

**Potential role of hormonally active environmental contaminants
in human reproductive tract developmental abnormalities**

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Abstract

Fetal development and early postnatal life are periods of rapid growth, organ differentiation and development and are thus considered to be a period of increased sensitivity to the adverse effects of environmental or other toxicants. Sexual differentiation is a complicated, hormone-dependent sequence of molecular and cellular processes that must occur with precision and coordination to develop the male reproductive system and associated secondary sexual characteristics. Sexual differentiation, organogenesis and development of the endocrine system are sensitive to exogenous insults during prenatal development. Experimental animal studies have shown that exposure to environmental or other toxicants during these relatively brief critical windows of susceptibility can disrupt interdependent developmental processes and cause persistent functional and structural changes. Research findings have also shown that some reproductive tract toxicants specifically interfere with normal endocrine function but most have multiple mechanisms of actions and targets. The objective of this review is to describe the potential role of hormonally active environmental toxicants in the etiology of birth defects and functional abnormalities of the reproductive tract.

Introduction

The objective of this review is to describe the potential role of hormonally active environmental toxicants in the etiology of birth defects and functional abnormalities of the reproductive tract. The term “hormonally active agent” (HAA) is used here to describe exogenous substances with hormone-like activity, regardless of mechanism (National Academy of Sciences, 1999). Most evidence for adverse hormonal effects of environmental contaminants on the reproductive tract comes from studies of animals as there are few relevant epidemiologic studies (Vos et al., 2000). Particularly strong evidence comes from genetically homogeneous experimental animals in which exposures can be carefully controlled and administered to randomly selected animals, permitting valid comparisons of adverse health outcomes in exposed and unexposed animals. Such studies, however, carry uncertainties related to the use of high doses (generally) and the need to extrapolate results to humans.

Concern about environmental HAAs is a relatively recent phenomenon but the ability of HAAs such as DDT/DDE to greatly reduce testicular growth and secondary sexual development in young roosters was reported over 50 years ago (Burlington and Lindeman, 1950). The discovery of developmental and reproductive abnormalities and a rare form of vaginal cancer among children of women who used the drug diethylstilbestrol (DES) during pregnancy showed the potential threat of prenatal exposure to exogenous HAAs. No known environmental contaminant, however, has potency and human exposure levels comparable to DES. Exposure to environmental or other toxicants during relatively brief critical windows of susceptibility during fetal and early postnatal life can disrupt interdependent developmental processes and cause persistent functional and structural changes. For instance, the male rodent reproductive tract is more sensitive to toxicants such as TCDD during early-life development than during adulthood (Foster, 1998). Some reproductive tract toxicants specifically interfere with normal endocrine function but most have multiple mechanisms of actions and targets.

Endocrine function during fetal and childhood reproductive tract development

Fetal and child growth and development depend on normal maternal, fetal and child endocrine function during critical developmental time periods. For example, a deficiency of thyroid hormones during prenatal and neonatal development can cause profound mental retardation and impaired growth. This section describes selected aspects of normal endocrine function during early child development, focusing on the male and female reproductive systems.

During early pregnancy, a Y-chromosome gene (SRY) directs male embryonic gonadal cells to produce Mullerian inhibitory hormone (MIH) and testosterone (T) and to down-regulate expression of CYP19, a gene that encodes the enzyme p450 aromatase that converts T to estradiol-17 β (E2). T and MIH induce the differentiation of the mesonephric ducts into the epididymis, seminal vesicles and prostate. T also induces male differentiation of external genitalia and likely plays a role in testicular descent (Pryor et al., 2000). MIH also causes regression of the Mullerian ducts that, in the female fetus, form the fallopian tubes, uterus and upper vagina. Testicular growth during early infancy depends on thyroid hormones that regulate the duration of testicular Sertoli cell proliferation, thereby determining Sertoli cell numbers and sperm-producing capacity first expressed at puberty. In the female, proliferation and differentiation of oogonia and initiation of the first meiotic division all occur prenatally. A female infant is born with about two million oocytes, a number that declines thereafter with a half life of about 5-6 years (Kacsoh B., 2000).

At puberty, increased production of hypothalamic gonadotropin-releasing hormone (GnRH) stimulates release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland. LH and FSH control production of gonadal and adrenal steroidogenic acute regulatory protein (StAR), the rate-limiting step in steroid hormone synthesis, and the level of several steroidogenic enzymes in the cytochrome P-450 family. LH binds to receptors in certain testicular and ovarian cells, stimulating production of T in males and E2 and progesterone in females. FSH stimulates proliferation and differentiation of granulosa cells in females and spermatogenesis in males. Prenatally, E2 stimulates female reproductive tract development while at puberty it stimulates breast and uterine development, body fat deposition and epiphyseal closure (the latter causes cessation of growth in stature). T stimulates spermatogenesis and growth of non-reproductive tissues including muscle. Progesterone binds to progesterone receptors (PR-A and PR-B) in target tissues and stimulates lobulo-alveolar breast development and glial cell myelin production in brain. Knockout mice missing the PR gene display anovulation, uterine hyperplasia and lack of mammary gland development (Chappell et al., 1997).

T, E2, progesterone, triiodothyronine (T3) and thyroxine (T4) are small, lipid-soluble molecules that bind to nuclear receptors in target tissues, activating hormone-responsive gene transcription and cellular processes including division and differentiation. Androgen receptors (ARs) are expressed ubiquitously in genital and non-genital tissues in both sexes. ARs mediate biological actions of T and dihydrotestosterone including transcription of androgen-responsive genes that control growth, differentiation and function of the male urogenital tract. Androgen receptor (AR)

gene polymorphisms and mutations may cause up to 10% of human male infertility (Yong et al., 2000). The two known estrogen receptor (ER) subtypes in rodents and humans (ER α and ER β) have tissue-specific patterns of expression. E2 has similar affinity for both receptors and the resulting E2-ER complexes induce expression of several estrogen-responsive genes (Kuiper et al., 1998). Human ER β decreases cell sensitivity to E2 and is widely expressed in the male reproductive tract. Sertoli cell proliferation depends on the thyroid hormones T4 and T3 and occurs only during the perinatal period. Fetal and early-life hypothyroidism disrupts testicular Sertoli cell proliferation.

This section has touched on a few of the many hormone-target site interactions that control prenatal and early-life time reproductive tract development. The multiplicity of these interactions raises the potential for interference by a wide range of toxicants including those with direct or indirect hormonal activity.

Hormonal activity of selected environmental contaminants

Several environmental contaminants bind to ARs or ERs with widely variable affinities and effects ranging from full agonist (stimulating gene expression) to full antagonist (inhibiting gene expression). The ER binding affinities and ER-dependent gene expression activities of various environmental contaminants span at least four orders of magnitude. The ER binding affinity of a given substance is not very informative as it is only weakly correlated with the ability to activate ER-dependent gene expression. ER agonists include o,p'-DDT, certain hydroxylated PCB metabolites, tris(4-chlorophenyl)methanol (TCPM, a global contaminant of unknown origin but structurally related to DDT) and brominated bisphenol A compounds. Although not environmental contaminants, hormonally active phytoestrogens are ubiquitous in plant foods such as soy-based infant formula (Setchell et al., 1997).

ρ,ρ' -DDE (the major DDT metabolite found in human tissues), vinclozolin and procymidone (fungicides), linuron (a herbicide), fenitrothion (an insecticide) and some polycyclic aromatic hydrocarbons (PAHs) inhibit human and/or animal AR-dependent transcriptional activity. Vinclozolin binds fairly weakly to AR but two of its metabolites (M1, M2) are stronger antiandrogens. Certain phthalate and fungicide metabolites inhibit fetal and neonatal T production. Triazine herbicides are not active in ER binding assays but are estrogenic *in vivo* in

rodents, the mechanism being unknown. Some HAAs have mixed effects; for instance, some methoxychlor metabolites are agonists of ER β and antagonists of ER β and AR.

The ability of certain phthalates to cause reproductive tract birth defects in rodents appears to depend on their ability to reduce T production in testicular Leydig cells rather than binding to AR (Parks et al., 2000). Because Sertoli cells are essential for adult spermatogenesis, exposure to Sertoli cell toxicants such as the phthalate metabolite mono-(2-ethylhexyl) phthalate can cause reduced adult sperm production (Richburg et al., 2000). Other toxicants including lead (Thoreux-Manlay et al., 1995) and 7,12-dimethylbenz[a]anthracene (Mandal et al., 2001) also reduce Leydig cell T production. TCDD and dioxin-like compounds have multiple effects in experimental animals including increased ER α and decreased AR levels in male reproductive organs (after perinatal exposure) and modulation of p450 cytochromes (may increase T and E2 inactivation and decrease T production) (Cavalieri et al., 2000). TCDD increases T4 excretion, causing increased thyroid stimulating hormone (TSH) levels and thyroid hyperplasia.

This section has described some of the complexity of mechanisms whereby HAAs can interfere with reproductive tract growth and development. As noted in the introduction, HAAs may exert hormonal effects by modulating hormone receptors or through diverse other mechanisms.

Exposure to hormonally active environmental contaminants

The US Centers for Disease Control and Prevention measured the levels of a few HAAs in urine samples collected from samples of the US population during NHANES III and IV (Centers for Disease Control and Prevention, 2001; Centers for Disease Control and Prevention, 2003). Some of the findings from NHANES IV (1999-2000) for hormonally active environmental contaminants potentially important in child health included:

Phthalates

- Urinary phthalate metabolite levels were highest for those derived from diethyl and dibutyl phthalate; their levels increased with age, especially among women (suggesting the potential for fetal exposure during critical prenatal development windows)
- Children age 6-11 years had higher urinary metabolite levels than older persons for dibutyl, benzylbutyl and di-2-ethylhexyl phthalate (the latter is the most widely used phthalate) but it is not known whether this reflects higher exposure or age-related differences in body size or

metabolism; these phthalates are used in many consumer products including cosmetics, pharmaceutical coatings, food containers/packaging and toys

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) – This potent toxicant was detected in 0.7% of serum samples from persons age 6 years or older (limit of detection was 4.8 pg/g lipid); observed levels were far below those associated with health effects in occupationally or accidentally exposed persons

Organochlorine pesticides

- Mirex, hexachlorobenzene and lindane were only detected in 0, 0.6 and 1.7% of serum samples
- The 95th percentile serum p,p'-DDT and p,p'-DDE levels were, respectively, 15-fold and fivefold lower compared to NHANES II (1976-1980)
- The 95th percentile urinary levels of 2,4,5- and 2,4,6-trichlorophenol (metabolites of several organochlorine pesticides including hexachlorocyclohexane, hexachlorobenzene and pentachlorophenol) were about 6-fold higher in NHANES IV adults compared to a non-random subsample of NHANES III adults

Bisphenol A concentrations in human maternal and fetal plasma (median 2-3 ng/mL) and placenta (median 13 ng/mL) included levels in the range of those in recent animal studies showing reproductive toxicity in male and female offspring (Schonfelder et al., 2002). Among women undergoing routine second trimester amniocentesis, about a third of amniotic fluid samples contained detectable levels of hormonally active environmental contaminants including α -hexachlorocyclohexane, p,p'-DDE and specific PCB congeners (Foster et al., 2000). Although contaminant concentrations were quite low, these findings show that the fetus is directly exposed to potentially harmful hormonally active toxicants. Because of potency and/or dose, the estrogenic activities of endogenous hormones and natural plant phytoestrogens greatly exceed those of hormonally active environmental contaminants.

Potential adverse health effects

Multiple male reproductive system abnormalities may comprise a testicular dysgenesis syndrome (Boisen et al., 2001). The syndrome of male genital tract birth defects, testicular cancer, semen quality, and subfertility may all involve disruption by HAAs of embryonic programming and fetal

gonadal development in genetically susceptible individuals. There is considerable animal evidence to support this hypothesis and some evidence from the few directly relevant epidemiologic studies.

Peer-review panels concluded that there were definite low-dose effects for several HAAs in experimental animals including estrogenic and immunologic effects of methoxychlor and nonylphenols (Melnick et al., 2002); possible low-dose effects included bisphenol (estrogenic) and vinclozolin (anti-androgenic). Indirect evidence of the potential importance of environmental HAAs in humans comes from reports of increasing incidence or prevalence rates of birth defects of the penis (hypospadias) and testes (cryptorchidism), altered timing of puberty and reduced semen quality.

Developmental abnormalities of the reproductive tract

Hypospadias

Hypospadias is a birth defect in which the urethra opens on the ventral surface of the penis. Birth prevalence rates of hypospadias vary substantially between and within countries. Data from Atlanta and the nationwide Birth Defects Monitoring Program both showed a doubling of hypospadias rates in the 1970s and 1980s with increases in all four regions of the United States. Severe hypospadias rates and the ratios of severe to mild cases both increased during 1968-1993, arguing against a reporting artifact (Paulozzi et al., 1997). Contrary to this in Finland where all children are examined at birth by a pediatrician, the cumulative prevalence of hypospadias to age eight years remained constant among boys born during 1970-1986 (Aho et al., 2000). Hypospadias birth prevalence and surgical repair rates were also constant in New York State during 1983-1995 (Choi et al., 2001).

Hypospadias has been linked to proximity to landfill sites (Dolk et al., 1998), to both maternal (Beral and Colwell, 1981) and maternal grandmother prenatal DES exposure (Klip et al., 2002), and to maternal prenatal use of progestins (Calzolari et al., 1986). Other risk factors include intrauterine growth retardation (IUGR) and preterm birth (Akre et al., 1999). Hypospadias was not associated with maternal serum DDE levels in a large birth cohort study (Longnecker et al., 2002).

Cryptorchidism

Testes spontaneously descend by age three months among about 70% of infants with neonatal cryptorchidism (undescended testicles). Clinical surveillance soon after birth tends to detect cases that would resolve spontaneously. Thus observed cryptorchidism rates depend on actual risk, diagnostic and reporting efficiency and age at examination. Reported rates during the past 30 years have varied considerably between countries and over time (Paulozzi, 1999). Rates of surgery for correction of cryptorchidism among boys age 0-14 years decreased by 33% in Great Britain during the 1990's, possibly because of decreased risk of cryptorchidism, more conservative diagnostic/treatment practices or both factors (Toledano et al., 2003). The absence of conclusive evidence concerning prevalence trends of male reproductive tract birth defects, however, does not eliminate a potential role for environmental contaminants in these conditions (see below).

Limited epidemiologic evidence indicates that cryptorchidism may be linked to prenatal parental pesticide exposure (Kristensen et al., 1997; Weidner et al., 1998), early childhood adipose tissue heptachlor and hexachlorobenzene levels (Hosie et al., 2000), increased free E2 and decreased T levels in maternal blood during early gestation (Bernstein et al., 1988; Key et al., 1996) and maternal smoking (McBride et al., 1991). There is mixed evidence for an association between cryptorchidism and maternal prenatal estrogen use (Beard et al., 1984; Depue, 1988). Cryptorchidism was not associated with maternal serum DDE levels in a large birth cohort study (Longnecker et al., 2002). Other risk factors include IUGR and preterm birth (Akre et al., 1999) and high maternal first trimester serum free E2 levels (Bernstein et al., 1988). In the face of conflicting results and the potential for major reporting artifacts, it is not possible to reach firm conclusions concerning hypospadias and cryptorchidism prevalence trends.

Other effects

In one of the rare epidemiologic studies to measure internal dose of environmental HAAs, there was a borderline association between high maternal serum DDE levels (the most persistent DDT metabolite) and accessory nipples in male infants (OR=1.9, CI 0.9-4.0) (Longnecker et al., 2002). This study used preserved maternal serum samples collected during 1959-1966, a time when

DDT levels were much higher than recently. Large, population-based case-control studies incorporating exposure biomarkers are needed to address the major knowledge gaps in this field.

Developmental reproductive toxicity of HAAs in experimental animals

Certain antiandrogenic pesticides (e.g., vinclozolin) cause developmental abnormalities in prenatally exposed experimental animals including feminization (e.g., reduced anogenital distance, retained nipples/areolas), reproductive tract malformations (e.g., cryptorchidism, hypospadias) and sexual dysfunction (e.g., inability after puberty to mate with sexually receptive females) (Gray et al., 2001). Prenatal exposure of experimental animals to high doses of several phthalate plasticizers can cause feminization of males and various reproductive tract malformations and other abnormalities (e.g., neural tube, skeletal and cardiac birth defects) (Agency for Toxic Substances and Disease Registry, 2000). Phthalates cause both Leydig cell and Sertoli cell toxicity, thereby inhibiting T synthesis and spermatogenesis. Testicular toxicity of phthalates is greatest prenatally and decreases with age postnatally. Low-dose prenatal bisphenol A caused feminization and increased prostate weight and AR levels in mice.

Single low prenatal doses of TCDD caused feminization, cryptorchidism and reduced testicular germ cell populations in males and genital clefting with hypospadias and vaginal threads in females. Low-dose prenatal bisphenol A caused increased body weight and earlier pubescence in female mice. Complex toxicant mixtures may have hormonal activity. Diesel exhaust, for instance, causes antiestrogenic effects in prenatally exposed rats including increased anogenital distances (both sexes) and reduced ovarian primary follicles (Watanabe and Kurita, 2001). There is mixed evidence for estrogenic effects of perinatal nonylphenol exposure in rodents.

Puberty

Precocious puberty involves the early appearance of secondary sex characteristics – breast development before age 8 years or menarche before age 9 years in girls and testicular volume ≥ 3 ml or genital hair growth before age 9 years in boys. Cases are about 10-fold more frequent in girls than boys (Partsch and Sippell, 2001). Although some cases of precocious puberty are caused by brain tumours, brain malformations, hormone-secreting tumours or genetic disorders, most have no known cause. Age at menarche in European females appeared to decrease during

the early decades of the 20th century in parallel with improved nutrition with little or no change during recent decades (de Muinck Keizer and Mul, 2001). Available data for the United States suggest that age at breast development may have decreased but there was little change in age at menarche during recent decades; in the absence of repeat standardized surveys, it was not possible to reach firm conclusions concerning such trends (Lee et al., 2001).

Sporadic cases of precocious puberty in girls have been linked to DES or estrogens in personal care products. Epidemics of precocious puberty in girls in Italy and Puerto Rico were thought to be related to consumption of estrogen-contaminated meat but definitive evidence is lacking (Partsch and Sippell, 2001). Early menarche in girls has been linked to prenatal/lactational exposure to polybrominated biphenyls (Blanck et al., 2000) and premature breast development was associated with current serum phthalate levels (Colon et al., 2000). Delayed genital development among Dutch boys and girls, respectively, was associated with current serum PCB and dioxin-like compound levels (Den Hond et al., 2002). There was, however, no association between prenatal and lactational PCB or DDE exposure indices and pubertal milestones in either sex in a North Carolina birth cohort study (Gladen et al., 2000).

Semen quality abnormalities

There is continuing controversy about the reality of declining human sperm quality and potential environmental links. Decreasing average sperm concentrations and semen volumes during recent decades (mainly before about 1970) were reported in several countries (Carlsen et al., 1992; Swan and Elkin, 1999). There is some evidence that semen quality has continued to decline during more recent years; for instance, semen quality declined in Canada during 1984-1996 (Younglai et al., 1998). A few small epidemiologic studies have examined the relation between semen quality and internal dose of contaminants. Sperm concentrations (both total sperm and sperm with rapid linear motility) were inversely associated with serum hydroxylated PCB levels but not with serum hexachlorobenzene or DDT/DDE levels (Dallinga et al., 2002). Other small studies showed inverse relationships between serum PCB levels and sperm quality (especially reduced motility and abnormal morphology) (Bush et al., 1986) (Hauser et al., 2002). One study showed similar associations between prenatal PCB exposure during the Yusho incident (involving PCB exposure) and sperm quality at about age 17 years (Guo et al., 2000).

Conclusion

Direct evidence of the potential effects of environmental HAAs on human reproductive tract development and function is scant because of the few epidemiologic studies conducted to date. The National Research Council concluded that there is insufficient toxicologic and epidemiologic evidence to attribute changes in hypospadias, cryptorchidism, testicular cancer and sperm counts to environmental HAAs (National Academy of Sciences, 1999). Further research on environmental HAAs is needed and should address not only estrogenic substances but also those that modulate thyroid and androgen-dependent processes (Foster, 1998). The U.S. longitudinal study of children, currently being developed, is the type of study needed to fill this important knowledge gap (National Institute of Child Health and Human Development, 2002).

Research on experimental animals indicates that high exposures to certain environmental HAAs can cause developmental and reproductive abnormalities including birth defects, functional deficits (reduced sperm quality/fertility, impaired ability of males to mate with receptive females) and premature or delayed puberty. Other effects observed in animals include impaired thyroid and immune system function and increased cancer risks. It is not clear that all of these effects depend solely on hormonal mechanisms (most environmental HAAs have other mechanisms of toxicity). Results from high-dose testing of single agents in animal systems must be interpreted with caution because human exposures are usually much lower. It is known, however, that humans are exposed to multiple low-level HAAs and other toxicants during critical prenatal and childhood development periods.

In the absence of definitive evidence that environmental HAAs have adverse human health effects, it would be prudent to minimize exposures and to monitor population exposure levels. The latter would enable identification of high-risk groups and evaluation of progress in reducing population exposures. At present, only the United States and Germany have initiated nation-wide surveys of population exposure levels employing direct measures of internal dose. Data from NHANES (1999-2000), for instance, showed that the highest phthalate metabolite levels in urine were those for two compounds produced in much lower quantities than the two leading compounds; phthalate metabolite levels were relatively high among reproductive-age women and low-income groups (Blount et al., 2000; Centers for Disease Control and Prevention, 2003; Koo et al., 2002). Organochlorines such as DDE and PCBs were detected in one-third of human amniotic fluid samples in Los Angeles (Foster et al., 2000). There is also a need for improved, population-based disease tracking – there is only limited, inconclusive evidence to assess reported

increased incidence rates of hypospadias and cryptorchidism and increased prevalence rates of reduced sperm quality.

Reference List

Agency for Toxic Substances and Disease Registry. 2000. Final phthalate expert panel reports (Center for the Evaluation of Risks to Human Reproduction) [Web Page]. Located at: <http://cerhr.niehs.nih.gov/news/index.html>.

Aho M, Koivisto AM, Tammela TL, Auvinen A. 2000. Is the incidence of hypospadias increasing? Analysis of Finnish hospital discharge data 1970-1994. *Environ Health Perspect* 108:463-5.

Akre O, Lipworth L, Cnattingius S, Sparen P, Ekblom A. 1999. Risk factor patterns for cryptorchidism and hypospadias. *Epidemiology* 10:364-9.

Beard CM, Melton LJ 3rd, O'Fallon WM, Noller KL, Benson RC. 1984. Cryptorchism and maternal estrogen exposure. *Am J Epidemiol* 120:707-16.

Beral V, Colwell L. 1981. Randomised trial of high doses of stilboestrol and ethisterone therapy in pregnancy: long-term follow-up of the children. *J Epidemiol Community Health* 35:155-60.

Bernstein L, Pike MC, Depue RH, Ross RK, Moore JW, Henderson BE. 1988. Maternal hormone levels in early gestation of cryptorchid males: a case-control study. *Br J Cancer* 58:379-81.

Blanck HM, Marcus M, Tolbert PE, Rubin C, Henderson AK, Hertzberg VS, Zhang RH, Cameron L. 2000. Age at menarche and tanner stage in girls exposed in utero and postnatally to polybrominated biphenyl. *Epidemiology* 11:641-7.

Blount BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, Lucier GW, Jackson RJ, Brock JW. 2000. Levels of seven urinary phthalate metabolites in a human reference population. *Environ Health Perspect* 108:979-82.

Boisen KA, Main KM, Rajpert-De Meyts E, Skakkebaek NE. 2001. Are male reproductive disorders a common entity? The testicular dysgenesis syndrome. *Ann N Y Acad Sci* 948:90-9.

Burlington H, Lindeman VF. 1950. Effect of DDT on testes and secondary sex characters of white leghorn cockerels. *Proc Soc Exp Biol Med* 74:48-51.

Bush B, Bennett AH, Snow JT. 1986. Polychlorobiphenyl congeners, p,p'-DDE, and sperm function in humans. *Arch Environ Contam Toxicol* 15:333-41.

Calzolari E, Contiero MR, Roncarati E, Mattiuz PL, Volpato S. 1986. Aetiological factors in hypospadias. *J Med Genet* 23:333-7.

Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. 1992. Evidence for decreasing quality of semen during past 50 years. *BMJ* 305:609-13.

Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D. 2000. Estrogens as endogenous genotoxic agents--DNA adducts and mutations. *J Natl Cancer Inst Monogr* :75-93.

Centers for Disease Control and Prevention. 2001. National report on human exposure to environmental chemicals [Web Page]. Located at: <http://www.cdc.gov/nceh/dls/report/PDF/CompleteReport.pdf>.

Centers for Disease Control and Prevention. 2003. Second national report on human exposure to environmental chemicals [Web Page]. Located at: <http://www.cdc.gov/exposurereport/>.

- Chappell PE, Lydon JP, Conneely OM, O'Malley BW, Levine JE. 1997. Endocrine defects in mice carrying a null mutation for the progesterone receptor gene. *Endocrinology* 138:4147-52.
- Choi J, Cooper KL, Hensle TW, Fisch H. 2001. Incidence and surgical repair rates of hypospadias in New York state. *Urology* 57:151-3.
- Colon I, Caro D, Bourdony CJ, Rosario O. 2000. Identification of phthalate esters in the serum of young puerto rican girls with premature breast development. *Environ Health Perspect* 108:895-900.
- Dallinga JW, Moonen EJ, Dumoulin JC, Evers JL, Geraedts JP, Kleinjans JC. 2002. Decreased human semen quality and organochlorine compounds in blood. *Hum Reprod* 17:1973-9.
- de Muinck Keizer SM, Mul D. 2001. Trends in pubertal development in Europe. *Hum Reprod Update* 7:287-91.
- Den Hond E, Roels HA, Hoppenbrouwers K, Nawrot T, Thijs L, Vandermeulen C, Winneke G, Vanderschueren D, Staessen JA. 2002. Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. *Environ Health Perspect* 110:771-6.
- Depue RH. 1988. Cryptorchidism, and epidemiologic study with emphasis on the relationship to central nervous system dysfunction. *Teratology* 37:301-5.
- Dolk H, Vrijheid M, Armstrong B, Abramsky L, Bianchi F, Garne E, Nelen V, Robert E, Scott JE, Stone D and others. 1998. Risk of congenital anomalies near hazardous-waste landfill sites in Europe: the EUROHAZCON study. *Lancet* 352:423-7.
- Foster W, Chan S, Platt L, Hughes C. 2000. Detection of endocrine disrupting chemicals in samples of second trimester human amniotic fluid. *J Clin Endocrinol Metab* 85:2954-7.
- Foster WG. 1998. Endocrine disruptors and development of the reproductive system in the fetus and children: is there cause for concern? *Can J Public Health* 89 Suppl 1:S37-41, S52, S41-6.
- Gladen BC, Ragan NB, Rogan WJ. 2000. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. *J Pediatr* 136:490-6.
- Gray LE, Ostby J, Furr J, Wolf CJ, Lambright C, Parks L, Veeramachaneni DN, Wilson V, Price M, Hotchkiss A and others. 2001. Effects of environmental antiandrogens on reproductive development in experimental animals. *Hum Reprod Update* 7:248-64.
- Guo YL, Hsu PC, Hsu CC, Lambert GH. 2000. Semen quality after prenatal exposure to polychlorinated biphenyls and dibenzofurans. *Lancet* 356:1240-1.
- Hauser R, Altshul L, Chen Z, Ryan L, Overstreet J, Schiff I, Christiani DC. 2002. Environmental organochlorines and semen quality: results of a pilot study. *Environ Health Perspect* 110:229-33.
- Hosie S, Loff S, Witt K, Niessen K, Waag KL. 2000. Is there a correlation between organochlorine compounds and undescended testes? *Eur J Pediatr Surg* 10:304-9.
- Kacsob B. 2000. *Endocrine Physiology*. United States: McGraw-Hill.
- Key TJ, Bull D, Ansell P, Brett AR, Clark GM, Moore JW, Chilvers CE, Pike MC. 1996. A case-control study of cryptorchidism and maternal hormone concentrations in early pregnancy. *Br J Cancer* 73:698-701.
- Klip H, Verloop 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* 359:1102-7.

- Koo JW, Parham F, Kohn MC, Masten SA, Brock JW, Needham LL, Portier CJ. 2002. The association between biomarker-based exposure estimates for phthalates and demographic factors in a human reference population. *Environ Health Perspect* 110:405-10.
- Kristensen P, Irgens LM, Andersen A, Bye AS, Sundheim L. 1997. Birth defects among offspring of Norwegian farmers, 1967-1991. *Epidemiology* 8:537-44.
- Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA. 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 139:4252-63.
- Lee PA, Guo SS, Kulin HE. 2001. Age of puberty: data from the United States of America. *APMIS* 109:81-8.
- Longnecker MP, Klebanoff MA, Brock JW, Zhou H, Gray KA, Needham LL, Wilcox AJ. 2002. Maternal serum level of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene and risk of cryptorchidism, hypospadias, and polythelia among male offspring. *Am J Epidemiol* 155:313-22.
- Mandal PK, McDaniel LR, Prough RA, Clark BJ. 2001. 7,12-Dimethylbenz[a]anthracene inhibition of steroid production in MA-10 mouse Leydig tumor cells is not directly linked to induction of CYP11B1. *Toxicol Appl Pharmacol* 175:200-8.
- McBride ML, Van den Steen N, Lamb CW, Gallagher RP. 1991. Maternal and gestational factors in cryptorchidism. *Int J Epidemiol* 20:964-70.
- Melnick R, Lucier G, Wolfe M, Hall R, Stancel G, Prins G, Gallo M, Reuhl K, Ho SM, Brown T and others. 2002. Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. *Environ Health Perspect* 110:427-31.
- National Academy of Sciences. 1999. *Hormonally active agents in the environment*. Washington, D.C.: National Academy Press.
- National Institute of Child Health and Human Development. 2002. *The National Children's Study* [Web Page]. Located at: <http://nationalchildrensstudy.gov/about/>.
- Parks LG, Ostby JS, Lambright CR, Abbott BD, Klinefelter GR, Barlow NJ, Gray LE Jr. 2000. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. *Toxicol Sci* 58:339-49.
- Partsch CJ, Sippell WG. 2001. Pathogenesis and epidemiology of precocious puberty. Effects of exogenous oestrogens. *Hum Reprod Update* 7:292-302.
- Paulozzi LJ. 1999. International trends in rates of hypospadias and cryptorchidism. *Environ Health Perspect* 107: 297-302.
- Paulozzi LJ, Erickson JD, Jackson RJ. 1997. Hypospadias trends in two US surveillance systems. *Pediatrics* 100:831-4.
- Pryor JL, Hughes C, Foster W, Hales BF, Robaire B. 2000. Critical windows of exposure for children's health: the reproductive system in animals and humans. *Environ Health Perspect* 108 Suppl 3:491-503.
- Richburg JH, Nanez A, Williams LR, Embree ME, Boekelheide K. 2000. Sensitivity of testicular germ cells to toxicant-induced apoptosis in gld mice that express a nonfunctional form of Fas ligand. *Endocrinology* 141:787-93.

- Schonfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I. 2002. Parent bisphenol A accumulation in the human maternal-fetal-placental unit. *Environ Health Perspect* 110:A703-7.
- Setchell KD, Zimmer-Nechemias L, Cai J, Heubi JE. 1997. Exposure of infants to phyto-oestrogens from soy-based infant formula. *Lancet* 350:23-7.
- Swan SH, Elkin EP. 1999. Declining semen quality: can the past inform the present? *Bioessays* 21:614-21.
- Thoreux-Manlay A, Le Goascogne C, Segretain D, Jegou B, Pinon-Lataillade G. 1995. Lead affects steroidogenesis in rat Leydig cells in vivo and in vitro. *Toxicology* 103:53-62.
- Toledano MB, Hansell AL, Jarup L, Quinn M, Jick S, Elliott P. 2003 . Temporal trends in orchidopexy, Great Britain, 1992-1998. *Environ Health Perspect* 111:129-32.
- Vos JG, Dybing E, Greim HA, Ladefoged O, Lambre C, Tarazona JV, Brandt I, Vethaak AD. 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. *Crit Rev Toxicol* 30:71-133.
- Watanabe N, Kurita M. 2001. The masculinization of the fetus during pregnancy due to inhalation of diesel exhaust. *Environ Health Perspect* 109:111-9.
- Weidner IS, Moller H, Jensen TK, Skakkebaek NE. 1998. Cryptorchidism and hypospadias in sons of gardeners and farmers. *Environ Health Perspect* 106:793-6.
- Yong EL, Lim LS, Wang Q, Mifsud A, Lim J, Ong YC, Sim KS. 2000. Androgen receptor polymorphisms and mutations in male infertility. *J Endocrinol Invest* 23:573-7.
- Younglai EV, Collins JA, Foster WG. 1998. Canadian semen quality: an analysis of sperm density among eleven academic fertility centers. *Fertil Steril* 70:76-80.