</td>
</tr>
<tr>
<td>WHS</td>
<td>Unexposed</td>
<td>522</td>
<td>509</td>
<td>0.506</td>
<td>1.00(^b)</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>200</td>
<td>202</td>
<td>0.498</td>
<td>0.97 (0.76–1.23)</td>
</tr>
</tbody>
</table>

\(^a\)Adjusted for maternal age at conception, year of child’s birth, and parity. \(^b\)Reference group for interaction tests.

### Table 4. Offspring sex ratio in relation to prenatal DES exposure, overall and by timing and dose, restricted to first births.

<table>
<thead>
<tr>
<th>No. of children</th>
<th>Proportion male</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)(^a)</th>
<th>( p )-Value, test for trend(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexposed</td>
<td>650</td>
<td>625</td>
<td>0.510</td>
<td>1.00(^b)</td>
</tr>
<tr>
<td>Exposed</td>
<td>1,339</td>
<td>1,155</td>
<td>0.537</td>
<td>1.12 (0.97–1.26)</td>
</tr>
<tr>
<td>DES dose (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;5)</td>
<td>229</td>
<td>197</td>
<td>0.538</td>
<td>1.12 (0.98–1.29)</td>
</tr>
<tr>
<td>(\geq5)</td>
<td>261</td>
<td>202</td>
<td>0.564</td>
<td>1.24 (1.00–1.54)</td>
</tr>
<tr>
<td>Gestational age at first exposure (weeks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\geq13)</td>
<td>347</td>
<td>284</td>
<td>0.550</td>
<td>1.17 (0.97–1.42)</td>
</tr>
<tr>
<td>(9–12)</td>
<td>299</td>
<td>265</td>
<td>0.521</td>
<td>1.05 (0.86–1.28)</td>
</tr>
<tr>
<td>(&lt;9)</td>
<td>277</td>
<td>205</td>
<td>0.553</td>
<td>1.19 (0.99–1.43)</td>
</tr>
<tr>
<td>Dose and timing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;5) g, (\geq13) weeks</td>
<td>54</td>
<td>49</td>
<td>0.524</td>
<td>1.06 (0.71–1.58)</td>
</tr>
<tr>
<td>(\geq5) g, (\geq13) weeks</td>
<td>48</td>
<td>50</td>
<td>0.490</td>
<td>0.92 (0.61–1.39)</td>
</tr>
<tr>
<td>(&lt;5) g, (&lt;13) weeks</td>
<td>147</td>
<td>130</td>
<td>0.531</td>
<td>1.09 (0.84–1.41)</td>
</tr>
<tr>
<td>(\geq5) g, (&lt;13) weeks</td>
<td>200</td>
<td>142</td>
<td>0.585</td>
<td>1.35 (1.06–1.72)</td>
</tr>
</tbody>
</table>

\(^a\)Adjusted for maternal age at conception, year of child’s birth, and cohort. \(^b\)Reference group for all column comparisons.
endocrine-disrupting compounds, we had a
Finally, in contrast to most studies of
rics of those who were and were not lost to
the 1994 questionnaire (87%), and no differ-
sure influenced the reporting of offspring sex,
ential misclassification of exposure. It is
both of which reduce the potential for differ-
attenuated ORs, as would be expected under
random exposure misclassification, but the
estimates were largely consistent with the
main results. Moreover, when analyses were
restricted to first births only—a sample that
may be less biased because it is not over-
represented by multiparous women—overall
dose-specific results were generally stronger. Nonetheless, our limited data on
dose and timing should be taken into account
when interpreting our results.

Strengths of the present study include the
verification of DES exposure status by medi-
cal record and the determination of exposure
status before reporting of birth outcomes,
both of which reduce the potential for differ-
cential misclassification of exposure. It is
unlikely that knowledge of one’s DES expo-
sure influenced the reporting of offspring sex,
as there is no information in the lay press
about the influence of DES on secondary sex
ratio. Given that similar proportions of
exposed and unexposed women completed
the 1994 questionnaire (87%), and no differ-
cences were found in the baseline character-
istics of those who were and were not lost to
follow-up (data not shown), selection bias is
also an unlikely explanation of our results.
Finally, in contrast to most studies of
endocrine-disrupting compounds, we had a
spectrum of data on dose and timing, which
allowed for an examination of dose–response
relations.

An association between in utero DES expo-
posure and secondary sex ratio in women is bio-
logically plausible. According to James (1987),
environmental toxicants may influence sec-
ondary sex ratio via changes in maternal hor-
monal concentrations around the time of
conception, with high concentrations of testos-
terone and estrogen increasing the probability
of a son and high concentrations of gonado-
tropins and progesterone increasing the proba-
bility of a daughter. Another theory postulates
that sex ratio is influenced by both oocyte mat-
uration and the quality of cervical mucus
(Jongbloet 2004). Given that both maturation and
cervical liquefaction are influenced by
estrogens before the midcycle, toxicals with
antiestrogenic properties might be expected
to increase the sex ratio.

Studies of prenatal DES exposure and its
long-term effects on endogenous hormones are
limited. The sole animal study of this relation
showed that in utero secretion of testosterone,
total estrogen, and progesterone in ovarian
tissue was significantly increased in female
mice exposed prenatally to DES (Haney et al.
1984). However, tissue production in utero
may not necessarily reflect total secretion of
these hormones in vivo, especially because the
ovaries of exposed mice were smaller than
those of unexposed mice. Only two human
studies have examined differences in
gonadotropins and sex hormones in associa-
tion with in utero DES exposure (Peress et al.
1982; Wu et al. 1980), reporting that DES
daughters had elevated levels of serum testos-
terone (Wu et al. 1980) but not estrogens
(Peress et al. 1982; Wu et al. 1980), prog-
testerone (Wu et al. 1980), or LH (Peress et al.
1982; Wu et al. 1980). Higher FSH levels
among DES-exposed women were found only by
Peress et al. (1982), but the FSH to LH ratio was
unaffected. In the study by Wu et al.
(1980), differences in testosterone were great-
est in the postovulatory and perimenstrual
phases of the menstrual cycle, suggesting that
the corpus luteum of DES daughters produces
more testosterone. Under James’ hormonal
hypothesis (James 1987), elevated testosterone
levels around the time of conception would be
expected to increase the proportion of male
births, as found in the present study. In con-
trast, the “over-ripeness ovopathy” hypothesis
(Jongbloet 2004) seems less plausible given
that no differences in plasma estrogen levels
were observed between DES-exposed and
unexposed women in either study (Peress et al.
1982; Wu et al. 1980).

The interpretation of the literature on sec-
ondary sex ratio has been subject to debate,
particularly with respect to the veracity and
significance of the declining sex ratios reported
in several countries worldwide (Bonde and
Wilcox 2007; Davis et al. 1998). Whereas
some epidemiologists have argued for the use
of sex ratio as a sentinel health indicator in
response to environmental exposures (Davis
et al. 1998), others have questioned its value,
citing its vulnerability both to false positive
reports and publication bias (Bonde and
Wilcox 2007).

In conclusion, although we found no
overall association between DES exposure in
utero and secondary sex ratio, a small
increase in the proportion of male births was
observed among women who were first
exposed earlier in gestation and to higher
doses. These results warrant confirmation in
other study populations with data on DES
dose and timing. If the association is real, it
grows to the concern held by some
epidemiologists that endocrine disruptors
may affect secondary sex ratio in humans.


