Testicular Dysgenesis Syndrome and the Estrogen Hypothesis: A Quantitative Meta-Analysis

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BACKGROUND: Male reproductive tract abnormalities such as hypospadias and cryptorchidism, and testicular cancer have been proposed to comprise a common syndrome together with impaired spermatogenesis with a common etiology resulting from the disruption of gonadal development during fetal life, the testicular dysgenesis syndrome (TDS). The hypothesis that in utero exposure to estrogenic agents could induce these disorders was first proposed in 1993. The only quantitative summary estimate of the association between prenatal exposure to estrogenic agents and testicular cancer was published over 10 years ago, and other systematic reviews of the association between estrogenic compounds, other than the potent pharmaceutical estrogen diethylstilbestrol (DES), and TDS end points have remained inconclusive.

OBJECTIVES: We conducted a quantitative meta-analysis of the association between the end points related to TDS and prenatal exposure to estrogenic agents. Inclusion in this analysis was based on mechanistic criteria, and the plausibility of an estrogen receptor (ER)-α-mediated mode of action was specifically explored.

RESULTS: We included in this meta-analysis eight studies investigating the etiology of hypospadias and/or cryptorchidism that had not been identified in previous systematic reviews. Four additional studies of pharmaceutical estrogens yielded a statistically significant updated summary estimate for testicular cancer.

CONCLUSIONS: The doubling of the risk ratios for all three end points investigated after DES exposure is consistent with a shared etiology and the TDS hypothesis but does not constitute evidence of an estrogenic mode of action. Results of the subset analyses point to the existence of unidentified sources of heterogeneity between studies or within the study population.

KEY WORDS: cryptorchidism, diethylstilbestrol, endocrine disruption, environment, estrogen, hypospadias, meta-analysis, oral contraceptives, testicular cancer, testicular dysgenesis. Environ Health Perspect 116:149–157 (2008). doi:10.1289/ehp.10545 available via http://dx.doi.org/ [Online 8 November 2007]
the body of research carried out since the formulation of the estrogen hypothesis. Inclusion in this analysis was based on mechanistic criteria, and the plausibility of an ER-α-mediated mode of action was specifically explored. Moreover, subset analysis has been applied to categories of compounds with estrogenic potencies differing by several orders of magnitude in an attempt to detect the existence of any potency–response trend. Most of the studies of sperm quantity or quality have been concerned with time trends rather than etiology, and this endpoint was not considered further here.

Material and Methods
Identification and selection of literature. A computerized search was conducted using the databases Pubmed (National Center for Biotechnology Information 2007) and Web of Science (ISI Web of Knowledge 2007) for the period 1970 to April 2007. The general search keywords were “estrogen,” “risk,” “dose,” and either “hypospadias,” “cryptorchidism,” or “testicular cancer.” A preliminary identification was performed by screening the titles and, if relevant, the abstracts of retrieved literature. The next stage was to check the citations and references of selected studies. This was an iterative process, repeated until no new study could be identified. A set of both inclusion and exclusion criteria was defined, and all relevant literature was then checked for eligibility. The inclusion criteria considered were (a) study design, namely, either a case–control, cohort, or clinical trial; (b) written in English; (c) exposure to one or a mixture of known estrogenic compounds; and (d) sufficient data reported to be used in meta-analysis.

The following exclusion criteria were used:
- Exposure to a group of compounds (suspected endocrine disruptors) for which mode of action was unspecified, for example, pesticides.
- Studies of exposure to phytoestrogens. Some phytoestrogens have been found to have a greater binding affinity for ER-β than for ER-α and can result in agonistic or antagonistic effects (Mueller et al. 2004).
- Studies of maternal endogenous hormones.
- Studies of the same cohort as this would bias the results towards the particular studies.
- Incomplete data.

Data extraction and quality rating. In addition to the number of exposed and non-exposed cases and controls, and risk ratios (RRs) with their confidence intervals (CIs), information regarding the study design, estrogenic agent, geographic location of the study, and year of publication were extracted from the selected literature to allow subset analysis to be carried out. When more than one RR was reported, the following priorities were set for choice:

- Adjusted RRs were used, except when the study provided only unadjusted estimates.
- When multiple estimates were given, the RR estimator on which the authors had relied for their assessment of causal association was used.
- Overall RRs were chosen instead of those derived from further stratifications. If an overall estimate was not provided, the RRs of the maximum duration of exposure or the maximum exposure concentration were chosen.

Several aspects of the quality of each study were also recorded according to a rating scheme adapted from those previously described (Altman 1991; Rushton 2000). Every criterion was assessed on a scale of 0 to 2, 0 suggesting that it was not present, 1 when it was unclear, and 2 when that criterion was satisfied. A maximum score of 50 and 52 could be assigned for retrospective (case–control) and prospective (cohort and clinical trials) studies, respectively. This enabled a quality sensitivity analysis to be performed to check the influence of studies with low quality on the pooled estimate.

Data analysis. Graphical representation. The RRs and CIs were plotted against the year of publication to determine whether any positive or negative trends in reporting RRs had occurred over time. Similarly, quality scores were plotted against the year of publication to investigate whether the quality of studies improved over time. To assess publication bias, a funnel plot (SE vs. RR) was produced based on the assumption that smaller studies are less precise in their RRs and thus have less weight and larger SE and should scatter more widely at the lower end of the graph, whereas larger studies will tend to be closer together (Sterne et al. 2001). Forest plots present the RRs against the reference of the study and help check homogeneity visually.

Statistical pooling. Pooled estimates and 95% CIs were calculated using both a fixed-effects model (Mantel–Haenszel method) and a random-effects model (DerSimonian–Laird method), allowing evaluation of the dependence of the conclusions of the analysis on the model assumptions. A summary estimate is considered statistically significant at the 0.05 level if its CI does not include unity.

The Mantel–Haenszel pooled effect estimate was used in a chi-square statistical test of homogeneity to assess the between-study variance. The magnitude of the test statistics depends on the weight of each study. When the number of studies is low or the studies themselves are small, the test statistic Q tends to be small. Tests of heterogeneity in meta-analyses are generally low in their power to reject the null hypothesis of homogeneity. For this reason, the chi-square statistical test of homogeneity was carried out at both 0.05 and 0.1 significance levels. Additionally, pooled estimates calculated using fixed effect and random effect models differ only if there is lack of homogeneity between studies. The estimates obtained by both methods were therefore compared to better assess potential heterogeneity between studies, in which case a single summary estimate of effect may be considered inappropriate.

Subset and sensitivity analyses. To investigate potential sources of heterogeneity between studies, we performed subset analyses for the study design, estrogenic agent, and geographic location.

Some studies exploring the influence of hormonal treatment during pregnancy did not specify the type of hormone. From what is known of the hormonal treatment of common conditions occurring during pregnancy, it was deemed reasonable to assume that they would have been likely to include estrogens, and these studies were included in the analysis. The validity of this assumption was tested by applying stricter criteria and calculating a summary estimate of effect excluding any study in which the hormone used had not been specified. Further sensitivity analysis was performed by excluding low-quality studies and extremes (exclusion of the studies with the largest and smallest RR estimators and exclusion of the studies with the largest and smallest weights) to verify that either the quality of the studies or one particular study did not have an excessive influence on the pooled estimate.

Results
A total of 50 studies were identified for the association between in utero exposure to estrogenic agents and hypospadias and/or cryptorchidism, including 16 that had not been included in previous systematic reviews. Sixteen studies, of which 8 were new studies, were included in the calculation of a summary estimate of effect for either or both endpoints (Table 1). Studies predating the formulation of the TDS hypothesis often were designed to explore the association of in utero exposure to a range of pharmaceuticals with birth malformations. Other than 2 recent studies for which pesticide exposure was determined by chemical analysis of specific compounds, assessment of exposure to pesticides is generally derived from the occupation of the mother and specific agents are not identified.

Of the 12 studies identified for the association with testicular cancer, only 3 were excluded from the calculation of a summary estimate of effect (Table 2).

Hypospadias. The data from studies included in the meta-analysis for hypospadias are summarized in Table 3. Three extreme values, two greater than and one lower than unity, can be identified visually from the forest plot of
the RRs and their CIs (Figure 1). These extremes correspond to studies with larger SEs, and the shape given to the funnel plot (Figure 2) by those smaller positive studies would be consistent with publication bias. These two extreme positive risk ratios were, however, reported after what is commonly referred to as “third-generation exposure” to DES, when the mother herself had been exposed to DES prenatally. It was recognized that the inclusion of such studies in the meta-analysis could have introduced heterogeneity, and the influence of this choice was investigated in the subset analysis. Plots of the quality score and RRs versus year of publication did not show any pattern that could be attributed to the inclusion of these studies.

Table 1. Studies identified for the association between in utero exposure to estrogenic agent and hypospadias and cryptorchidism.

| Reference                  | End point               | Comment                                                        | Previous reviews |
|----------------------------|-------------------------|                                                               |                 |
| Aarskog 1970               | Hypospadias             | Data on progestins treatment only                            | R-W, T, S       |
| Beard et al. 1984          | Cryptorchidism          | Study too small to calculate risk ratio                       | Sx              |
| Beral and Colwell 1981     | Cryptorchidism          | Use of clomiphene before pregnancy recognized                | S               |
| Berkowitz and Lapinski 1996| Cryptorchidism          | Maternal endogenous hormones                                 | R-W, S          |
| Bernstein et al. 1988      | Cryptorchidism          | No control data for documented abnormalities                 | R-W, Sx         |
| Bhatia et al. 2005         | Hypospadias             | Occupational exposure of fathers to pesticides               | R-W             |
| Bianca et al. 2003         | Cryptorchidism          | Maternal endogenous hormone levels                            | S               |
| Burton et al. 1987         | Hypospadias             | Oral contraceptive use before pregnancy recognized          | R-Wx, S         |
| Calzolari et al. 1986      | Cryptorchidism          | No genitourinary abnormalities in exposed infants            | T               |
| Cosgrove et al. 1977       | Hypospadias             | Progesterone treatment                                       | R-Wx            |
| Creizel et al. 1979        | Cryptorchidism          | Ecological study design                                       |                 |
| Creizel et al. 1999        | Hypospadias             | Oral contraceptive use before pregnancy recognized          | S               |
| Davies et al. 1986         | Cryptorchidism          | Same cohort as Depue (1988)                                  | R-Wx, S         |
| Depue 1984                 | Cryptorchidism          | Included                                                       |                 |
| Depue 1988                 | Hypospadias             | Included                                                       |                 |
| Flores-Luevano et al. 2003 | Hypospadias             | Included                                                       |                 |
| Garcia-Rodriguez et al. 1996| Cryptorchidism         | Ecological study design                                       | V, S            |
| Gill et al. 1977           | Cryptorchidism          | Cryptorchidism in men with testicular hypoplasia             | T, S            |
| Gill et al. 1979           | Cryptorchidism          | All cases exposed to progesterone                             | R-W             |
| Harlap and Eldridge 1980   | Cryptorchidism          | No cases after oral contraceptive use during pregnancy        | R-W             |
| Harlap et al. 1985         | Hypospadias             | Included                                                       | R-W             |
| Heinonen et al. 1977       | Hypospadias             | Included                                                       |                 |
| Hemminki et al. 1999       | Hypospadias             | No exposed controls                                           | T, V            |
| Henderson et al. 1976      | Cryptorchidism          | No unexposed cases                                            | R-Wx            |
| Janerich et al. 1980       | Hypospadias             | Included                                                       | R-W             |
| Källén 1989                | Hypospadias             | Included                                                       | R-Wx            |
| Källén and Winberg 1982    | Hypospadias             | No exposed controls                                           | R-Wx, S         |
| Källén et al. 1991         | Hypospadias             | Included                                                       | R-W, S          |
| Key et al. 1996            | Cryptorchidism          | No exogenous hormone use                                      | S               |
| Klip et al. 2002           | Hypospadias             | Included                                                       |                 |
| Kristensen et al. 1997     | Cryptorchidism          | Exposure to unspecified pesticides                            | V, S            |
| Longnecker et al. 2002     | Hypospadias             | DDE is antiandrogenic                                         | V, S            |
| McBride et al. 1991        | Cryptorchidism          | Included                                                       | R-W, S          |
| Monteleone-Neto et al. 1981| Hypospadias             | Included                                                       | R-W, V, S       |
| North and Golding 2000     | Hypospadias             | Phytoestrogens                                                 | S               |
| Palmer et al. 2005         | Hypospadias             | Included                                                       |                 |
| Pieniak et al. 2004        | Cryptorchidism          | Phytoestrogens, unspecified pesticides, or EDCs              | R-W, S          |
| Polednak and Janerich 1983 | Hypospadias             | Included                                                       |                 |
| Pons et al. 2005           | Hypospadias             | Included                                                       | R-W, S          |
| Restrepo et al. 1990       | Cryptorchidism          | Unspecified pesticides                                         | V               |
| Rothman and Louik 1978     | Hypospadias             | Oral contraceptive use before pregnancy recognized          | S               |
| Sorensen et al. 2005       | Hypospadias             | Clomiphene is estrogenic but does not act via ER              | R-Wx, S         |
| Stoll et al. 1990          | Hypospadias             | Oral contraceptive use before pregnancy recognized          | R-W             |
| Swee et al. 1974           | Cryptorchidism          | No exogenous estrogens during pregnancy                       | R-W, S          |
| Torfs et al. 1981          | Hypospadias             | Same cohort as Bhatia et al. (2005)                          | R-Wx            |
| Vessey et al. 1983         | Cryptorchidism          | Included                                                       |                 |
| Vrijheid et al. 2003       | Hypospadias             | No unexposed cases                                            | S               |
| Weidner et al. 1998        | Cryptorchidism          | Included                                                       |                 |
| Whitehead and Leiter 1981  | Hypospadias             | Exposure to unspecified pesticides                            | V, S            |
| Whitehead and Leiter 1981  | Cryptorchidism          | No controls                                                   | T               |

*The letters R-W, T, V, and S refer to Raman-Wilms et al. (1995), Toppari et al. (1996), Vidaeff and Sever (2005), and Storgaard et al. (2006), respectively, where the suffix “x” indicates study was excluded from that review.
not suggest any significant trends in quality of the studies or estimates of effect over time (not shown).

The pooled estimates of effect by both the Mantel–Haenszel and DerSimonian–Laird methods are very close to unity, and no relationship between in utero exposure to estrogenic agents and hypospadias could be detected (Table 4). None of the chi-square tests allowed the rejection of the null hypothesis of homogeneity between the studies at the 0.05 or 0.1 level of statistical significance. The subsets of studies in which exposure to DES and pharmaceutical estrogens were investigated yielded statistically significant risk ratios with both models, although the modest discrepancy between the fixed-effects and random-effects estimates suggests heterogeneity. Summary estimates for the latter subset were no longer significant when studies that included DES exposure were excluded. Although these results were based on four studies that all addressed in utero exposure to oral contraceptives, some heterogeneity between studies remained. Excluding the studies of third-generation exposure to DES, values for the summary estimate of effect were found to be 1.33 (95% CI, 0.63–2.83) by the Mantel–Haenszel method and 1.31 (95% CI, 0.52–3.26) by the DerSimonian–Laird method, a very modest and nonsignificant increase in risk. Excluding third-generation exposure from the DES subset yielded estimates of 2.02 (95% CI, 1.12–3.65) by the Mantel–Haenszel method and 2.00 (95% CI, 0.97–4.15) by the DerSimonian–Laird method, on the basis of two studies investigating exposure to any estrogenic drug during the first trimester of pregnancy. The difference between the results obtained by the two models for studies of third-generation exposure to DES was reduced only slightly by excluding the study by Klip et al. (2002); the Mantel–Haenszel method yielded an estimate of 2.46 (95% CI, 0.91–6.67) and the DerSimonian–Laird method of 2.18 (95% CI, 0.64–7.46). The latter study’s cohort had been recruited in a fertility clinic, and whether results obtained with subfertile women are generalizable to all women exposed to DES in utero has been questioned (Hernandez-Diaz 2002).

Although the equality of the results obtained by both methods for the environmental estrogens subset suggests those results are robust, the influence of the weight of the study by Vrijheid et al. (2003) cannot be underestimated, as shown by the sensitivity analysis. Exclusion of this study from the analysis yielded a statistically significant Mantel–Haenszel estimate but a lower and not statistically significant DerSimonian–Laird estimate, revealing heterogeneity. A statistically significant estimate was obtained for prospective studies by the Mantel–Haenszel method, but the wide difference with the estimate using the random effect model was suggestive of heterogeneity. Geographic subsets point to a higher risk in Latin America, although the pooled estimates for this location were based on only two studies and did not reach statistical significance.

Table 2. Studies identified for the association between in utero exposure to estrogenic agent and testicular cancer.

| Reference | Comment | Previous reviews |
|-----------|---------|------------------|
| Brown et al. 1986 | Included | T, S |
| Degue et al. 1983 | Included | T, S |
| Dieckmann et al. 2001 | Maternal endogenous hormone levels | |
| Gershman and Stolley 1988 | Included | S |
| Hardell et al. 2004 | Included | T, S |
| Hemminki et al. 1999 | No cases | |
| Henderson et al. 1979 | Included | T, S |
| Moss et al. 1986 | Included | T, S |
| Schottenfeld et al. 1980 | Included | T, S |
| Strohsnitter et al. 2001 | Included | S |
| Walcott et al. 2002 | Phytoestrogens | Included |
| Weir et al. 2000 | Included | S |

*The letters T and S stand for Toppari et al. (1994) and Storgaard et al. (2006), respectively.

Table 3. Summary of data used for the meta-analysis of the association between prenatal estrogenic agents and hypospadias.

| Reference | Design | Agent | Location | Cases | Controls | RR (95% CI) | SE | Weight | Quality score |
|-----------|--------|-------|----------|-------|----------|-------------|-----|--------|--------------|
| Bhatia et al. 2005 | Case–control | DOT | California, USA | 9 | 34 | 0.79 (0.33–1.89) | 0.38 | 7.07 | 41 |
| Flores-Luevano et al. 2003 | Case–control | DOT | Mexico City | 8 | 33 | 1.13 (0.24–5.29) | 0.65 | 2.39 | 37 |
| Harlap et al. 1985 | Cohort | Oral contraceptives | North Carolina, USA | 3 | 98 | 3.29 (1.00–10.30) | 0.64 | 2.47 | 36 |
| Heimonen et al. 1977 | Cohort | Estrogenic drugs | United States | 4 | 184 | 2.57 (1.52–4.33) | 0.69 | 2.12 | 45 |
| Källén et al. 1989 | Case–control | Oral contraceptives | Sweden | 5 | 43 | 2.11 | 0.79 | 1.58 | 23 |
| Källén et al. 1991 | Case–control | Oral contraceptives | B countries | 16 | 830 | 1.36 (0.64–2.92) | 0.43 | 5.40 | 30 |
| Klip et al. 2002 | Cohort | DES (mother exposed prenatally) | Netherlands | 4 | 8 | 2.30 (1.60–7.00) | 2.34 | 0.18 | 27 |
| Monteleone-Neto et al. 1981 | Case–control | Sex hormones | Latin America | 21 | 252 | 2.20 (1.04–4.91) | 0.44 | 5.11 | 24 |
| Palmer et al. 2005 | Cohort | DES (mother exposed prenatally) | United States | 10 | 3 | 1.70 (1.40–6.80) | 0.72 | 1.95 | 36 |
| Polesnak and Janerich 1983 | Case–control | DES (mother exposed prenatally) | New York, USA | 1 | 98 | 0.33 | 0.82 | 1.48 | 27 |
| Pons et al. 2005 | Case–control | DES (mother exposed prenatally) | Paris, France | 3 | 44 | 4.99 (1.20–16.80) | 1.30 | 0.59 | 17 |
| Vrijheid et al. 2003 | Case–control | Phthalates (occupational) | United Kingdom | 147 | 3,324 | 0.90 (0.74–1.10) | 0.09 | 129.31 | 31 |

Abbreviations: E, exposed; NE, nonexposed.
In addition to the results of the sensitivity analysis presented in Table 4, a pooled estimate of effect was calculated when a stricter inclusion criterion was applied, namely, excluding results from the study by Monteleone-Neto et al. (1981). This had little influence on the overall result, generating summary estimates of 0.97 (95% CI, 0.83–1.13) for the fixed effect model or 0.93 (95% CI, 0.80–1.09) for the random effect model.

**Cryptorchidism.** Data for the six studies included in the meta-analysis for cryptorchidism can be found in Table 5. The results of only two studies significantly differ from unity, as illustrated by the forest plot (Figure 3). The small number of eligible studies renders detection that any of the subsets were significantly heterogeneous. Excluding studies method yielded a no longer statistically significant estimate of 1.34 (95% CI, 0.84–2.14) and the DerSimonian–Laird method an estimate of 1.27 (95% CI, 0.72–2.23).

Applying a stricter exclusion criterion to studies examining hormonal treatment did not affect which studies were included in the meta-analysis of cryptorchidism. The study with the highest weight appears to lower the overall estimates, whereas increasing quality seems to reduce heterogeneity and lower the estimate of effect toward unity. These variations did not, however, influence the overall conclusion that aside from the DES studies subset, summary estimates did not detect any association between *in utero* exposure to estrogenic substances and cryptorchidism.

**Testicular cancer.** Nine studies were included in the meta-analysis of testicular cancer and the data used are summarized in Table 6. The Mantel–Haenszel and DerSimonian–Laird methods are marginally superior to unity, and their relative heterogeneity, as shown by the sensitivity analysis. Excluding the study by Depue (1988) reduced the difference between estimates by both methods, the Mantel–Haenszel estimate then calculated as 1.29 (95% CI, 0.87–1.91) and that by the DerSimonian–Laird method as 1.23 (95% CI, 0.81–1.86). This was also observed for the American subset of studies. When the Depue (1988) study is omitted, the Mantel–Haenszel method yielded an estimate of 1.34 (95% CI, 0.84–2.14) and the DerSimonian–Laird method an estimate of 1.27 (95% CI, 0.72–2.23).

Table 4. RRs (95% CIs) of the summary estimate of effect, subsets, and sensitivity analyses for the association between hypospadias and prenatal exposure to estrogenic agents.

| Subset of studies | No. of studies included | Mantel–Haenszel method (fixed effects) | DerSimonian–Laird method (random effects) |
|-------------------|-------------------------|----------------------------------------|------------------------------------------|
| All studies       | 12                      | 1.02 (0.88–1.19)                        | 1.16 (0.83–1.62)                         |
| Excluding DES     | 7                       | 0.93 (0.79–1.09)                        | 0.97 (0.78–1.22)                         |
| Studies including DES | 5                   | 2.49 (1.54–4.02)                         | 2.14 (1.15–3.98)                         |
| Mothers exposed to DES | 3                | 3.73 (1.58–8.80)                         | 2.54 (0.78–8.33)                         |
| Pharmaceutical estrogens only | 9            | 1.85 (1.30–2.64)                         | 1.54 (1.00–2.36)                         |
| Pharmaceutical estrogens excluding DES | 4 | 1.27 (0.74–2.19) | 1.13 (0.61–2.10) |
| Environmental estrogens only | 3 | 0.90 (0.76–1.06) | 0.90 (0.76–1.06) |
| European studies  | 4                       | 0.96 (0.81–1.14)                        | 0.96 (0.72–1.27)                         |
| North American studies | 5            | 1.03 (0.63–1.68)                        | 0.93 (0.56–1.55)                         |
| Latin American studies | 2           | 1.08 (0.99–3.48)                         | 1.78 (0.67–3.64)                         |
| Excluding highest risk ratio | 11 | 1.00 (0.98–1.16) | 0.99 (0.82–1.20) |
| Excluding lowest risk ratio | 11 | 1.03 (0.87–1.20) | 1.02 (0.84–1.25) |
| Excluding highest weight | 11 | 1.18 (1.13–2.11) | 1.29 (0.90–1.85) |
| Excluding lowest weight | 11 | 1.00 (0.86–1.16) | 0.99 (0.82–1.20) |
| Case–control studies only | 8 | 0.98 (0.84–1.15) | 1.00 (0.78–1.38) |
| Cohort studies only | 4 | 2.10 (1.14–3.95) | 1.46 (0.59–3.57) |
| Excluding studies with quality score < 30 | 7 | 0.94 (0.80–1.10) | 0.93 (0.79–1.08) |
| Excluding studies with quality score < 35 | 5 | 1.11 (0.69–1.77) | 1.06 (0.65–1.73) |

Table 5. Summary of data used for the meta-analysis of the association between prenatal exposure to estrogenic agents and cryptorchidism.

| References    | Design       | Agent                  | Location    | Cases | Controls |
|---------------|--------------|------------------------|-------------|-------|----------|
| Beard et al. 1984 | Case–control | Estrogenic drugs | Minnesota, USA | 9    | 104      | 15 211 | 2.20 (0.70–7.20) | 0.47 | 4.60 | 34 |
| Bhatia et al. 2005 | Case–control | Estrogenic drugs | California, USA | 11 32 | 42 117   | 0.95 (0.43–2.07) | 0.39 | 6.65 | 41 |
| Depue 1988     | Case–control | Estrogenic drugs | United States | 5    | 380      | 3 765 | 5.15 | 1.01 | 0.99 | 29 |
| Harlap et al. 1985 | Cohort     | Oral contraceptives | North Carolina, USA | 6   | 196     | 844 27,595 | 1.10 (0.10–3.90) | 0.42 | 5.78 | 36 |
| McBride et al. 1991 | Case–control | Oral contraceptives | British Columbia, Canada | 18 226 | 34 454   | 1.10 | 0.31 | 10.50 | 38 |
| Vessey et al. 1983 | Clinical trial | DES | United Kingdom | 6   | 6 138    | 126 | 0.91 | 0.58 | 3.00 | 18 |

Abbreviations: E, exposed; NE, nonexposed.

Figure 3. Forest plot risk estimates and their 95% CIs from the studies included in the meta-analysis of the association between prenatal exposure to estrogenic agents and cryptorchidism.

Figure 4. Funnel plot of the risk estimate studies included in the meta-analysis of the association between prenatal exposure to estrogenic agents and cryptorchidism and their SEs.
Table 7. Of these, 4 had not been included in the summary estimate previously calculated by Toppari et al. (1996). The lack of homogeneity between studies is evident from the forest plot (Figure 5). Further, the funnel plot (Figure 6) also illustrates the relatively small size of the included studies. Although a positive trend over time was found for the quality of the included studies (Figure 7), no significant time trend could be detected for the effect size (not shown).

Both the fixed and random effect models yield a statistically significant estimate; however, the discrepancy between the two results is suggestive of heterogeneity despite the result from the chi-square test (Table 8). Conversely, the subset analysis was limited by the similarity of the question addressed by the studies included. Eight of the nine studies were interested in hormonal exposure and were conducted in the United States. Despite this, statistically significant heterogeneity between the studies was detected at the 0.1 level. Pooling the two studies examining DES exposure specifically produced a raised but statistically nonsignificant result. Despite the unexplained heterogeneity, all estimates that were calculated point to a doubling of the risk of developing testicular cancer after exposure to estrogenic agents in utero. The work on chlorinated biphenyls (PCBs) by Hardell et al. (2004) was the only study examining environmental estrogens. Its size was relatively small, and it did not detect such an effect.

Applying a stricter exclusion criterion to studies examining hormonal treatment excluded four studies from the meta-analysis; namely, Brown et al. (1986), Gershman and Stolley (1988), Henderson et al. (1979), and Weir et al. (2000). This resulted in a slightly lower Mantel–Haenszel estimate of 1.98 (95% CI, 1.23–3.18) and if the DerSimonian–Laird estimate remained equal to 1.39, because of the wider confidence interval (95% CI, 0.93–2.69), statistical significance was no longer achieved. The sensitivity analysis is consistent with some heterogeneity between the studies, the estimates obtained being relatively sensitive to the exclusion of particular studies varying above and below a risk estimate of 2. The quality of the studies seemed to explain at least some of this heterogeneity.

Discussion

While it is clear that hypospadias, cryptorchidism, and testicular cancer are all positively associated with prenatal exposure to DES, this meta-analysis was unable to produce evidence that such effects were associated with environmental estrogens or even accidental use of oral contraceptives during pregnancy. This is consistent with the results obtained in earlier meta-analyses (Raman-Wilms et al. 1995; Toppari et al. 1996).

The main limitations of meta-analysis are (a) the susceptibility of its summary results to publication bias, (b) the influence of the quality of studies, (c) the possibility of including multiple results from the same study, and finally, (d) heterogeneity between studies that could lead to invalid conclusions. The methodology employed in this present review attempts to address these issues. Additionally, the importance of carrying out and reporting a sensitivity analysis was illustrated by the case of hypospadias where the weight attributed to one particularly large study had a nonnegligible influence on the results. In this particular case, the study by Vrijheid et al. (2003) inferred exposure to phthalates from registry data about occupation, and although such an approach can allow the analysis of a great number of cases, assessment of exposure is much more likely to be prone to confounding. The number of studies included in meta-analyses lies typically between 5 and 15, and the results presented here also fall within this range. The size of the homogeneity test statistic depends on both the number and size of individual studies. The funnel plots offer a good visual representation of the precision and size of individual studies, and it is clear that most studies published on the association between estrogenic agents and the probable end points of a TDS were found to be relatively small. The chi-square tests had, therefore, a relatively low power to detect heterogeneity. However, in the absence of statistical

Table 6. RRs and 95% CIs of the summary estimate, subsets and sensitivity analyses for the association between cryptorchidism and prenatal exposure to estrogenic agents.

| Subset of studies | No. of studies included | Mantel–Haenszel method (fixed effects) | χ² | DerSimonian–Laird method (random effects) |
|------------------|-------------------------|----------------------------------------|----|-----------------------------------------|
| All studies      | 6                       | 1.34 (0.98–1.87)                       | 0.44| 1.22 (0.86–1.73)                        |
| Excluding DES exposure | 3                   | 1.06 (0.70–1.59)                       | 0.95| 1.05 (0.70–1.59)                        |
| Studies including DES | 3                    | 2.09 (1.13–3.96)                       | 0.24| 1.80 (0.83–3.93)                        |
| Pharmaceutical estrogens | 5                    | 1.44 (0.99–2.10)                       | 0.37| 1.31 (0.87–1.96)                        |
| Pharmaceutical estrogens excluding DES | 2           | 1.10 (0.49–2.49)                       | 1   | 1.10 (0.49–2.49)                        |
| Case–control studies | 4                  | 1.45 (0.98–2.15)                       | 0.24| 1.38 (0.81–2.34)                        |
| Cohort studies   | 2                       | 1.04 (0.53–2.02)                       | 0.79| 1.03 (0.53–2.00)                        |
| American studies | 4                       | 1.55 (1.00–2.39)                       | 0.24| 1.40 (0.82–2.41)                        |
| Excluding highest risk ratio | 5               | 1.21 (0.80–1.72)                       | 0.66| 1.16 (0.81–1.66)                        |
| Excluding lowest risk ratio | 6          | 1.39 (0.97–1.97)                       | 0.34| 1.27 (0.88–1.87)                        |
| Excluding highest weight | 5           | 1.46 (0.97–2.19)                       | 0.32| 1.30 (0.82–2.66)                        |
| Excluding lowest weight | 5          | 1.21 (0.86–1.72)                       | 0.66| 1.16 (0.81–1.66)                        |
| Excluding studies with quality score < 30 | 4  | 1.25 (0.86–1.80)                       | 0.53| 1.19 (0.82–1.73)                        |
| Excluding studies with quality score < 35 | 3  | 1.06 (0.70–1.59)                       | 0.95| 1.05 (0.70–1.59)                        |

Figure 5. Forest plot risk estimates and their 95% CIs from the studies included in the meta-analysis of the association between prenatal exposure to estrogenic agents and testicular cancer.

Table 7. Summary of data used for the meta-analysis of the association between prenatal estrogenic agents and testicular cancer.

| Reference                  | Design          | Agent            | Location         | Cases | Controls | RR (95% CI) | SE  | Weight | Quality score |
|----------------------------|-----------------|------------------|------------------|-------|----------|-------------|-----|--------|--------------|
| Brown et al. 1986          | Case–control    | Sex hormones     | Washington, DC, USA | 4    | 198      | 0.80 (0.20–3.50) | 0.64| 2.43   | 30           |
| Depue et al. 1983          | Case–control    | Estrogenic drugs | Los Angeles, USA  | 8    | 88       | 8.00 (1.30–49)   | 1.07| 0.88   | 32           |
| Gershman and Stolley 1988  | Case–control    | DES              | Connecticut, USA  | 4    | 75       | 0.80 (0.10–4.50)  | 0.65| 2.37   | 27           |
| Hardell et al. 2004        | Case–control    | Estrogenic PCBs  | Sweden            | 29   | 29       | 1.30 (0.50–3.00)  | 0.37| 7.31   | 39           |
| Henderson et al. 1979      | Case–control    | Hormone treatment| Los Angeles, USA  | 6    | 72       | 5.00           | 1.47| 0.46   | 29           |
| Moss et al. 1986           | Case–control    | DES or other hormones | California and Nevada, USA | 7  | 202      | 0.90 (0.30–2.60)  | 0.55| 2.89   | 34           |
| Schottenfelder et al. 1980 | Case–control    | DES or other hormones | United States     | 11   | 170      | 3.05           | 0.79| 1.61   | 26           |
| Strohsnitter et al. 2001   | Cohort          | DES              | United States     | 6    | 2        | 3.05 (0.65–21.98) | 1.01| 0.99   | 37           |
| Weir et al. 2000           | Case control    | Hormone treatment| Ontario, Canada    | 15   | 310      | 4.90 (1.70–13.90) | 0.61| 2.66   | 40           |

Abbreviations: E, exposed; NE, nonexposed.
heterogeneity, the results of the fixed effect and random effect models should be virtually identical, and the comparison of results obtained by applying both the Mantel–Haenszel and DerSimonian–Laird models enabled the exploration of sources of heterogeneity despite this low statistical power.

If the quality of the studies was found to explain some of the heterogeneity observed, particularly in the case of testicular cancer, the remaining heterogeneity could not be explained solely by the fact that environmental, and therefore generally much weaker, estrogens were included in the analysis. The systematic review of published literature yielded relatively few studies examining the association of male urogenital abnormalities or testicular cancer with environmental estrogens specifically; a number of studies concerned with an association with broad categories of putative endocrine disruptor, most often pesticides, were excluded from the meta-analyses. This illustrates the difficulties associated with assessment of exposure, pesticide exposure often being inferred from parental occupation rather than direct measurement. Furthermore, there is increasing evidence that, in accordance with pharmacokinetic theory, the effects of xenobiotics acting via the same mechanism can be predicted fairly accurately by concentration addition (Zhu et al. 2006). Accurately accounting for combined exposure or adjusting for the confounding introduced by environmental exposures will probably require the development of mechanism-specific biomarkers of exposure.

When DES is excluded, there is no conclusive evidence of an effect of pharmaceutical estrogens. Exposure to such estrogens is related mainly to the accidental use of oral contraceptives during pregnancy or hormonal pregnancy tests. Such estrogen pharmaceuticals often are given in combination with progestagens, and it is legitimate to question whether unopposed estrogens would have the same effects as opposed estrogens. This also highlights another difficulty associated with exposure assessment, that of critically sensitive periods of development and the ascertainment of whether exposure took place during a “window” of susceptibility to hormone disruption. Nonetheless, studies in which maternal levels of hormones were measured in the first and third trimester of pregnancy do not support an association with elevated estrogen levels but rather indicate that a lower estrogen/androgen ratio and/or higher levels of α-fetoproteins may be beneficial (McGlynn et al. 2005; Zhang et al. 2005). If in animals both estrogenic and antiandrogenic compounds have been associated with end points consistent with those of human TDS (Fisher 2004; Vereramachaneni 2000), epidemiologic evidence remains elusive. Alternatively, the doubling of the risk estimates of all three effects associated with DES exposure would be consistent with a shared etiology and the TDS hypothesis. It does not constitute conclusive evidence of an estrogenic mode of action, however, as common etiologic factors could be related to the underlying condition for which DES was prescribed. Furthermore, hypospadias, cryptorchidism, and testicular cancer have all been found to be associated with low birth weight, suggesting a potential association with an underlying placental defect.

The understanding of the importance of endogenous estrogens in normal adult testicular function is becoming clearer. Their roles during fetal life, however, remain relatively unclear, but those mediated by the ER-α or ER-β have been shown to differ (Habert et al. 2006). Interestingly, DES has been found to have similar affinity for both receptors, whereas estradiol has only a slightly stronger affinity for ER-α compared with ER-β (Mueller et al. 2004). ER-α has been detected in undifferentiated gonads as early as 10 days postconception in the mouse and found to be localized in the Leydig cells of fetal testis in rodents (Habert et al. 2006). Studies of the expression of ER-α and ER-β in human and nonhuman primates have so far yielded inconsistent results. Gaskell et al. (2003) reported that ER-α could not be detected in human fetal testes between weeks 12–19 of gestation, whereas Shapiro et al. (2005) found that ER-α was apparent by week 12, its levels peaked at 16 weeks before diminishing, and it was localized in Leydig cells. Current research focus has shifted to the role played by testosterone, anti-Müllerian hormone and insulin-like factor 3 produced by the fetal testes during masculinization. In the male rat, exposure to high levels of estrogens has been shown not only to suppress testosterone production but also to downregulate the expression of the androgen receptor protein in reproductive target tissues including the testes, Wolffian duct, and prostate (Sharpe 2006). Further research in this area may help shed light on possible mechanisms of injury or relevance of the rodent model.

The subset analyses did not generate many clues to explain the heterogeneity of the collected data. This is, however, consistent with the wide geographic variability in the incidence of the conditions of interest (Boisen et al. 2004; Richiard et al. 2004). Interactions between genetic susceptibility and the environment have been the focus of research in this area (Martin et al. 2007), and advances in genomics have allowed the identification of polymorphisms associated with hypospadias, cryptorchidism, and testicular cancer (Releza-Meireles et al. 2006; Kurahashi et al. 2005; Starr et al. 2005; Yoshida et al. 2005). Such discoveries may,

Table 8. RRs and 95% CIs of the summary estimates, subsets and sensitivity analyses for the association between testicular cancer and prenatal exposure to estrogenic agents.

| No. of studies included | Mantel–Haenszel method (fixed effects) | χ² | p-Value | DerSimonian–Laird method (random effects) |
|-------------------------|----------------------------------------|----|---------|------------------------------------------|
| All studies             | 9                                      | 2.14 [1.48–3.10]            | 0.12 | 1.59 [1.04–2.43]                           |
| DES exposure exclusively| 2                                      | 2.53 [0.79–8.09]            | 0.77 | 2.47 [0.61–10.00]                          |
| Pharmaceutical estrogens| 8                                      | 2.57 [1.66–3.99]            | 0.09 | 1.94 [0.98–3.87]                          |
| Case–control studies only| 8                                      | 2.10 [1.43–3.07]            | 0.09 | 1.71 [0.92–3.17]                          |
| North American studies  | 8                                      | 2.57 [1.66–3.99]            | 0.09 | 1.94 [0.98–3.87]                          |
| Excluding highest risk ratio| 8                              | 1.89 [1.29–2.78]            | 0.21 | 1.56 [0.93–2.61]                          |
| Excluding lowest risk ratio| 8                              | 2.31 [1.56–3.40]            | 0.14 | 1.94 [1.08–3.48]                          |
| Excluding highest weight | 8                                      | 2.57 [1.66–3.99]            | 0.09 | 1.94 [0.98–3.87]                          |
| Excluding lowest weight | 8                                      | 2.08 [1.42–3.03]            | 0.10 | 1.68 [0.95–2.97]                          |
| Excluding studies with quality score < 30 | 6                      | 2.16 [1.42–3.29]            | 0.08 | 1.79 [0.91–3.52]                          |
| Excluding studies with quality score < 35 | 3                      | 2.33 [1.39–3.91]            | 0.13 | 2.23 [0.98–5.05]                          |
however, give rise to as many questions as they offer to answer. This is well illustrated by the recent identification of the association of a variant of the gene for the ER-α with hypospadias and cryptorchidism in Japanese cohorts (Watanabe et al. 2007; Yoshida et al. 2005) that has now been found to be associated with a decreased incidence of hypospadias in a European cohort (Galan et al. 2007).

Conclusion
The modest increase in risk for all three end points associated with DES exposure is consistent with a shared etiology and the TDS hypothesis, whereas the results of the subset analyses suggest the existence of yet unidentified sources of heterogeneity between studies or within the study populations. Although 10 years of further research on the potential effects of endocrine disruptors on male reproductive health have provided some clues regarding the etiology and mechanism of conditions such as hypospadias, cryptorchidism, and testicular cancer, there is still no conclusive evidence of the role played by environmental estrogens.

REFERENCES
Aarskog D. 1970. Clinical and cytogenetic studies in hypospadias. Acta Paediatr Scand 203(suppl 203):1–62.
Altman DG. 1991. Practical Statistics for Medical Research. such as hypospadias, cryptorchidism, and testicular health have provided some clues regarding the etiology and mechanism of conditions such as hypospadias, cryptorchidism, and testicular cancer, there is still no conclusive evidence of the role played by environmental estrogens.

Cospogro MD, Benton B, Henderson BE. 1977. Male genitalitory abnormalities and maternal diethylstilbestrol. J Urol 117:220–222.
Cous JF, Dixon D, Yates M, Moore AB, Ma L, Maas R, et al. 2001. Estrogen receptor-α[alpha] knockout mice exhibit resistance to the developmental effects of neonatal diethylstilbestrol exposure on the female reproductive tract. Dev Biol 238:224–238.
Czeizel AE, Toth J, Erodi E. 1979. Aetiological studies of hypospadias in Hungary. Hum Herb 29:166–171.
Czeizel AE, Hegedu S, Timar L. 1999. Congenital abnormalities and indicators of genital malformations in the vicinity of an acrylonitrile producing factory. Mut Res 427:105–123.
Dawson TW, Williams DRR, Whicker RH. 1986. Risk factors for undescended testis. Int J Epidemiol 15:197–201.
Depeu RH. 1984. Maternal and gestational factors affecting the risk of cryptorchidism and inguinal hernia. Int J Epidemiol 13:415–420.
Depeu RH. 1988. Cryptorchidism, an epidemiologic-study with emphasis on the relationship to central nervous-system dysfunction. Teratology 37:301–305.
Depeu RH, Pike MC, Henderson BE. 1983. Estrogen exposure during gestation and risk of testicular cancer. J Natl Cancer Inst 71:1151–1155.
Dieckmann KP, Endins G, Pichlmeier U. 2001. How valid is the diagnosis of testicular dysgenesis syndrome? Reprod BioMed Online 17:373–379.
Fischler J. 2004. Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. Reprod BioMed Online 17:305–315.
Garcia-Rodriguez J, Garcia-Martin M, Nogueras-Ocana M, de Amigo MA, Ruiperez M, Casado F, et al. 1997. Congenital abnormalities and maternal diethylstilbestrol exposure. J Reprod Med 42:571–576.
García-Ocaña A, Olea N, Bornstein S, Schmid B, Dourado M, et al. 2004. Analysis of environmental endocrine disruptors and risk of hypospadias. A case-control pilot study. Salud Publica Mex 46:420–438.
Galan JJ, Guarducci E, Nati F, Gonzalez A, Ruiz M, Ruiz A, et al. 2007. Molecular analysis of estrogen receptor alpha gene AGATA haplotype and SNP12 in European populations: potential protective effect for cryptorchidism and lack of association with maternal hormone exposure. Toxicol Sci 98:244–448.
Garcia-Rodriguez J, Garcia-Martin M, Nogueras-Ocana M, de Dios Luna-del-Castillo J, Espinaceras G, Olea N, et al. 1996. Exposure to pesticides and cryptorchidism: geographical evidence of a possible association. Environ Health Perspect 104:1090–1095.
Gaskell TL, Robinson LLL, Groome NP, Andersen RA, Saunders PTK. 2003. Differential expression of two estrogen receptor beta isoforms in the human fetal testis during the second trimester of pregnancy. J Clin Endocrinol Metab 88:424–432.
Gershman ST, Stolley PD. 1988. A case-control study of testicular cancer using Connecticut tumour registry data. Int J Epidemiol 17:738–742.
Gil WB, Schumacher GFB, Bibbo M. 1977. Pathological semen and anatomical abnormalities of the genital tract in human male subjects exposed to diethylstilbestrol in utero. J Urol 117:477–480.
Gil WB, Schumacher GFB, Bibbo M, Strauss FH, Schoenberg H, Schoenberg HV. 1978. Association of diethylstilbestrol exposure in utero with cryptorchidism, testicular hypoplasia and semen abnormalities. J Urology 122:36–39.
Gray LE, Otby J, Fur J, Price M, Veeramachaneni DNR, Parks L. 2000. Perinatal exposure to the phthalates DEHP, DBP, and DINP, but not DEP, DOP, or DTT, alters sexual differ- entiation of the male rat. Toxicol Ind Health 15:48–68.
Habert R, Delbes G, Duquenne C, Livier G, Levacher C. 2006. Effets des estrogènes sur le développement du testicule pendant la vie foetale et néonatale [in French]. Genèse 34:970–977.
Hardell L, Malmqvist N, Dhillon CG, Westberg H, Eriksson M. 2004. Testicular cancer and occupational exposure to polyvinyl chloride plastics: a case-control study. Int J Cancer 108:560–564.
Hemminki K. 2004. Familial risk in testicular cancer as a clue to a heritable and environmental aetiology. Br J Cancer 90:1765–1770.
Hemminki K. 2004. Familial risk in testicular cancer as a clue to a heritable and environmental aetiology. Br J Cancer 90:1765–1770.
Henderson BE, Benton B, Cospogro M, Baptista J, Aldrich J, Townsend D, Littledine CD, Publishing Sciences Group. 1986. Familial risk in testicular cancer as a clue to a heritable and environmental aetiology. Br J Cancer 90:1765–1770.
Joffe M. 2001. Are problems with male reproductive health caused by endocrine disruption? Occup Environ Med 58:281–288.
Källén B. 1988. Case-control study of hypospadias, based on reg- istry information. Teratology 37:45–50.
Källén B, Mastra-Luevanos S, Khan MA, Romano-Riquer P, Weber JP, Dewailly E, et al. 2003. DDT/DOE concentra- tions and risk of hypospadias. A case-control pilot study. J Epidemiol Commun H 35:155–160.
Källén B, Winberg J. 1982. An epidemiological study of hypospadi- as in Sweden. Acta Paediatr Scand 71:155–159.
Klip H, Verpoort J, van Gool JD, Koster ME, Burger CW, van Leeuwen FE. 2002. Hypospadias in sons of women exposed to diethylstilbestrol in utero: a cohort study. Lancet 359:1102–1107.
Kristensen F, Jorgensen J, Andersen A, Byae AS, Sundheim L. 1997. Birth defects among offspring of Norwegian farmers, 1967–1991. Epidemiology 8:537–544.
Kurashashi N, Sata F, Kasa S, Shibata T, Moriyuki K, Yamada H, et al. 2005. Maternal genetic polymorphisms in DPY1A1, GSTM1 and GSTT1 and the risk of hypospadias. Mol Hum Reprod 11:93–98.
Longnecker MP, Kliebhan MF, Brock JW, Zhou HB, Gray KA, Needham LL, et al. 2002. Maternal serum level of 1,1- dichloro-2,2-bis(p-chlorophenyl)ethylene and risk of cryp- toorchidism, hypospadias, and polythelia among male offspring. Am J Epidemiol 155:113–119.
Martin OV, Lester JN, Voulvoulis N, Boobis AR. 2007. Human health and endocrine disruption: a simple multicriteria framework for the qualitative assessment of end point-spe- cific risks in a context of scientific uncertainty. Toxicol Sci 98(2):332–347.
McBride ML, Vandenstreek N, Lamb CW, Gallagher RP. 1991. Maternal and gestational factors in cryptorchidism. Int J Epidemiol 20:964–970.
McGlany KA, Graubard BI, Nam JM, Stanczyk FZ, Longnecker MP, Kliebhan MF, Brock JW, Zhou HB, Gray KA, Needham LL, et al. 2002. Maternal serum level of 1,1- dichloro-2,2-bis(p-chlorophenyl)ethylene and risk of cryp- toorchidism, hypospadias, and polythelia among male offspring. Am J Epidemiol 155:113–119.
Martin OV, Lester JN, Voulvoulis N, Boobis AR. 2007. Human health and endocrine disruption: a simple multicriteria framework for the qualitative assessment of end point-spe- cific risks in a context of scientific uncertainty. Toxicol Sci 98(2):332–347.
Moscovici DF, Stone D, Shapiro S. 1977. Birth Defects and Drugs in Pregnancy Littleton, CO:Publishing Sciences Group.
Hemminki E, Gissler M, Toukomaa H. 1999. Exposure to female hormone drugs during pregnancy: effect on malformation and cancer. Br J Cancer 80:1092–1097.
Hemminki K. 2004. Familial risk in testicular cancer as a clue to a heritable and environmental aetiology. Br J Cancer 90:1765–1770.
Henderson BE, Benton B, Cospogro M, Baptista J, Aldrich J, Townsend D, Littledine CD, Publishing Sciences Group. 1986. Familial risk in testicular cancer as a clue to a heritable and environmental aetiology. Br J Cancer 90:1765–1770.
Sharpe RM. 2006. Pathways of endocrine disruption during male sexual differentiation and masculinisation. Best Pract Res Clin Endocrinol Metab 20:91–110.

Sharpe RM, Skakkebæk NE. 2003. Male reproductive disorders and the role of endocrine disruption: advances in understanding and identification of areas for future research. Pure Appl Chem 75:2033–2038.

Skakkebæk NE, Rajaert-De Meys E, Main KM. 2001. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 16:972–978.

Sorensen HT, Pedersen L, Skrivner MV, Naergaard M, Norgard B, Hatch EE. 2005. Use of clomifene during early pregnancy and risk of hypospadias: population based case-control study. Br Med J 330:126–127.

Starr JR, Chen C, Doody DR, Hsu L, Ricks S, Weiss NS, et al. 2005. Risk of Testicular germ cell cancer in relation to variation in maternal and offspring cytochrome p450 genes involved in catechol estrogen metabolism. Cancer Epidemiol Biomarkers Prev 14:2183–2190.

Sterne JAC, Egger M, Smith GD. 2001. Investigating and dealing with publication and other biases. In: Systematic Reviews in Health Care: Meta Analysis in Context, 2nd ed. (Egger M, Smith GD, Altman DG, eds). London:BMJ Publishing Group.

Stoll C, Alemby, Roth MP, Dott B. 1990. Genetic and environmental factors in hypospadias. J Med Genet 27:559–563.

Storgaard L, Einarson TR, Koren G. 2004. Maternal and paternal risk factors for cryptorchidism and hypospadias: a case-control study in newborn boys. Scand J Work Environ Health 30:107–113.

Strohsnitter WC, Noller KL, Hoover RN, Robboy SJ, Palmer JR, Wise LA, Wise LA, Robboy SJ, Titus-Ernstoff L, Noller KL, North K, Golding J. 2000. A maternal vegetarian diet in pregnancy—report from a Symposium at the 10th International-Congress of ISPOG. J Psychosom Obstet Gynecol 14:71–88.

Pierik FRH, Burdorf A, Deddens JA, Juttmann RE, Weber RFA. 2004. Maternal and paternal risk factors for cryptorchidism and hypospadias: a case-control study in newborn boys. Environ Health Perspect 112:1570–1576.

Poleynek AP, Janerich DT. 1983. Maternal characteristics and hypospadias—a case-control study. Teratology 28:67–73.

Pons JC, Papiernik E, Billon A, Hessabi M, Duyme M. 2005. Hypospadias in sons of women exposed to diethylstilbestrol in utero. Prenat Diagn 25:417–426.

Raman-Wilms L, Tseng AL, Wijhardi S, Einarson TR, Koren G. 1995. Fetal genital effects of first-trimester sex-hormone exposure—a meta-analysis. Obstet Gynecol 85:141–149.

Restrepo M, Munoz N, Day N, Parra JE, Hernandez C, Blettner M, et al. 1990. Birth defects among children born to a population occupationally exposed to pesticides in Colombia. Scand J Work Environ Health 16:239–246.

Richiardl L, Bellocco R, Adami HO, Torrango A, Barllo L, Hakulinen T, et al. 2004. Testicular cancer incidence in eight Northern European countries: secular and recent trends. Cancer Epidemiol Biomarkers Prev 13:2157–2166.

Rothman KJ, Louik C. 1978. Oral contraceptives and birth defects in human offspring. Am J Epidemiol 113:563–574.