Hypospadias and Endocrine Disruption: Is There a Connection?

Laurence S. Baskin,[1] Katherine Himes,[2] and Theo Colborn[3]

[1]Department of Urology, University of California, San Francisco, California, USA; [2]Harvard University Medical School, Boston, Massachusetts, USA; [3]Wildlife and Contaminants Program, World Wildlife Fund, Washington, D.C., USA

Hypospadias is one of the most common congenital anomalies in the United States, occurring in approximately 1 in 250 newborns or roughly 1 in 125 live male births. It is the result of arrested development of the urethra, foreskin, and ventral surface of the penis where the urethral opening may be anywhere along the shaft, within the scrotum, or in the perineum. The only treatment is surgery. Thus, prevention is imperative. To accomplish this, it is necessary to determine the etiology of hypospadias, the majority of which have been classified as idiopathic. In this paper we briefly describe the normal development of the male external genitalia and review the prevalence, etiology, risk factors, and epidemiology of hypospadias. The majority of hypospadias are believed to have a multifactorial etiology, although a small percentage do result from single gene mutations. Recent findings suggest that some hypospadias could be the result of disrupted gene expression. Discoveries about the antiandrogenic mechanisms of action of some contemporary-use chemicals have provided new knowledge about the organization and development of the urogenital system and may provide additional insight into the etiology of hypospadias and direction for prevention. ***Key words*****: antiandrogens, differentiation, external genitalia, gene expression, urogenital development.** ***Environ Health Perspect*** **109:1175–1183 (2001). [Online 7 November 2001]**
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Hypospadias is one of the most common congenital anomalies in the United States; it occurs in approximately 1 in 250 newborns or roughly 1 in 125 live male births (*1,2*). Hypospadias can be defined as an arrest in normal development of the urethral, foreskin, and ventral aspect of the penis. This results in a wide range of abnormalities, with the urethral opening being anywhere along the shaft of the penis, within the scrotum, or even in the perineum (*3*). The more severe forms of hypospadias are associated with penile curvature. Left uncorrected, patients with severe hypospadias may need to sit down to void and tend to shun intimate relationships because of the fears related to abnormal sexuality. Babies born with severe hypospadias and penile curvature may have "ambiguous genitalia" in the newborn period, making an immediate accurate sex assignment difficult.

The only treatment for hypospadias is surgical repair of the anatomical defect (*3*). Reconstruction, if performed by an experienced surgeon, generally involves a single outpatient procedure (*3,4*). Occasionally, however, extensive surgery is required, or patients may face "redo" surgeries to improve suboptimal results (*5*). There is significant morbidity associated with some surgical procedures to correct hypospadias as well as potential psychosocial consequences of having an abnormal genital (*6,7*). In addition to the difficulty of surgery, the emotional and physical stress for the parents of patients with ambiguous genitalia must be considered (*8*).

In this paper we explore the hypothesis that hypospadias may in part be the result of exposure to synthetic and/or natural chemicals that can perturb normal male development. The fetus is especially sensitive to these chemicals known as endocrine disruptors that can mimic or interfere with the natural hormones that control development. We provide a brief description of normal development of the male external genitalia and review the prevalence, etiology, risk factors, and epidemiology of hypospadias. We also present evidence concerning the effects of recently discovered xenobiotic antiandrogens on the development of the male urogenital system.

Classification of Hypospadias

Hypospadias is classified depending on the location of the urethral opening (meatus) (Figure 1) (*3*). Anterior hypospadias is described as glandular (meatus on the inferior surface of the glans penis; Figure 1A), coronal (meatus in the balanopenile furrow; Figure 1B), or distal (in the distal third of the shaft; Figure 1C). Middle hypospadias is along the middle third of the penile shaft. Posterior hypospadias extends through the proximal third of the penile shaft to the perineum and is described as posterior penile (at the base of the shaft), penoscrotal (at the base of the shaft in front of the scrotum; Figure 1D), scrotal (on the scrotum or between the genital swellings; Figure 1E), or perineal (behind the scrotum or behind the genital swellings; Figure 1F). Chordee or penile curvature is a downward curvature of the penis that typically accompanies the more severe forms of hypospadias. Standard classification of hypospadias does not take into account the associated penile curvature. A patient with severe curvature and an anterior urethral meatus may in fact require a more extensive surgery to correct both the curvature and the abnormal urethra.

Development of the Male External Urogenital System

Formation of the external male genitalia is a complex developmental process involving genetic programming, cell differentiation, hormonal signaling, enzyme activity, and tissue remodeling. By the end of 4 weeks of gestation, the hindgut and future urogenital system reach the surface of the embryo at the cloacal membrane on the ventral surface. During this indifferent stage up to the eighth week, the cloacal membrane, under the genital tubercle, is divided into the anal and anterior halves, the latter of which is composed of the urogenital membrane. The urogenital membrane is flanked on each side by two genital swellings, forming the urethral groove. At this point, masculinization of the external genitalia commences under the influence of testosterone converted to 5α-dihydrotestosterone (DHT) in response to a surge of luteinizing hormone from the pituitary. One of the first signs of masculinization is an increase in the distance between the anus and the genital structures. This is followed by elongation of the phallus, formation of the penile urethra from the urethral groove beginning from the anus at about 11 weeks, and development of the prepuce (*9,10*). The entire male urethra is formed by dorsal growth into the genital tubercle and ventral growth and fusion of the urethral folds (*3,4,11*).

The future prepuce begins to form at the same time as the urethra and is dependent on normal urethral development. At about the eighth week of gestation, low preputial folds (foreskin) appear on both sides of the penile shaft, which join dorsally to form a flat ridge at the proximal edge of the corona. The ridge does not entirely encircle the glans. The

Address correspondence to T. Colborn, Wildlife and Contaminants Program, World Wildlife Fund, 1250 24th Street NW, Washington, DC 20037 USA. Telephone: (202) 778-9643. Fax: (202) 530-0743. E-mail: colborn@wwfus.org

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foreskin is transported distally by active growth of mesenchymal tissue. The process continues until the foreskin covers all of the glans. The fusion is usually present at birth. If the genital folds fail to fuse, the preputial tissues do not form ventrally. Consequently, in hypospadias preputial tissue is absent on the ventrum, and it is excessive dorsally (Figure 1) (*3,4*).

At the molecular and cellular level, at approximately 8 weeks, the chronology of penile differentiation commences. The undifferentiated embryo proceeds along a female pattern of differentiation until it is altered by testosterone released by the fetal testis, which develops from genes encoded on the Y chromosome. It is at this time that testosterone is converted to DHT by the microsomal enzyme, type 2 5α-reductase, for complete differentiation of the penis with a male-type urethra and glans.

Prevalence of Hypospadias

In 1997, the Centers for Disease Control and Prevention (CDC) reported a doubling of hypospadias from 1968 to 1993 in the United States (*1*). Seven European countries, including Norway, Sweden, England and Wales, Hungary, Denmark, Italy, and France, also reported increasing rates of hypospadias during the 1960s, 1970s, and 1980s according to the International Clearinghouse for Birth Defects Monitoring Systems (*12*). These results did not demonstrate a worldwide trend. Increases were most notable in the United States, Norway, and Denmark. Also, it was determined that increases were not seen in the less affluent and less industrialized nations (gross domestic product was used as a marker of affluence and industrialization) for which data were available. Increasing trends in England, Canada, and the northern Netherlands appeared to be leveling off after 1985 (*12*). Between 1970 and 1986, there appeared to be no increase in hypospadias in Finland (*13*).

It is difficult to draw conclusions from international birth defects monitoring because different registries have different reporting requirements and diagnostic criteria as well as varying degrees of physician compliance with reporting. However, two independent surveillance systems in the United States with consistent and unchanging diagnostic criteria also reported significant increases in hypospadias over 30 years (*1*). Data from the Metropolitan Atlanta Congenital Defects Program (MACDP), a population-based registry that uses active case ascertainment in 22 hospitals and clinics in the Atlanta, Georgia, area, indicated that the total hypospadias rate almost doubled from 1968 to 1993 ($p < 10^{-6}$) at an annual rate of increase of 2.9% (*2*). No single hospital in the Atlanta metropolitan region was responsible for the observed increases. Between 1968 and 1990, severe cases increased from 1.1 to 2.7 per 10,000 live births (includes both males and females) and by 1993 to 5.5 per 10,000 births per year ($p < 10^{-6}$) (*1*). Severe cases in this registry included the urethral opening on the shaft of the penis, on the scrotum, or perineum.

The Birth Defects Monitoring Program (BDMP), a program that gathered diagnoses recorded on newborn discharge summaries from hospitals nationwide, also reported an increase in hypospadia; it increased from 20.2 per 10,000 live births in 1970 to 39.7 per 10,000 live births in 1993 ($p < 10^{-6}$) (*1*). Both independent surveillance programs indicate a near doubling in reported rates of hypospadias. It is unlikely that this increase is due to greater sensitivity in the surveillance programs because no major changes in case ascertainment has occurred in the MACDP or the BDMP during that period. It is possible that physicians' reporting habits of hypospadias have changed over time, particularly in increased reporting of mild hypospadias. This is not consistent, however, with reports from the MACDP, which indicate that the ratio of mild-to-severe hypospadias decreased from 4.2 in 1968–1982 to 2.6 in 1983–1993 (*1*), and the unclassified hypospadias decreased. This raises the question whether the mild cases are underreported. Nonetheless, these longitudinal studies support an increase in hypospadias in the United States over a 14-year period.

Etiology

Reports of increasing prevalence of hypospadias have raised questions concerning etiology, treatment, and prevention. To date, there is no comprehensive understanding of the etiology of hypospadias that can inform primary prevention efforts and improve therapeutics. The etiology of many hypospadias is often assumed to be multifactorial, implicating some combination of genes and environment in the development of the anomaly. Efforts to define a clear etiology have been unsuccessful. For example, 33 patients with severe (scrotal or penoscrotal) hypospadias were evaluated with a range of diagnostic techniques including clinical assessment, ultrasonography, karyotyping, endocrine evaluation, and molecular genetic analysis of the androgen receptor (AR) and 5α-reductase genes to classify and determine the cause of the hypospadias. In 12 patients, diagnoses were determined. The remaining 64% of patients were classified as hypospadias of unknown etiology (*14*).

Genetic impairment. Theoretically, genetic alterations in any of the genes involved in development of the male urogenital system could result in hypospadias. However, currently only a small percentage of

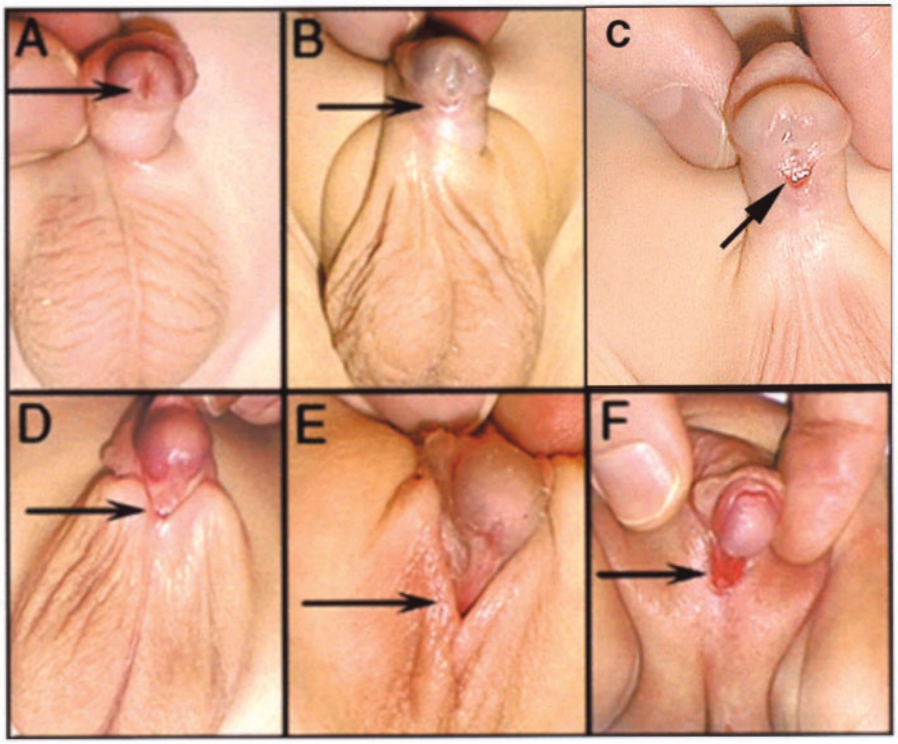

Figure 1. Classes of hypospadias by location of the meatus. (*A*) Anterior, on the inferior surface of the glans penis. (*B*) Coronal, in the balanopenile furrow. (*C*) Distal, on the distal third of the shaft. (*D*) Penoscrotal, at the base of the shaft in front of the scrotum. (*E*) Scrotal, on the scrotum or between the genital swellings. (*F*) Perineal, behind the scrotum or genital swellings.

hypospadias has been linked to genetic or chromosomal damage (*15–17*). One in nine patients with severe hypospadias had a single amino acid replacement of the AR (*17*). Single-strand conformational polymorphism analysis revealed a missense mutation of exon 2 of the *AR* gene in 1 of 40 patients with distal hypospadias (*18*). Several other authors concluded that mutations in the *AR* gene are rarely associated with hypospadias (*19–21*), implying that other factors are responsible.

Homeobox (*HOX*) genes are transcription factors that play a role in embryonic organization and patterning. Genes of the *Hoxa* and *Hoxd* clusters are expressed in regionalized domains along the axis of the urogenital tract. Transgenic mice with loss of function of single *Hoxa* or *Hoxd* genes exhibit homeotic transformations and impaired morphogenesis of the urogenital tract (*22–25*). Human males with hand–foot–genital syndrome, an autosomal dominant disorder characterized by mutations in *HOXA13*, exhibit hypospadias of variable severity, suggesting that *HOXA13* may be important in normal patterning of the penis (*26–28*).

Fibroblast growth factor (*FGF*) genes have been demonstrated to play a role in genital tubercle development (*29*). As with Hoxa-13, Fgf-10 and insulin-like growth factor receptor (Igfr) knockout mice have been shown to develop hypospadias. More specifically, the condition of the external genitalia in Fgf-10 knockout mice suggests impairment in the development of the glans penis.

Genetic mutations might also interfere with epithelial–mesenchymal interactions necessary for normal embryogenesis (*30*). The Sonic hedgehog (*Shh*) gene is expressed in the epithelium of the male urogenital sinus and is not regulated by testosterone. *Shh* has also been shown to be critical for prostate development; however, it has not been studied in relation to hypospadias (*31*). Genetic impairment of *Shh* during development may be involved in hypospadias and is consistent with the well-established role of *Shh* in limb development (*32*).

Indirect effects of genetic impairment. Genetic mutations could theoretically interfere indirectly with fetal testis and adrenal testosterone production and with the adequate virilization of the urogenital sinus and external genitalia during embryogenesis if the conversion of testosterone to DHT by 5α-reductase is interrupted. In addition, any errors in the activity of enzymes involved in converting cholesterol to testosterone could indirectly affect urogenital virilization. Aaronson et al. (*16*) determined the incidence of defects in three major enzymes in the biosynthetic pathway leading to the production of testosterone (3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, and 17,20-lyase) in 30 boys with fully descended testes but with penoscrotal or proximal shaft hypospadias. One-half of the boys had evidence of impaired function of one or more of these enzymes, suggesting that there was an underlying defect in the biosynthesis of testosterone (*16*).

Attempts have also been made to link hypospadias to low numbers of ARs. No deficiencies in either AR or 5α-reductase levels were found in preputial skin from boys with hypospadias (*33*). Bentvelsen et al. (*34*) demonstrated that androgens induce proliferation of ARs as well as increase AR levels within cells. They did not find significant differences in mean AR content and measured AR expression in foreskins of boys with hypospadias and age-matched controls (*15*). However, they did not measure the mean AR expression in the preputial folds during gestation when hypospadias is determined.

Other risk factors. A number of maternal and paternal risk factors have been suggested. Maternal age and primiparity have been significantly associated with hypospadias, although some studies have questioned the maternal age effect (*35*). Fisch et al. (*36*), using data from New York (1983–1996) and California (1983–1989, 1990–1995), found that advanced maternal age increased the risk factor for hypospadias by 20%. They also found a 50% increase in severe cases in sons of the older mothers (> 35 years).

Paternal risk factors associated with hypospadias include abnormalities of the fathers' scrotum or testes (*37*) and low spermatozoa motility and abnormal sperm morphology (*38*). Fritz and Czeizel (*38*) suggested that perhaps the recent increase in hypospadias reflects the improvement in fertility treatment, where the number of children born to subfertile men is increasing.

In addition to parental risk factors, lower birth weight has been associated with hypospadias (*39*). Fredell et al. (*39*) examined discordant monozygotic twins and found that the the birth weight of the twin with hypospadias was 78% of the birth weight of the twin without hypospadias. The birth weight difference was still significant when compared with birth weight difference between healthy monozygotic twins. Another study found that boys with hypospadias have a lower placental weight than control boys (*40*). Growing evidence suggests that androgens play a role in the lower birth weight of girls compared to boys (*41*). Exposure to an agent that compromises the weight-gaining advantage of androgen during gestation could play a role in lowered birth weight and development of hypospadias.

Prenatal exposure to progestins or combined progestins and estrogens led to a 4-fold increase in hypospadias (8.3% among cases vs. 1.8% among controls) (*42*). In another study, there were two cases with hypospadias among 53 males conceived via *in vitro* fertilization; both of the cases were exposed to progesterone administration up to the eighth week of gestation (*43*). In a study in Maryland, Silver et al. (*44*) found a 5-fold increase in risk of hypospadias in boys conceived by *in vitro* fertilization, again supplemented with progesterone through the eighth week, with an incidence of 1.5%. In both studies, advanced age and prior fertility problems confound the associations. However, advancing age has been associated with advancing body burden of persistent, bioaccumulative xenobiotics. The women in these studies were ≥ 35 years of age. On the other hand, in an 846 case–control pair study using data from eight countries, Kallen et al. (*45*) found no association between contraceptive use and hypospadias. A meta-analysis of first trimester exposure to progestins and oral contraceptives also showed no increased risk for hypospadias (*46*). Exposure to the pharmaceutical diethylstilbestrol (DES) was excluded in this study. Cosgrove et al. (*47*) reported one case of hypospadias among 11 DES-exposed males compared with none in 4 controls. This is hardly evidence for a causal relationship. In their larger survey (225 cases and 111 controls), a risk ratio of 7.2 for urination problems was discovered, suggesting a urethral problem (*47*). Gill et al. (*48*), in an extensive follow-up of DES offspring, report no finding of hypospadias.

North and Golding (*49*) found that mothers (n = 7,928 male births) who took a codeine preparation during the first trimester had significantly more sons born with hypospadias than mothers who did not (2.3% vs. 0.5%). The odds for developing hypospadias were 2.07 [95% confidence interval (CI), 1.00–4.32; p = 0.041] if the mother took iron supplements during the first 18 weeks of pregnancy and 3.19 (95% CI, 1.50–6.78; p = 0.002) if the mother had influenza in the first trimester (*49*).

Environmental factors. In the past, environmental factors were generally ruled out as causes for hypospadias (*35,40*). More recently, however, multicausality models include environmental contaminants to determine risk of developing a given phenotype. For example, familial clustering of hypospadias among first-degree relatives has traditionally been perceived as evidence of a strong genetic component in the etiology of hypospadias. In light of the growing number of endocrine disruptors reported in human tissue (*50,51*), exposure to environmental contaminants is now being considered in familial clusters because of the high probability of shared exposure. In those cases where the effects are the most profound, genetic

predisposition exacerbated by environmental exposure should be considered (*38*).

The increases of multiple end points of male dysgenesis over the past 50 years co-occurring with increasing production and use of synthetic chemicals has raised concerns that environmental factors may play a role in the etiology of these problems (*52,53*). Increasing rates of hypospadias have paralleled reports of other untoward end points related to male reproductive health, including increasing rates of testicular cancer (*54*) and cryptorchidism, and decreasing semen and sperm quality (*55*). Cheng et al. (*56*) found that 8% of patients (n = 252) with undescended testes also had urogenital anomalies and over 50% of those were hypospadias. Prener et al. (*57*) found that testicular cancer risk increases in cases with undescended testicles [relative risk (RR) = 5.2; 95% CI, 2.1–13.0] and hypospadias (RR = 4.2; 95% CI, 0.4–42.7) as well as reduced sperm production and quality. The authors point out that this suggests there may be a common causal agent.

Changes in gene expression in the presence of xenoantiandrogens. Hypospadias as the result of disrupted gene expression during embryogenesis provides a potential explanation for some of the unexplained cases among individuals who do not have genetic mutations. Several environmental antiandrogens have been discovered since 1994, each having unique mechanisms of action that interfere with differentiation and function (*58*). Under normal conditions, testosterone dissociates from its carrier proteins in plasma and enters cells via passive diffusion (*59*). Once in the cell, testosterone binds to the AR and induces conformational changes that protect the complex from degradation by proteolytic enzymes (*59*). This conformational change is also required for AR dimerization and DNA binding—steps necessary for the effects of testosterone to be expressed. The AR complex then binds the androgen response element along the DNA and activates transcription of genes leading to development of the male gonad from the gene products. Androgen binding also displaces heat shock proteins, possibly relieving constraints on receptor dimerization or DNA binding. DHT also binds the AR with enhanced androgenic activity, in part because of its slow dissociation rate from the AR (*59*).

Antiandrogens can interfere with the proper conformational change necessary to stabilize the AR that allows DNA binding. They can also inhibit AR binding DNA because of increased AR degradation, or increase the failure of mixed-ligand AR dimer binding DNA because of inappropriate dimer conformation. They can also interfere with the ability to release receptor-associated heat shock proteins (*58*). *In utero* exposure to *p,p′*-DDE, the persistent, lipophilic metabolite of DDT, can lead to feminization of the developing male fetus. *p,p′*-DDE inhibits androgen binding to the AR and inhibits transcription in androgen-responsive genes. Pregnant rats gavaged with *p,p′*-DDE produced pups that exhibited reduced anogenital distance (AGD), hypospadias, and cryptorchidism (*58*). The doses used in this study were within the range of human exposure (*60,61*). The authors discovered that *p,p′*-DDE is 1/10th as potent as flutamide, a pharmaceutical used to treat adults with prostate cancer (*58*).

Another pharmaceutical, finasteride, provides a different model for an antiandrogen. It inhibits human type 2 5α-reductase, responsible for converting 5α testosterone to DHT (*62*). This drug is used to treat benign prostatic hyperplasia because it decreases circulating and tissue levels of DHT.

Normal urogenital differentiation also relies on the interdependency of testosterone with epidermal growth factor (EGF), a potent mitogen. The AR mediates EGF's role in male sexual differentiation (*63*). The content of EGF increases in the fetal genital tract of mice with advancing differentiation (*64*). EGF alone induces partial virilization of the external genitalia *in vivo*, and in the presence of anti-EGF serum, differentiation is inhibited. Full differentiation, however, requires the presence of testosterone (*63*), similar to the role of EGF to promote growth and differentiation of the mouse uterus and vagina (*65*). Reduced EGF density in foreskins was discovered in 16 children undergoing hypospadias surgery compared with 22 children undergoing circumcision (p = 0.001), although there was no reduction in mean EGF receptors (EGFR) (*66*). This suggests interference with receptor binding. EGF has a wound-healing effect in the genitourinary tract, leading these authors to suggest that the lack of EGF may reflect some of the wound-healing problems associated with hypospadias surgery.

Although 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) has not been demonstrated in the laboratory to cause hypospadias in males, it induces c-Src kinase activity and reduces EGFR binding during testicular development following a single intraperitoneal dose of dioxin (0.1, 1.0, 5.0, and 10.0 µg/kg/body weight) in 21-day-old rats (*67*). Dioxin, as well as furans, polychlorinated biphenyls (PCBs), and some chlorinated pesticides (e.g., *p,p′*-DDE, dieldrin, heptachlor, chlordane, toxaphene, lindane, fenarimol) are cytochrome P450 (CYP450) isozyme agonists that induce one or more of the testosterone and benzo[*a*]pyrene hydroxylases (*68–70*). Each of these chlorinated products has its own pattern of action. For example, the herbicide fenarimol both induces and suppresses CYP450 activity depending on dose, sex, and tissue studied (*70*). In the case of dioxin, changes were found at doses that are environmentally relevant (*68*). More embryonic studies are needed to determine if perturbation of these enzyme systems interferes with imprinting or morphogenesis.

Several synthetic chemicals that act as xenoandrogens profoundly affect the developing reproductive tract and consistently induce hypospadias in male offspring exposed *in utero* (Table 1) (*71,72*). Vinclozolin, a commonly used fungicide on soft fruits and vegetables, induces female-like AGD, retained nipples (areolas), cleft phallus, and hypospadias in 100% of male offspring exposed during sexual differentiation (100 mg/kg/day to pregnant rats) (*71,73*). AGD and areolas were reported at the lowest dose administered, 3.125 mg/kg/day; hypospadias was reported at 50 mg/kg/day. Gray and colleagues (*71,73*) point out that target tissue responses differ depending on varying AR numbers, the presence or amount of nuclear coactivators and repressors, the availability of androgen response elements on androgen-dependent genes, and tissue metabolism. The parent compound, vinclozolin, is inert and acts through two active metabolites that competitively bind and inhibit the AR with different potency (*74*). The metabolites shift active ARs to inactive ARs by binding to only a small percentage of cellular ARs, thus preventing maximal DNA binding, reducing androgen gene expression and protein synthesis, and ultimately altering morphologic development.

Procymidone, another antiandrogenic fungicide, induces hypospadias in all male offspring of pregnant rats fed 200 mg/kg/day during sexual differentiation (*75*). At the lowest dose administered, 25 mg/kg/day, hypospadias, areolas, AGD, and reduced weight of androgen-dependent tissues,

Table 1. Environmental endocrine disruptors that cause hypospadias in laboratory animals.

Type	Chemical
Agricultural and public health	
Insecticide	*p,p′*-DDE (breakdown product of DDT)[a]
Fungicides	Vinclozolin[a] Procymidone[a]
Herbicide	Linuron[a]
Industrial	
Plastic components	DBP[a] DEHP[a]
Persistent organochlorines	Dioxin (TCDD)[b] PCB 169[b]

Data from Gray et al. (*71*) and Hurst et al. (*72*).
[a]Reduced anogenital distance, the most sensitive end point. [b]Only in females; in all other cases, females have not been examined to date.

including the glans penis, were reported. *In vitro*, procymidone inhibits DHT-induced transcriptional activation in cell lines transfected with human AR. The range of effects is similar to those associated with vinclozolin and *p,p′*-DDE (*71*).

Male rats perinatally exposed to dibutyl phthalate (DBP) and diethylhexyl phthalate (DEHP) have reduced AGD, retained nipples, epididymal agenesis, undescended testes, and hypospadias. Gray et al. (*76*) never found a no-effect level for the phthalates. They also believed that phthalates exerted their antiandrogenic action through a mechanism other than AR antagonism (*71*). Follow-up studies found that DBP, DEHP, and diisononyl phthalate (which is weaker) inhibit fetal testosterone production rather than competitively binding AR (*77,78*). Gray et al. (*73*) propose that for some end points in male development, especially AGD, there may be no threshold dose of an active compound.

Linuron, a widely used herbicide, is a weaker AR antagonist than procymidone and vinclozolin. It also causes reduced AGD, retained nipples, and low incidence of epispadias (1:13). The other lesions of the male reproductive tract are not similar to those caused by procymidone and vinclozolin (*71*).

Changes in gene expression in the presence of xenoestrogens. There are currently no known xenoestrogens that have been associated with hypospadias, nor is there clear evidence in the literature on how xenoestrogens might cause hypospadias. However, the sons and daughters exposed *in utero* to DES provide an excellent model for interrupted gene expression during development. They suffer a broad range of reproductive tract problems, although hypospadias has rarely been reported (*79,80*). Abnormalities of the urethra (4.4% vs. 0%; $p = 0.017$) and problems passing urine (12.9% vs. 1.8%; $p = 0.0003$) were significantly higher in DES sons than in controls (*79*). Although data do not support an association between DES and hypospadias in humans, in male rat pups exposed to DES on gestation days 13, 16, 18, and 20, hypospadias was observed at all doses administered (0.015, 0.03, and 0.60 mg/kg subcutaneous) (*81*).

There are a number of possible mechanisms by which xenoestrogens might alter development of the penis and urogenital tract, from influencing specific areas of the brain to directly affecting the development of the reproductive organs. In addition, each xenoestrogen can manifest a unique range of molecular mechanisms that differ depending on the stage of development and tissue involved. For example, the widely used insecticide methoxychlor is both estrogenic and antiandrogenic, depending on time of exposure and target tissue involved. It must be metabolized to be active, and its metabolites at the molecular level bind both the estrogen receptor and the AR (*82*).

As mentioned earlier, *Hox* genes play an essential role in organization of the urogenital system. The expression of these abdominal *Hoxa* genes in embryonic mice is under control of estrogen (and progesterone) (*82*). DES inhibits expression of *Hoxa-10* gene in developing female reproductive tissue in mice. Some anomalies induced by perinatal administration of DES to mice resembled morphology in *Hoxa-10*, *Hoxa-11*, and *Hoxd-13* mutant mice. Thus, in addition to a possible primary defect in *Hox* genes, improper regulation or expression of hormonal factors during embryogenesis could disrupt normal expression of *Hox* genes as well, and lead to reproductive tract anomalies. If it is determined that environmental endocrine disruptors with estrogenic activity can repress abdominal *Hox* gene expression in the developing fetus, this mechanism provides an explanation of how transient *in utero* exposure to an endocrine disruptor(s) could lead to a permanent anomaly such as hypospadias.

Soy-based diets, rich in phytoestrogens, can affect male sexual differentiation in laboratory animals and cause male genital tract tumors or developmental disorders. Santti et al. (*84*) hypothesized that phytoestrogens and structurally related compounds that have a weak affinity for the estrogen receptor but are present in large quantities in the diet could act as antiestrogens. To date, there are no studies revealing a mechanism of phytoestrogens that could lead to hypospadias.

Exposure

Little quantitative, empirical data on human embryonic or fetal exposure to xenobiotics exist, even though these individuals are the most vulnerable to endocrine disruptors (*85*). Humans are continually in contact with endocrine-disrupting chemicals: for example, pesticides; concentrated food products containing natural plant estrogens; and by-products and end products of modern technology, including plastics and plastic components, detergents, pharmaceuticals, perfumes and cosmetics, among others (*86,87*). Some of these substances do not degrade rapidly, and because of their persistence, they accumulate in human tissue over a lifetime (*50*). Diet, lifestyle choices, and occupation play a large role in determining individual exposure to synthetic estrogens and antiandrogens, which varies significantly between individuals and populations based on cohort age and geographic location. By the time a woman reaches reproductive age, she shares her accumulation of the persistent chemicals with her offspring in the womb and through breast-feeding. For example, in 1998, *p,p′*-DDE was the most frequently recovered contaminant in amniotic fluid of women ≥ 35 years of age (range 0.1–0.63 ng/mL; $n = 41$), even though agricultural use of DDT in the United States was restricted in 1972 (*60*). PCBs and phytoestrogens (demonstrated estrogens) have also been recovered in amniotic fluid (*60*). On a broader scale, a CDC nationwide survey of contaminants in urine found that women between 20 and 40 years of age had significantly higher voiding concentrations of the metabolites of DEHP (demonstrated antiandrogens) than any other cohort. The main route of exposure in this case was thought to be inhalation (*87*).

Total cumulative worldwide use of DDT is estimated to be 1,500,000 metric tons since it was first produced in 1938. The breakdown products of DDT have a half-life of 58 years in temperate climates, which means that more than one-half of the DDT produced and its degradation products are still in the environment (*88*). Figures for use of vinclozolin, linuron, and procymidone are more difficult to find. The data are patchy, dependent upon weather conditions from year to year, crops under tillage, regional growing patterns, and compulsory reporting of use. However, it is estimated that 1 billion pounds of phthalates are produced each year. It is difficult to define what percentages of that are DBP and DEHP (*89*).

Human Epidemiology

A limited number of human epidemiologic studies have examined the risk of hypospadias in offspring of parents based on regional agricultural and industrial background exposure and lifestyle (Table 2). A study in Minnesota found an increased risk of urogenital anomalies in the general population when crop regions were compared. The odds ratio was 1.56 in the corn/soybean region compared with 2.25 in the wheat/sugar beet/potato region of the state (*90*). The latter region was considered a high-use region based on poundage applied of fungicides and chlorphenoxy herbicides. Not only are offspring of farmers in this region at greater risk but children of nonagriculturally employed parents living in the same region are as well. Children conceived in the spring were at greatest risk for all birth defects ($p < 0.01$), which coincided with the season of heaviest pesticide use. Hypospadias was not distinguished among the urogenital effects in this study.

A Canadian study comparing birth defects among four communities found a significant increase in urogenital defects between one community and three others that were not as industrialized (*91*). Hypospadias was the only end point that was significantly different among the communities. No association was

made with a specific industry in the community. However, three industries in the high-risk community (producers of polyvinyl chloride, aluminum, and paper and pulp) are among those associated with the release of dioxin and dioxin-like compounds. As with the Minnesota study (*90*), the urogenital defects were not categorized.

Hypospadias has been correlated with TCDD in boys born after an explosion in Seveso, Italy, in 1976. Exposure decreased across four zones extending distally from the factory site, based on soil contamination (*92*). Zone A was the area of highest exposure (TCDD = 192.8 μg/m^2). Zones B and R had decreasing concentrations of TCDD that varied from 3 to 43.8 μg/m^2 in Zone B and 0.9 μg/m^2 to 9.7 μg/m^2 in Zone R. Zone Non-ABR (not affected by the explosion) was included in the study for comparison. Zone A had two mild birth defects (n = 26) and no hypospadias. Soon after the explosion, Zone A was affected by abortions (spontaneous and recommended) and stillbirths. There were 4 cases of hypospadias in 435 births in Zone B (~1:100 births or 1:54 male births), which decreased to 4 cases in 2,439 births in Zone R (~1:602 live births or 1:305 male births) and to 41 cases in 12,391 births in Zone Non-ABR (~1:300 live births or 1:150 male births). It is not clear if all hypospadias cases were reported. As with most epidemiologic studies looking for differences across a large number of birth defects, the data specific to hypospadias are inconclusive.

Dolk et al. (*93*) reported a small and marginally significant increase in hypospadias among sons of families living near hazardous-waste landfill sites in Europe (p = 0.06). In a Danish study looking at cryptorchidism and hypospadias in the offspring of farmers and women gardeners, Weidner et al. (*94*) found no risk for hypospadias but an increase for cryptorchidism. A Norwegian study looking at 192,417 births between 1967 and 1991, where the parents were identified as farmers, revealed an odds ratio of 1 for hypospadias (*95*). However, between 1967 and 1971, the odds increased to 2.06 among tractor sprayers. Prevalence was greatest throughout the study for April–June conceptions and grain farming.

A longitudinal pregnancy study (n = 7,928; p = 0.001) in the United Kingdom concluded that boys born of vegetarian mothers have an odds ratio for hypospadias of 4.99 (95% CI, 2.1–11.88) (*49*). Sons of vegetarian mothers who consumed only organic produce had no hypospadias, where 1.07 cases were expected. Although these boys represented a very small fraction of the study population, this raises the question whether pesticides used on fruits and vegetables may be involved, rather than phytoestrogens. Mothers who drank soy milk and ate soy products delivered a larger proportion of boys with hypospadias, although this was not statistically significant. Mothers who were vegetarians before their pregnancies but became omnivores throughout pregnancy were no more likely to have a son with hypospadias than those mothers who were never vegetarians (*49*).

Evidence in Wildlife

Although hypospadias has been reported in domestic animals (*96*), it has never been reported in wildlife, perhaps because of the difficulties associated with examination. However, a 1.5-year-old zoo polar bear was recently discovered with hypospadias. The captive-bred bear was presumed to be a female until it was examined for a urinary problem (*97*). Recent findings of abnormal baculum among mink and river otters on the lower Columbia River (*98*) and unusual external genitalia and pseudohermaphroditism among black and polar bears (*99,100*) suggest that gonadal development in wild mammals may currently be affected by xenobiotics. With the exception of the black bears that were not monitored for contaminants, all of the above animals were carrying elevated levels of organochlorine chemicals (*97–100*), and in the case of the river otters, there was a dose–response relationship with the intensity of the problem (*98*).

Estrogenic and antiandrogenic contaminants have been associated with impaired phallus (penile) development in the American alligator in several lakes in Florida (*101*). Male mosquito fish from the same habitat have gonopodia that are 25% smaller than those of fish in a reference lake (*102*). The alligators carry a range of known endocrine disruptors in their tissue (e.g. dieldrin, endrin, mirex, *p,p′*-DDE, oxychlordane, *trans*-nonachlor, hexachlorobenzene, heptachlor epoxide, heptachlor) (*101*). Although it has been demonstrated that 17β-estradiol disrupts postnatal penile development in mice (*103*), the normal process of penile development is poorly understood at the cellular and molecular levels, and little is known about how or whether exogenous estrogens perturb penile development. It could very well be that the animals are exhibiting the result of exposure to a vast number of mixtures of xenobiotics that interfere with both estrogen and androgen control of development.

Conclusion

Hypospadias is an anomaly with multiple etiologies. Table 3 summarizes the mechanisms that have been proposed as possible causes, some of which have been demonstrated in the laboratory and others that have been hypothesized to account for the observed effects. Perhaps, as the human genome project progresses and more is revealed about the genes controlling male development, other genetic causes will be discovered to account for some cases of hypospadias that are currently classified as idiopathic.

Over the past decade, however, rapid advances in integrated cellular, molecular, physiologic, biochemical, and toxicologic research have revealed several stages of urogenital development that are vulnerable to endocrine-disrupting chemicals. To date, the activity of xenoestrogens and their feminizing effects on males do not explain hypospadias. However, since environmental antiandrogens were first reported in 1995 (*58*), several stages of male urogenital development have clearly been revealed where specific synthetic chemicals can impede normal molecular and biochemical activity leading to frank expression of hypospadias. Despite these new discoveries, the lack of a putative causal agent

Table 2. Urogenital anomalies and hypospadias in offspring of mothers and/or fathers exposed to natural and synthetic endocrine-disrupting substances.

Exposure/geographic location	Outcome	Identified risks	Reference
Father's occupation as farmer, pesticide applicator, Minnesota	Urogenital anomalies	OR = 1.69; 95% CI, 1.06–2.64; p = 0.06	(*90*)
Parents reside in four communities with different industrial activity, Quebec, Canada	Urogenital anomalies	One community had significantly more urogenital anomalies than the other three	(*91*)
Parents exposed to dioxin after an industrial explosion, Seveso, Italy	Hypospadias	Increasing number of hypospadias in dose–response exposure to TCDD (dioxin)	(*92*)
Within a 3-km radius of a hazardous-waste landfill, Europe	Hypospadias	OR = 1.96; 95% CI, 0.98–3.92; p = 0.06	(*93*)
Parent occupation as gardener or farmer in year of conception, Denmark	Urogenital anomalies	Mother OR = 1.27; 95% CI, 0.81–1.99 Father OR = 1.19; 95% CI, 0.96–1.49	(*94*)
Parent occupation as farmers, Norway	Hypospadias	OR = 1.00; 95% CI, 0.75–1.34 OR = 2.06; 95% CI, 1.00–4.23 for tractor applier (1967–1971)	(*95*)
Vegetarian mothers, United Kingdom	Hypospadias	OR = 4.99; 95% CI, 2.10–11.88; p = 0.001	(*49*)

OR, odds ratio.

Table 3. Proposed explanations for the etiology of hypospadias.

Explanation	References
Defective gene (direct)	
Hox: organization and patterning	(*25–28*)
Shh: tissue to tissue signaling	(*30*)
Fgf family: growth and development	(*32*)
Igfr: growth and development	(*104*)
Enzymes: steroidogenesis and metabolism	(*16*)
Receptors: missense or frameshifts	(*18*)
Defective gene (indirect)	
Steroidogenesis precursors	(*16*)
Receptors	(*17,18*)
Perturbed gene expression (direct)	
AR antagonist	(*59,72,74*)
Increased AR degradation	(*59*)
Estrogen receptor agonist	(*80*)
Estrogen receptor antagonist	(*83*)
Perturbed gene expression (indirect; successional or developmental cascade effects)	
Inhibition of	
Fetal testosterone	(*77,78*)
DHT production	(*62,71,75*)
Induction of	
c-Src kinase leading to reduced EGFR	(*64–66*)
CYP450 isoenzyme agonists interfering with steroid homeostasis	(*68–70*)
Interference with	
Release of heat shock proteins	(*58,59*)
AR conformational change during binding	(*58,59*)

for hypospadias in humans continues to pose a problem. This will become more of a problem if the list of antiandrogens continues to grow. It will also increase the difficulty of making personal and public health decisions about reducing exposure of reproductive age individuals.

In the meantime, more new epidemiologic approaches are urgently needed to determine whether endocrine disruptors are involved in the etiology of hypospadias. We hope that technology will continue to improve and broaden the scope of detection of contaminants in human tissue, and surveillance programs will be established. Surveys of urine and other tissue, such as breast milk, from childbearing-age females are needed, followed by laboratory confirmation that the parent products and metabolites in the tissue do or do not cause developmental problems. Similarly, in epidemiologic studies where the risk more than doubles for hypospadias and where there are only associations between exposure with suspected classes or groups of agricultural or industrial chemicals, laboratory confirmation of the safety of the suspected chemicals is needed. To date, almost none of the 15,000 high volume chemicals, widely used and found in the environment, have been tested during development for their possible endocrine-disrupting effects, either at high or background exposure doses.

The human embryo/fetus is exposed to endocrine disruptors from conception to birth via placental transfer from the mother. The concentration of persistent xenobiotics transferred to the unborn is dependent on maternal daily exposure as well as accumulated maternal body burden, which varies among individuals. Even before the moment of conception, the embryo is exposed to its mother's background burden of persistent chemicals such as PCBs, dioxins, furans, and DDT (*105*). In addition, many xenobiotics are not persistent and their exposure is transient, increasing the difficulty of determining exposure *in utero*. In light of the human suffering associated with hypospadias, determining the etiology and exercising prevention should be major goals for public health authorities and clinicians, respectively.

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