Endocrine disrupters and child health

Possible developmental early effects of endocrine disrupters on child health

World Health Organization

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Contents

1. Introduction

In the 1960s, congenital malformations caused by drugs used during pregnancy alerted the medical community to the fragility of the developing fetus. The thalidomide tragedy changed the attitude to developmental toxicology. Only a decade later, another sad story of pregnancy-related medication started to unravel when an association between fetal exposure to diethyl stilbestrol (DES) and vaginal clear cell adenocarcinoma in teen-aged girls became evident. Later on, several other adverse effects of DES were found both in boys and girls. These unfortunate 'human experiments' could have been avoided, if the drugs had been properly tested and the results given proper attention. DES is a potent synthetic estrogen that has been linked to cryptorchidism, hypospadias and reduced sperm production after fetal and perinatal exposure in both the human and the mouse. It may also increase the risk of testicular cancer. Data from numerous reproductive and developmental toxicity tests that were increasingly performed after the 1960s brought to light a large number of chemicals that affected the endocrine system and showed adverse effects in the reproductive organs. The rapid increase in the incidence of testicular cancer and deteriorating semen quality plus the emerging problems in reproduction of wild animals were linked to possible developmental endocrine disruption, and the chemical compounds having this kind of effects in experimental animals were called endocrine disrupters (or disruptors). According to WHO, endocrine disrupting chemicals are substances that alter one or more functions of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations (WHO, International Programme on Chemical Safety). Estrogenic endocrine disrupters received much of the early attention, but soon anti-androgenic and thyroid hormone disrupting compounds came into the focus of endocrine research. Adverse effects of endocrine disrupters on adipose tissue, the adrenal glands and the endocrine pancreas have further widened this research area.

There is ample evidence of endocrine disruption in wildlife, and the mechanisms of action of endocrine disrupters have been elucidated in experimental animals, but there is limited knowledge of the association of human disorders with exposure to endocrine disrupters. Accumulating data suggest that many adult diseases have fetal origins, but the causes have remained unexplained. Reproductive disorders, especially those

of adult men, are strongly associated with congenital disorders such as cryptorchidism and hypospadias. These disorders, together with testicular cancer and impaired semen quality, form the testicular dysgenesis syndrome (TDS) that by definition has a developmental origin. Epidemiological studies on TDS components and other endocrine-related disorders have often suffered from poor exposure assessment or inaccurate case ascertainment particularly in registry-based studies. It is difficult to envisage how epidemiological studies alone could either confirm or refute the role of endocrine disrupters in common childhood (or adult) disorders. It is becoming clear that we need to combine biological data on endocrine signalling, chemical exposure data (including data on mixtures), genetics and proper epidemiological methods by the means of systems biology to advance the recognition of endocrine disrupters and the prevention of adverse health effects.

The present document is a short summary of the current knowledge of the effects of endocrine disrupters on child health. We focus on the congenital disorders, cryptorchidism and hypospadias, which have a clear endocrine connection, on thyroid hormone-related problems, and on puberty. Some of the endocrine disrupters, such as polychlorinated biphenyls (PCBs) also have adverse effects on neurocognitive development. However, that is a topic of an entirely different large body of literature that is not related to endocrine disruption and therefore not presented here. Even endocrine disruption itself is a huge research area, and we have not been able to include all studies here. We hope that this serves as an introduction to new studies and aids in better understanding of the developmental effects of endocrine disrupters on child health.

a. Endocrine system

The endocrine system regulates the metabolism and function of the body. Endocrine glands secrete hormones that act on their target organs through cognate receptors. The targets are in many cases also endocrine organs that secrete hormones acting on the next level and also inhibiting the upper level via negative feedback. We will focus only on the hormones that are essential in the regulation of development of the brain and reproductive organs. Sexual differentiation and reproductive functions are specifically under hormonal control. Thyroid hormones are essential for brain development and normal metabolism of the whole body. The regulatory system of both reproductive hormones and thyroid hormones involves the hypothalamus

in the brain, the pituitary gland connected to the hypothalamus and the peripheral thyroid gland and gonads. Hypothalamic gonadotropin releasing hormone (GnRH) neurons stimulate pituitary gonadotropins to secrete gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH) that act on the gonads. FSH stimulates inhibin production in the testis and ovary, which inhibits FSH production in the pituitary. LH stimulates testosterone production, which serves an inhibitory function in the upper level. Both gonadotropins influence estrogen secretion from the ovary, and that has both inhibitory and, before ovulation, stimulatory effect on GnRH neurons and the pituitary. This hypothalamo-pituitary-gonadal (HPG) axis (Figures 1A and 1B) has also yet another regulatory network

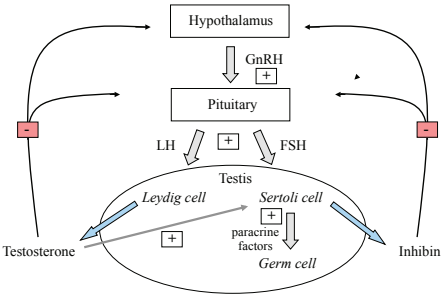


Figure 1A. Schematic representation of the hypothalamo-pituitary-testis axis. GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone.

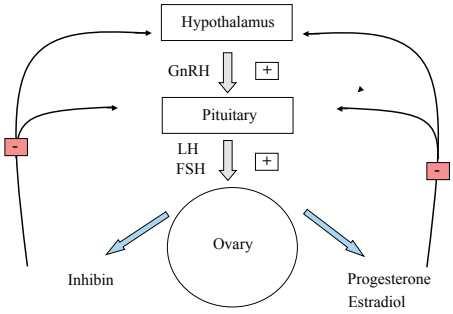


Figure 1B. Schematic representation of the hypothalamo-pituitary-ovary axis. GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone

in the brain controlling the GnRH neurons. In an analogous fashion, thyreotropin releasing hormone (TRH) from the hypothalamus stimulates pituitary thyrotropic cells to secrete thyroid stimulating hormone (TSH) which in turn stimulates the thyroid gland to produce thyroxin (Figure 2).

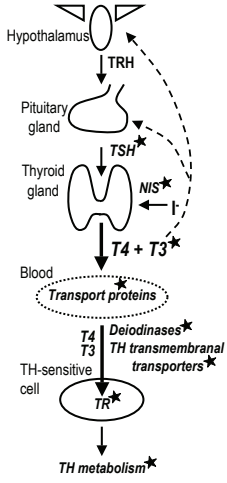


Figure 2. Schematic representation of the hypothalamo-pituitary-thyroid axis. TRH, thyreotropin releasing hormone; TSH, thyroid stimulating hormone; NIS, sodium-iodide symporter; T4, thyroxine; T3, triiodo-thyronine;

This inhibits TSH secretion to maintain a balance, called euthyroidism. Two common diseases disturb this hypothalamo-pituitary-thyroid (HPT) axis. In autoimmune hypothyroidism, the thyroid gland is affected by auto-antibodies, which leads to low thyroxin levels and very high TSH levels. In autoimmune hyperthyroidism (Graves disease) the thyroid gland is stimulated by immunoglobulins that activate TSH receptors, which leads to very high thyroxin levels and low TSH levels. Normal function of both HPG and HPT axes is essential for normal development.

b. Endocrine regulation of development

i. Gonadal hormones – sex differentiation

In the early embryo the two sexes are indistinguishable before the gonadal sex is determined by a genetic programme involving *SRY* gene in the Y chromosome. In the presence of *SRY* and several down stream genes the gonad is directed to become a testis, whereas in the absence of *SRY* other genes guide the gonad towards ovarian development. The fetal ovary stays hormonally inactive, whereas fetal testis is producing large amounts of hormones soon after testicular differentiation in gestational weeks 8-16. Somatic Sertoli cells in the testis produce anti-Müllerian hormone (AMH) that induces involution of the paramesonephric ducts (also called Müllerian ducts) that in the absence of AMH develop into the oviducts, the uterus and the upper part of the vagina. Therefore male newborns do not have these structures, whereas females do. Testicular Leydig cells produce testosterone that stimulates fetal mesonephric ducts (also called Wolffian ducts) to develop to epididymides, ejaculatory ducts and seminal vesicles. These structures disappear in female fetuses, because the ovaries do not secrete testosterone. Testosterone is further metabolized by 5-alpha-reductase enzyme to dihydrotestosterone (DHT) in the genital area. DHT is needed for the development of the prostate and masculinization of the external genitalia, i.e. development of scrota and the penis. If the DHT is missing, fusion of the urethral folds can be insufficient resulting in hypospadias and the penis may remain very small. In worst cases scrotal fusion may also be deficient with the result that the 46,XY newborn looks like a female. Leydig cells secrete also insulin like peptide 3 (INSL3) that together with testosterone regulates testicular descent from the abdomen to the scrotum.

Exposure of female fetuses to androgens leads to their masculinization, whereas exposure of male fetuses to anti-androgens results in under-masculinization (feminization) (Welsh *et al.*, 2008; Rey and Grinspon, 2011). Since the development of a male-type reproductive system is dependent on multiple hormones, male fetuses are more susceptible to endocrine disruption than females. Developmental disorders that appear in newborn males include penile defects (hypospadias, micropenis) and defects of testicular descent to the scrota (cryptorchidism). There is strong evidence that testicular cancer, which appears several years later in young adulthood, also has its origin in fetal life (Rajpert-De Meyts, 2006). Furthermore, sperm production capacity may be largely determined during early development (Sharpe *et al.*, 2003). However, that can be measured only after pubertal maturation. It is unknown whether the timing of pubertal development is affected by fetal programming.

Although male fetuses appear more affected by endocrine disrupters, female fetuses are also vulnerable. Androgen exposure can cause masculinization when the doses are high, but lower doses have been suggested to be associated with the development of the polycystic ovarian syndrome later in adulthood (Pasquali *et al.*, 2011). Breast development is another sensitive target for endocrine disruption that may have serious late-onset consequences (McLachlan, Simpson and Martin, 2006).

ii. Thyroid hormones – significance in brain development

It is well established that thyroid hormones are of special importance in the development of the brain. Numerous *in vitro* and animal studies have shown that the absence of thyroid hormones reduces neuronal growth and differentiation in the cerebral cortex, hippocampus, and cerebellum (Nicholson and Altman, 1972; Auso *et al.*, 2004; Lavado-Autric *et al.*, 2003). This is of special importance in fetal life, as development of the brain *in utero* is dependent upon normal levels of thyroid hormones.

The fetal thyroid gland develops from the third gestational week and thyroid follicles are formed and iodine concentration begins at approximately the 12th gestational week. However, the gland is not under feedback control by TSH and fully functioning until approximately the 20th gestational week. Thus, in the first trimester of gestation, before development and function of the fetal thyroid gland, the fetus is dependent on transplacental supply of maternal thyroxin (T4), and consequently on the ability of the maternal

thyroid gland to increase the hormone production during pregnancy in order to meet the needs of both fetus and mother.

Thyroid function is regulated by a finely tuned endocrinological homeostasis maintaining relatively stable serum levels of thyroid hormones. Thyroid hormone serum levels are monitored by a negative feedback mechanism mediated by the effects of circulating thyroid hormones at the hypothalamic and pituitary levels. In response to low levels of thyroid hormones in the blood, the pituitary secretes thyroid stimulating hormone (TSH), which stimulates the synthesis and release of triiodothyronine ($T_{3)}$ and thyroxine (T_4). In serum, these hormones are transported to the tissues bound to transport proteins, among which thyroxine binding globulin (TBG) is the most important thyroid hormone transport protein in humans, whereas transthyretin (TTR) is the major transport protein in many animals. T_4 is converted to the active hormone T_3 in the liver or in local tissues by iodothyronine deiodinases. The highly sensitive feedback regulation results in a remarkably stable concentration of TSH in blood (except for known diurnal variations) and consequently of circulating thyroid hormones in an individual.

Interference with thyroid homeostasis can take place on many different levels of the HPT-axis and may result in alterations of thyroid hormones available for the TH-receptors. In cases of markedly reduced hormone production capacity in both maternal and fetal glands, e.g. in iodine-deficient countries, severe brain damage may occur. Similarly, normal levels of thyroid hormones are important for postnatal neurological development in early childhood. Consequently, children who are born with congenital hypothyroidism and not treated with substitution therapy from the neonatal period develop severe central nervous system damage.

Minor changes in the thyroid homeostasis may also affect neurological development. Epidemiological studies have documented that even a marginally low thyroxine level in a pregnant women may give rise to reduction of cognitive functions of the offspring (Haddow *et al.*, 1999; Pop *et al.*, 2003; Berbel *et al.*, 2009). In this way, exposure to thyroid-disrupting chemicals may result in decreases of serum hormone levels and consequently neurological damage.

Additionally, a normal thyroid function presupposes a successful development of the thyroid gland itself and establishment of a well-functioning HPT-axis. Thyroid homeostasis may be disturbed by

hyperthyroidism or the presence of thyroid autoantibodies. However, it is not yet clear whether some environmental chemicals may interfere with thyroid function through these pathways.

2. Endocrine disrupters (recognized on the basis of experimental work in vitro and in vivo)

a. Sex hormone disrupters

The list of chemical compounds affecting the synthesis, transport, metabolism and action of sex hormones is growing, and it is not possible to include all studies in a review, since there are several hundreds of studies of each of them. The US National Toxicology Program (NTP) and the WHO International Programme on Chemical Safety (IPCS) among others have published comprehensive reviews on individual chemicals. Tables 1 and 2 provide short summaries of the main findings relevant to reproductive development.

Hypospadias and cryptorchidism in experimental animals can be induced by several endocrine disrupters that are either anti-androgenic or estrogenic (Toppari, 2008). Examples of anti-androgens are the fungicides vinclozolin and procymidone and DDE, the persistent congener of estrogenic dichlorodiphenyltrichloroethane (DDT), that act as androgen receptor antagonists (Gray *et al.*, 2006), and phthalate esters, dibutyl phthalate and diethyl hexyl phthalate that disturb androgen biosynthesis (Mylchreest *et al.*, 2002; Fisher *et al.*, 2003). Some compounds disrupt both receptor action and biosynthesis, e.g. linuron and prochloraz (Gray *et al.*, 2006). Dioxins act via aryl hydrocarbon receptors and interfere with several nuclear receptors, causing genital malformations (Peterson, Theobald and Kimmel, 1993). Penta-brominated diphenyl ethers are also anti-androgenic (Stoker *et al.*, 2005; Lilienthal *et al.*, 2006), while some polybrominated diphenyl ether metabolites can stimulate aromatase activity in cells derived from human adrenocortical carcinoma (Song *et al.*, 2008), which also disturbs the androgen-estrogen balance. These chemicals show additivity of the effects in low doses making the mixtures harmful even when none of the individual compounds is present higher than the no observed adverse effect level (NOAEL) (Kortenkamp and

TABLE 1 Effects of endocrine disrupters observed in the human reproductive system

Contaminant	Sex	Observation	References
Diethylstilbestrol (DES)	Male	Increased risk of hypospadias	Brouwers et al., 2006; Klip et al., 2002
		Tendency towards smaller testes	Bibbo et al., 1977; Gill et al., 1977, Ross et al., 1983,
		Increased prevalence of cryptorchidism	Palmer et al., 2009
		Capsular induration of testis	Bibbo et al., 1977; Gill et al., 1977
		Severe sperm abnormalities	Bibbo et al., 1977; Gill et al., 1977
		Epididymal cysts	Bibbo et al., 1977; Gill et al., 1977; Palmer et al., 2009
		Infection/inflammation of testis	Palmer et al., 2009
	Female	Increased risk of breast cancer	Palmer et al., 2006
		Vaginal adenosis	Bibbo et al., 1977; Sherman et al., 1974
		Oligomenorrhea	Bibbo et al., 1977
		Increased risk of clear cell adenocarcinoma of the vagina and cervix	Herbst et al., 1971; Herbst et al., 1979; Verloop et al., 2010
		Increased frequency of preterm delivery, first-trimester spontaneous abortion, second-trimester pregnancy loss and ectopic pregnancy	Kaufman et al., 2000
Phthalate esters (DBP, DMP,BBP,DEHP, DEP, DOP)	Male	Associated with anogenital index	Swan et al., 2005
		Positive correlation with increased serum LH/testosterone ratio	Main et al., 2006a
Flame retardants (Polybrominated diphenyl ethers)	Male	Associated with cryptorchidism	Main et al., 2007
Phytoestrogens	Male	Associated with hypospadias	North et al., 2000
Dioxins	Female	Increased probability of female births	Mocarelli et al., 1996; Mocarelli et al., 2000
Polychlorinated biphenyls (PCBs)	Male	Higher percentage of oligospermia, abnormal morphology and reduced sperm capacity of binding and penetration to hamster oocyte	Hsu et al., 2003

TABLE 2 Effects of endocrine disrupters observed in the reproductive system of animals

Contaminant	Sex	Observation	References
Diethylstilbestrol (DES)	Male	Sterility	McLachlan, 1977
		Epididymal cysts	McLachlan, 1977
		Cryptorchidism	McLachlan, 1977
		Reduction in testis weight	Fisher et al., 1999; Lewis et al., 2003; McKinnell et al., 2001
		Testicular lesions	McLachlan, 1977
		Inflammatory disease of the accessory sex glands	McLachlan, 1977
		Reduction in the number of spermatogonia with multinucleate cells in lumina of testis	McLachlan, 1977
		Nodular enlargements of the seminal vesicles and/or prostate	McLachlan, 1977
		Distension and overgrowth of the rete testis	Fisher et al., 1999; McKinnell et al., 2001; Rivas et al., 2002
		Distension and reduction in epithelial height of the efferent ducts	Fisher et al., 1999; McKinnell et al., 2001; Rivas et al., 2002
		Underdevelopment of the epididymal duct epithelium	McKinnell et al., 2001
		Reduction in epithelial height in the vas deferens	McKinnell et al., 2001; Rivas et al., 2002
		Convolution of the extra-epididymal vas	McKinnell et al., 2001;
		Decreased testosterone levels	Rivas et al., 2002; Yamamoto et al., 2003
		Increased gonadotrophin levels	Yamamoto et al., 2003
		Decreased AR expression in testis, epithelium of the rete testis, caput and cauda epididymis and vas deferens	McKinnell et al., 2001
	Female	Decrease in reproductive capacity	McLachlan, 1977
		Impaired ovarian function	McLachlan, 1977
		Increased uterus weight	Lewis et al., 2003
		Squamous metaplasia in the oviducts, uterus and cervix	McLachlan, 1977
		Increased the size of sexually dimorphic nucleus of the preoptic area	Faber and Hughes, 1991; Lewis et al., 2003
		Cystic hyperplasia of the endometrium and uterine adenocarcinoma	McLachlan, 1977
		Epidermoid tumors of the cervix and vagina	McLachlan, 1977
		Glandular elements and cellular atypia in the vaginal epithelium	McLachlan, 1977

Diethylstilbestrol (DES)	Female	Advanced development of primary and secondary follicles in the ovary	Yamamoto et al., 2003
		Decreased pituitary responsiviness to GnRH	Faber and Hughes, 1991
		Increased pubertal FSH levels	Yamamoto et al., 2003
Tributyltin	Male	Increased anogenital distance	Adeeko et al., 2003
		Reduced the number of Sertoli cells and gonocytes in fetal testis	Kishta et al., 2007
	Female	Reduced the number of germ cells in fetal ovaries	Kishta et al., 2007
		Increased post-implantation loss	Adeeko et al., 2003
Phtytoestrogens (Genistein, Daidzein)	Male	Impaired erectile function	Pan et al., 2008
		Decreased plasma testosterone levels	Pan et al., 2008
		Increased testis weight	Fisher et al., 1999
		Reduction in epithelial height of the efferent ducts	Fisher et al., 1999
		Increased pituitary response to GnRH	Faber and Hughes, 1991
	Female	Decreased pituitary responsiviness to GnRH	Faber and Hughes, 1991
		Increased the size of sexually dimorphic nucleus of the preoptic area	Faber and Hughes, 1991; Lewis et al., 2003
		Increased the weight of uterus	Lewis et al., 2003
		Decreased the weight of uterus	Awoniyi et al., 1998
		Decreased the weight of ovaries	Awoniyi et al., 1998
		Reduced serum estradiol levels	Awoniyi et al., 1998
		Reduced serum progesterone levels	Awoniyi et al., 1998; Lewis et al., 2003
		Irregular estrus cycle	Nagao et al., 2001
		Histopathological changes in the ovaries and uterus	Nagao et al., 2001
		Induced permanent estrus	Lewis et al., 2003
		Decreased the age of vaginal opening	Lewis et al., 2003
Alkyl phenol ethoxylates (p-tert-octylphenol, p-nonylphenol)	Male	Increased testis weight	Fisher et al., 1999
		Decreased testis weight	de Jager et al., 1999; Pocock et al., 2002
		Decreased seminiferous tubule diameter	de Jager et al., 1999; Pocock et al., 2002
		Decreased epididymal weight	de Jager et al., 1999
		Decreased total cauda epididymal sperm count	de Jager et al., 1999
		Reduction in epithelial height of the efferent ducts	Fisher et al., 1999
	Female	Post-implantation embryonic loss	Harazono and Ema, 2001
		Irregular estrus cycle	Katsuda et al., 2000; Pocock et al., 2002

Alkyl phenol ethoxylates (p-tert-octylphenol, p-nonylphenol)	Female	Increased sexual motivation towards a female teaser	Pocock et al., 2002
		Decreased the weight of ovaries	Pocock et al., 2002
		Increased the size of sexually dimorphic nucleus of the preoptic area	Herath et al., 2001
		Decreased the age of vaginal opening	Katsuda et al., 2000
		Persistent estrus	Katsuda et al., 2000
		Increased relative uterine weight	Katsuda et al., 2000
		Decreased serum gonadotrophin levels	Katsuda et al., 2000
		Decreased serum progesterone levels	Katsuda et al., 2000
		Increased serum inhibin levels	Katsuda et al., 2000
Phthalate esters (DEHP, BBP, DINP, DBP)	Male	Nipple retention	Barlow et al., 2004; Borch et al., 2004; Gray et al., 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000
		Decreased testis weight	Gray et al., 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000; Parks et al., 2000
		Reduced anogenital distance	Borch et al., 2004; Barlow et al., 2004; Gray et al., 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000; Parks et al., 2000
		Cryptorchidism	Gray et al., 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000
		Reduced accessory sex organ weights	Andrade et al., 2006; Barlow et al., 2004; Gray et al, 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000
		Lesion of the rete testis	Barlow et al., 2004
		Hemorrhagic testis	Gray et al., 1999b; Gray et al., 2000
		Cleft phallus and hypospadias	Barlow et al., 2004; Gray et al., 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000
		Multinucleated gonocytes	Gray et al., 2000; Parks et al., 2000
		Agenesis of the seminal vesicles and coagulating glands	Gray et al., 2000; Mylchreest et al., 2000
		Agenesis of bulbourethal glands	Gray et al., 2000
		Agenesis of ventral prostate	Barlow et al., 2004; Gray et al., 2000
		Agenesis of gubernacular cords	Gray et al., 2000
		Agenesis of epididymis and vas deferens	Barlow et al., 2004; Gray et al., 1999b; Mylchreest et al., 1999; Mylchreest et al., 2000
		Histopathological changes of testis	Barlow et al., 2004; Mylchreest et al., 1999; Mylchreest et al., 2000; Parks et al., 2000

Phthalate esters (DEHP, BBP, DINP, DBP)	Male	Delayed preputial separation	Gray et al., 1999b; Mylchreest et al., 1999
		Reduced fertility	Gray et al., 1999b
		Reduced fecundity	Gray et al., 1999b
		Reduced cauda epididymal sperm numbers	Gray et al., 1999b
		Reduced daily sperm production	Andrade et al., 2006
		Reduced plasma and/or testicular testosterone levels	Borch et al., 2004; Parks et al., 2000
		Increased serum testosterone levels	Andrade et al., 2006
		Reduced serum inhibin B levels	Borch et al., 2004
		Increase plasma LH levels	Borch et al., 2004
	Female	Uterine abnormalities	Gray et al., 1999b
		Reduced fertility	Gray et al., 1999b
Chlorinated pesticides (DDE)	Male	Nipple retention	Gray et al., 1999b; Kelce et al., 1995; You et al., 1998
		Hypospadias	Gray et al., 1999b
		Reduced accessory sex organ weights	Gray et al.,1999b; Kelce et al., 1995
		Reduced anogenital distance	Kelce et al., 1995; You et al., 1998
		Delayed preputial separation	Kelce et al., 1995
		Abnormally small penis	Guillette et al., 1994
		Poorly organized testis	Guillette et al., 1994
		Decreased plasma testosterone levels	Guillette et al., 1994
	Female	Increased plasma estradiol levels	Guillette et al., 1994
		Abnormal ovarian morphology with large number of polyovular follicles and polynuclear oocytes	Guillette et al., 1994
Dioxins	Male	Reduced accessory sex organ weights	Gray et al., 1995; Mably et al., 1992a; Mably et al., 1992b; Ohsako et al., 2001; Simanainen et al., 2004
		Decreased testis weight	Gray et al., 1995; Mably et al., 1992b
		Delayed preputial separation	Gray et al., 1995a
		Reduced anogenital distance	Gray et al., 1995; Mably et al., 1992a; Ohsako et al., 2001; Simanainen et al., 2004
		Delayed testis descent	Mably et al., 1992a
		Epididymal malformations	Gray et al., 1995; Simanainen et al., 2004
		Altered sex behavior	Gray et al., 1995
		Decreased sperm numbers	Gray et al., 1995; Mably et al., 1992b; Simanainen et al., 2004

Dioxins	Male	Decerased daily sperm production	Mably et al., 1992b
		Dose-related tendencies to decrease plasma testosterone and DHT	Mably et al., 1992a
	Female	Delayed puberty	Gray and Ostby, 1995
		Clef phallus	Gray and Ostby, 1995
		Vaginal thread	Gray and Ostby, 1995
		Reduced ovarian weight	Gray and Ostby, 1995
		Enhanced incidences of constant estrus	Gray and Ostby, 1995
		Cystic endometrial hyperplasia	Gray and Ostby, 1995
		Decreased fertility rate	Gray and Ostby, 1995
		Reduced fecundity	Gray and Ostby, 1995
Polychlorinated biphenyls (PCBs; PBC 77, 118, 126, 132, 169)	Male	Reduced accessory sex organ weights	Faqi et al., 1998; Gray et al., 1999b; Hsu et al., 2007; Kuriyama and Chahoud, 2004
		Decreased testis weight	Gray et al., 1999b; Kuriyama and Chahoud, 2004
		Increased testis weight	Faqi et al., 1998
		Increased epididymis weight	Faqi et al., 1998
		Reduced anogenital distance	Faqi et al., 1998
		Increased anogenital distance	Kuriyama and Chahoud, 2004
		Delay in onset of spermatogenesis, preputial separation and sex accessory growth	Gray et al., 1999b
		Decreased sperm number and total motile sperm count	Gray et al., 1999b; Hsu et al., 2007; Kuriyama and Chahoud, 2004
		Increased daily sperm production	Faqi et al., 1998
		Decreased serum testosterone levels	Faqi et al., 1998
		Increased the number of abnormal sperm	Kuriyama and Chahoud, 2004
		Altered sex behavior	Faqi et al., 1998
	Female	Vaginal thread	Gray et al., 1999b
		Mild hypospadias	Gray et al., 1999b
		Delayed the timing of vaginal opening	Faqi et al., 1998
Dicarboximide Fungicides (Vinclozolin, Procymidone)	Male	Hypospadias with cleft phallus	Gray et al., 1994; Gray et al., 1999a; Gray et al., 1999b; Hellwig et al., 2000; Ostby et al., 1999
		Reduced anogenital distance	Cowin et al., 2010; Elzeinova et al., 2008; Gray et al., 1994; Gray et al., 1999a; Gray et al., 1999b; Hellwig et al., 2000; Ostby et al., 1999
		Decreased testis weight	Elzeinova et al., 2008; Hellwig et al., 2000
		Cryptorchidism	Gray et al., 1994; Hellwig et al., 2000; Ostby et al., 1999

Dicarboximide Fungicides (Vinclozolin, Procymidone)	Male	Increased the number of apoptotic germ cells in testis	Cowin et al., 2010
		Nipple retention	Gray et al., 1994; Gray et al., 1999a; Hellwig et al., 2000; Ostby et al., 1999
		Reduced accessory sex organ weights	Cowin et al., 2010; Elzeinova et al., 2008; Gray et al., 1994; Gray et al., 1999a; Gray et al., 1999b; Hellwig et al., 2000; Ostby et al., 1999
		Glandular atrophy and chronic inflammation of prostate	Cowin et al., 2010; Gray et al., 1999b; Hellwig et al., 2000; Ostby et al., 1999
		Reduced secretion and chronic inflammation of seminal vesicles	Hellwig et al., 2000
		Epididymal granulomas	Gray et al., 1994; Gray et al., 1999a; Ostby et al., 1999
		Chronic inflammation of epididymis	Hellwig et al., 2000
		Agenesis of prostate	Gray et al., 1994
		Spermatogenic granuloma	Hellwig et al., 2000
		Decreased sperm number and daily sperm production	Elzeinova et al., 2008; Gray et al., 1994; Gray et al., 1999a
		Increased sperm head abnormalities	Elzeinova et al., 2008
		Reduced elongated spermatid content per testis	Cowin et al., 2010
		Low ejaculated sperm count	Gray et al., 1999a
		Abnormal morphology of seminiferous tubules	Elzeinova et al., 2008; Gray et al., 1994
		Decreased fertility	Gray et al., 1994
		Reduction of erections during the ex copula penile reflex test	Colbert et al., 2005
		Increase in seminal emissions during the ex copula penile reflex tests	Colbert et al., 2005
		Decreased serum testosterone levels	Gray et al., 1994
Herbicides (Linuron)	Male	Nipple retention	Gray et al., 1999b
		Reduced accessory sex organ weights	Gray et al., 1999b
		Delayed preputial separation	Gray et al., 1999b
		Decreased testis weight	Gray et al., 1999b
		Reduced spermatid number	Gray et al., 1999b
		Decreased anogenital distance	Gray et al., 1999b
		Epispadias	Gray et al., 1999b
		Testicular and epididymal malformations	Gray et al., 1999b

Lead	Male	Reduced accessory sex organ weights	Ronis et al., 1996
		Decreased testis weight	Ronis et al., 1996
		Enlarged prostate weight	McGivern et al., 1991
		Reduced serum testosterone levels	Ronis et al., 1996
		Decreased sperm counts	
		Reduced serum LH levels	Ronis et al., 1996
		Reduced volume of the sexually dimorphic mucleus of the preoptic area	McGivern et al., 1991
		Less masculine sex behavior	McGivern et al., 1991
		Irregular release pattern of gonadotrophins	McGivern et al., 1991
	Female	Delayed the timing of vaginal opening and the day of first diestrus	Dearth et al., 2002; Kimmel et al., 1980; McGivern et al., 1991; Ronis et al., 1996
		Prolonged and irregular periods of diestrus	McGivern et al., 1991;
		Disruption of estrus cycling	Ronis et al., 1996
		Suppressed serum levels of IGF-1, LH and/or estradiol	Dearth et al., 2002; Ronis et al., 1996
		Irregular release pattern of gonadotrophins	McGivern et al., 1991

Cadmium	Male	Time- and dose-dependent decrease in sperm motility	Benoff et al., 2008
		Partial or entire evacuation of the seminiferous tubules	Toman et al., 2002
		Increased the diameter of seminiferous tubules	Toman et al., 2002
		Reduced epithelial volume and increased lumen of tubule in the epididymis	Toman et al., 2002
		Hyperemic testes with extensive haemorrhaging, destruction of all of the presperm spermatogenic cells, and general necrosis and shrinkage of the seminiferous tubules	Foote, 1999
		Decrease in sperm output	Foote, 1999
		Reduced size of the testis	Tam and Liu, 1985
		Reduced number of differentiating germ cells in 16.5-day embryos	Tam and Liu, 1985
		Spermatozoa had poor ability to capacitate in vitro and showed a low fertilizing capability	Tam and Liu, 1985
	Female	Perturbed estrus cycles	Ishitobi and Watanabe, 2005
		Reduced number of differentiating germ cells and the size the ovary in 16.5-day embryos	Tam and Liu, 1985
		Tendency towards delayed timing of vaginal opening	Ishitobi and Watanabe, 2005
		Earlier onset of vaginal opening	Johnson et al., 2003
		Increased the epithelial area and the number of terminal end buds in the mammary glands and decreased the number of alveolar buds	Johnson et al., 2003
Manganese	Male	Increased serum gonadotrophin levels	Lee et al., 2006
		Increased serum testosterone levels	Lee et al., 2006
		Increased daily sperm production and efficiency of spermatogenesis	Lee et al., 2006
	Female	Increased serum gonadotrophin levels	Pine et al., 2005
		Increased serum estradiol levels	Pine et al., 2005
		Earlier onset of vaginal opening	Pine et al., 2005

Faust, 2010). Thus, when animals are exposed to the chemicals at levels that never cause hypospadias, they can together elicit hypospadias in 100% of offspring (Jacobsen *et al.*, 2010; Rider *et al.*, 2010).

b. Thyroid hormone disrupters

Numerous chemicals have been shown to interfere with thyroid function in experimental studies. Several groups of chemicals, e.g. dioxin-like compounds and certain flame retardants, have a high degree of structural similarity with the thyroid hormones T3 and T4, thus competing with the hormones for the TH-receptor and transport proteins.

PCBs and dioxins

Polychlorinated biphenyls (PCBs), dioxins (PCDDs) and furans (PCDFs) are widespread, persistent and toxic environmental pollutants from industrial production or production of herbicides. They comprise a group of highly persistent lipophilic chemicals that can be detected in samples from human and wildlife populations, although banned for decades in most countries. Many of these compounds, especially the hydroxylated metabolites, which are also biologically active, have a high degree of structural resemblance to thyroxine (T4).

The negative effect of PCB-exposure on peripheral thyroid hormone levels is well documented by studies in laboratory animals. Thus, PCBs and dioxins decrease the levels of circulating thyroid hormones in rats, especially T4 (Gauger *et al.*, 2004; van der Plas *et al.*, 2001; Hallgren *et al.*, 2001; Hallgren and Darnerud, 2002; Martin and Klaassen, 2010; Viluksela *et al.*, 2004; Nishimura *et al.*, 2002). Similarly, monkeys exposed to PCBs showed dose-dependent reductions of thyroid hormone levels (van den Berg, Zurcher and Brouwer., 1988). Mixtures of dioxin-like compound also decrease levels of T4 in an additive manner (Crofton *et al.*, 2005).

There is substantial evidence that perinatal exposure to PCBs and their hydroxylated metabolites decreases thyroid hormones in the offspring. This has been shown for exposure to PCBs in rats (Crofton *et al.*, 2000; Meerts *et al.*, 2002; Donahue, Dougherty and Meserve, 2004; Meerts *et al.*, 2004; Zoeller *et al.*, 2000), in sled dogs (Kirkegaard *et al.*, 2010), and exposure to dioxins in rats (Nishimura *et al.*, 2003; Seo *et al.*, 1995). Mouse studies have demonstrated accumulation of hydroxylated metabolites in the fetal compartment (Darnerud *et al.*, 1996).

Negative correlations between serum levels of PCBs or other organochlorine pollutants and thyroid hormones are reported among wildlife, including polar bears (Skaare *et al.*, 2001), seals (Chiba *et al.*, 2001; Sormo *et al.*, 2005), and nestling eagles (Cesh *et al.*, 2010).

In conclusion, experimental and wildlife observations point towards subtle, but significant, effects of exposure to dioxin-like chemicals and PCBs on mammalian thyroid function.

Flame retardants

The industrial use of flame retardants is abundant and this group of chemicals is found in a wide range of products such as electronic equipment, plastics, paints and synthetic textiles. This group of chemicals includes different compounds such as tetrabromobisphenol A (TBBPA), polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs), of which TBBPA and PBDEs show an even closer structural relationship to T4 than PCBs.

Numerous, but not all (Van den Steen *et al.*, 2010), studies in rats have demonstrated that PBDEs and commercial mixtures of flame retardants decrease the levels of circulating thyroid hormones (Fowles *et al.*, 1994; Zhou *et al.*, 2001; Stoker *et al.*, 2004; Hallgren et al., 2001; Lee *et al.*, 2010).

Perinatal maternal exposure of rats to different mixtures and congeners of PBDEs similarly reduced thyroid hormones in the fetuses (Zhou et al., 2002; Kodavanti et al., 2010; Kim et al., 2009), and this has been confirmed in other species including kestrels (Fernie *et al.*, 2005) and minks (Zhang *et al.*, 2009). Recently, several studies have demonstrated that even low doses of maternal PBDE exposure, comparable to levels of human environmental exposure, may similarly disrupt thyroid homeostasis in rat pups (Kuriyama *et al.*, 2007) or lambs (Abdelouahab *et al.*, 2009).

Pesticides

Innumerable different chemicals are used as pesticides and are part of potentially widespread human exposure. Many animal and toxicological studies suggest that multiple pesticides may have thyroid-disrupting properties. Both persistent organochlorine pesticides and non-persistent pesticides such as organophosphates, carbamates and pyrethroids, may interfere with thyroid function. The persistent chemicals dichlorodiphenyltrichloroethane (DDT) (and the metabolite DDE),

hexachlorobenzene (HCB), and nonylphenol (NP; a surface active substance used in pesticide aerosols) are among the most studied with regard to thyroid-disrupting effects. Although use of these chemicals has long been banned in many countries, they are still present in the environment due to their long environmental half-lives and continuous use in some countries.

Exposures to DDT (Scollon, Carr and Cobb, 2004), HCB (Rozman *et al.*, 1986; van Raaij *et al.*, 1993a; van Raaij *et al.*, 1993b; Foster et al., 1993; Alvarez *et al.*, 2005), and different mixtures of pesticides (den Besten *et al.*, 1993; Rawlings, Cook and Waldbillig, 1998) decrease serum levels of thyroid hormones in rats. Similarly NP decreases the level of T4 in studies of salmon (McCormick *et al.*, 2005) and lambs (Beard *et al.*, 1999).

Perfluorinated chemicals

The use of perfluorinated chemicals (PFC) in industrial and consumer products is increasing due to their surface protection properties, which are exploited in products such as stain- and oil-resistant coatings, but also in floor polishes and insecticide formulations. The group comprises several chemicals, e.g. perfluorooctanoic acid (PFOA) as well as perfluorooctane sulfonate (PFOS), which is also the metabolic end product of other PFCs. PFCs are extremely persistent in the environment.

Exposure to PFOS and PFOA decreased levels of T_4 after both short-term (Martin and Klaassen, 2007; Chang *et al.*, 2007) and long-term exposure (Yu, Liu and Jin, 2009). A study of monkeys showed reduction of T_3 after exposure to PFOS (Seacat *et al.*, 2003).

Perinatal exposure to PFOS also reduced serum levels of T_4, both in pregnant dams (Thibodeaux *et al.*, 2003) and in the offspring (Lau *et al.*, 2003; Luebker *et al.*, 2005). Cross-over studies of rats exposed in utero or/and in lactation document that both prenatal and postnatal exposure to PFOS may reduce thyroid hormone levels in the offspring (Yu *et al.*, 2009).

Phthalates

Phthalates are widely used as plastic emollients and additives in various industrial and consumer products, and exposure to phthalates is inevitable. For certain groups, such as hospitalized neonates and premature babies, exposure may be massive. In these patients, changes in thyroid hormone levels as a result of exposure to phthalates may be transient, but could

nonetheless have permanent effects on the development of the central nervous system, if changes occur in a developmentally critical phase.

Studies of the thyroid-disrupting effects of phthalates and their monoester metabolites are scarce. In rats, di-n-butyl phthalate (DBP) decreased T_3 and T_4 in rats in a dose-dependent manner (O'Connor, Frame and Ladics, 2002), and several studies have shown histopathological changes in the thyroid after exposure to phthalates (Howarth *et al.*, 2001; Poon *et al.*, 1997). In vitro studies indicated antagonistic properties of DBP and DEHP (Sugiyama *et al.*, 2005; Shen *et al.*, 2009).

Bisphenol A

Bisphenol A (BPA, 4,4'-isopropylidenediphenol) is widely used to manufacture numerous plastic products including food can linings and clear plastic bottles and several population studies have reported a high degree of human exposure (Calafat *et al.*, 2008; Ye *et al.*, 2008). Young children can be particularly exposed via baby bottles and plastic baby products. Several countries have banned BPA from baby products following the precautionary principle.

Despite the current debate on reproductive effects of BPA, only a few animal studies of thyroid-disrupting effects of BPA exist. BPA fed to pregnant rats was associated with a significant increase of T_4 in the pups, compatible with thyroid resistance syndrome (Zoeller *et al.*, 2005). However, other studies have found no or contrasting effects on thyroid hormone levels (Nieminen *et al.*, 2002a; Nieminen *et al.*, 2002b; Xu *et al.*, 2007) after exposure to BPA.

Ultraviolet filters

Several ultraviolet (UV) filters used in sunscreens are suspected to have thyroid-disrupting properties. 4-methylbenzylidene-camphor (4-MBC) and octyl-methoxycinnamate (OMC), and benzophenone 2 (BP2) decreased serum levels of thyroid hormones in rats (Seidlova-Wuttke *et al.*, 2006; Klammer *et al.*, 2007; Jarry *et al.*, 2004; Schmutzler *et al.*, 2007).

3. Early effects, child health problems putatively associated with endocrine disruption

a. Cryptorchidism

i. Epidemiology

Congenital cryptorchidism is defined as a condition in which one or both testes are not located at the bottom of the scrotum at the time of birth. Figure 3 describes the clinical classification of testicular position in cryptorchidism (non-palpable testis excluded).

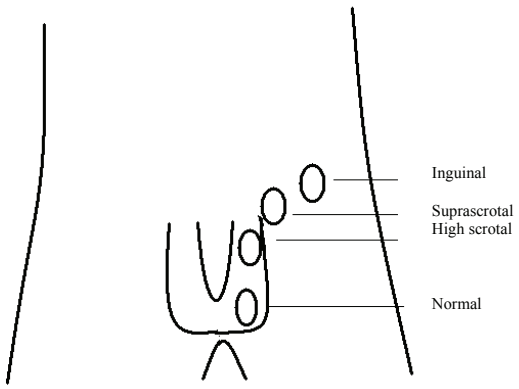


Figure 3. Clinical classification of testicular position in cryptorchidism (non-palpable testis excluded).

Testes descend to the scrota normally during the last trimester of pregnancy. Preterm boys are often bilaterally cryptorchid, because they have not yet reached the age at which the testes descend, and their testes usually descend spontaneously before the due date. However, the incidence of cryptorchidism at the expected time of delivery is still higher in this group than in full-term babies. Therefore the incidence rates are usually given separately for full-term and preterm infants, and the weight of < 2.5 kg is often used as a proxy for being preterm. In addition to maturity of the baby, the exact position of the testis at examination is an important determinant in the ascertainment of cryptorchidism. This can be assessed reliably only in prospective clinical studies, whereas registry- and interview-based epidemiological studies tend to misclassify cases as normal. Registries are unreliable sources of data for cryptorchidism (Toppari *et al.*, 2001). Interestingly, the reported prevalence of cryptorchidism can vary

from 1 to 9% in the same population, depending on the data source (1% orchidopexy rate, 2% hospital discharge registry, 4% mothers' interview, 9% clinical examination at birth; Boisen *et al.*, 2004; Strandberg-Larsen *et al.*, 2009).

Scorer (1964) used the distance of the testis from the pubic bone as a criterion to classify the testis as descended or undescended. The position of the undescended testis can be abdominal, inguinal, suprascrotal or high scrotal. Non-palpable testes are either absent or abdominal or sometimes deep in the inguinal canal or they may be ectopic, which means that they are outside their normal route of descent, e.g. above the pubic bone or in the thigh. Normal testes locate at the bottom of the scrotum, whereas retractile testes move freely up and down, but can be manipulated to the bottom at least for some time. The high scrotal testes may locate in the upper part of the scrotum or they may also be manipulated down, but return immediately back to their higher position (Boisen *et al.*, 2004). Clear definitions of cryptorchidism have been used in several prospective clinical studies, which makes them comparable with other studies using similar definitions (Table 3). Table 3 demonstrates that there are large regional differences and adverse trends. The incidence of cryptorchidism at birth is much lower in Finland than in Denmark, and an increasing rate can be seen in the United Kingdom and Denmark.

The majority of cryptorchid testes (up to 75%) descend spontaneously during the first three months of life (Boisen *et al.*, 2004) when the hypothalamo-pituitary-testicular axis is very active (Andersson *et al.*, 1998). After that, the testes may reascend and also new cases of (acquired) cryptorchidism appear (Hack *et al.*, 2003a; Wohlfahrt-Veje *et al.*, 2009). Congenital and acquired cases are mixed in all epidemiological studies that use the hospital discharge registries and interviews as data sources. The cause of both congenital and acquired cryptorchidism remains elusive in most cases, but it is most likely that the aetiology is different for these conditions, which further complicates all association studies that do not assess them as distinct outcomes. Entrapment of the testis into the inguinal scar after previous operation (Eardley, Saw and Whitaker, 1994), improper elongation of the spermatic cord during childhood (Clarnette and Hutson, 1997) or spasticity of the cremaster muscle e.g. in patients with cerebral palsy (Smith *et al.*, 1989) have been proposed to cause acquired cryptorchidism. Previous retractility of the testes has also been reported in some cases (Lamah *et al.*, 2001). In the Danish cohort study,

Table 3. Rate of congenital cryptorchidism in prospective clinical studies using clearly defined criteria of cryptorchidism

Country	Reference	Number of subjects	Diagnosis based on	Rate of cryptorchidism at birth
U.S., Rochester Minnesota, St. Mary's Hospital	(Harris and Steinberg, 1954)	n=4474	position (testis cannot be manipulated into the scrotum)*	1.3% (BW>2500g), 1.5% of all boys
Denmark, Copenhagen, Rigshospitalet	(Buemann et al., 1961)	n=2701)	position	1.8% (BW>2500g), 4.1% of all boys
U.K., West London, Hillingdon Hospital	(Scorer, 1964)	n=3612	distance measurement	2.7% (BW>2500g), 4.2% of all boys
India, Kanpur, Dufferin Hospital and U.I.S.E Maternity Hospital	(Mital and Garg, 1972)	n=2850	distance measurement	1.6% of full-term single born boys
Taiwan, Provincial Tao-Yuan Hospital	(Hsieh and Huang, 1985)	n=1208	position (presence or absence of testes in the scrotum)*	4.1% in preterm boys, 1.4% in mature boys
Korea, 38 hospitals	(Choi et al., 1989)	n=7990	position	0.7% of all boys
U.K., Oxford, John Radcliffe Hospital	(Group, 1992)	n=7400	position distance measurement	3.8% (BW≥2500g), 4.9% of all boys (excluding boys with severe congenital malformations) 4.1% (BW≥2500g), 5.0% of all boys (excluding boys with severe congenital malformations)
U.S., New York, Mount Sinai Hospital	(Berkowitz et al., 1993)	n=6935	distance measurement	2.2% (BW≥2500g), 3.7% of all boys
Malaysia, Kuala Lumpur, University Hospital	(Thong et al., 1998)	n=1002	position	2.4% (BW≥2500g), 4.8% of all boys
Italy, Pisa, S. Chiara Hospital and Division of Neonatology at the University of Pisa	(Ghirri et al., 2002)	n=10730	position	3.5% (BW≥2500g), 6.9% of all boys
Denmark, Copenhagen, Rigshospitalet	(Boisen et al., 2004)	n=1046	position	8.4% (BW≥2500g), 9.0% of all boys
Finland, Turku, Turku University Hospital	(Boisen et al., 2004)	n=1455	position	2.1% (BW≥2500g), 2.4% of all boys
Lithuania, Panavėžys City Hospital	(Preiksa et al., 2005)	n=1204	position	4.6% (BW≥2500g), 5.7% of all boys
UK, Cambridge Baby Growth Study	(Acerini et al., 2009)	n=742	position	5% (BW≥2500g), 5.9% of all boys

*Does not seem to include high scrotal testis as cryptorchid testis

0.8% and 1.4% (accumulated rate) of boys had acquired cryptorchidism (ascending testis) at the age of 18 and 36 months, and 0.6% and 0.8% of boys, respectively, had recurrent cryptorchidism (spontaneous descent at 3 months and reascent thereafter) (Wohlfahrt-Veje *et al.*, 2009). In the Cambridge cohort study, the prevalence of acquired cryptorchidism was 7.0% at 2 years of age (Acerini *et al.*, 2009). In the Netherlands, prevalence rates of up to 2.2% for acquired cryptorchidism between 6 to 13 years of age were reported (Hack *et al.*, 2007a). The Dutch have suggested a wait-and-see policy in the treatment and follow-up of these cases because >75% have spontaneous descent at puberty (Hack *et al.*, 2003b; Hack *et al.*, 2007b). In the Nordic countries early orchidopexy is recommended to all cryptorchid boys, because the possible adverse effects that delay may cause are unknown (Ritzen *et al.*, 2007). Semen quality is better in men with early orchidopexy than in those with a later operation (Virtanen *et al.*, 2007; Canavese *et al.*, 2009) and postpubertal orchidopexy may be associated with a higher risk of testicular cancer than prepubertal operation (Pettersson *et al.*, 2007; Walsh *et al.*, 2007), although a large Danish cohort based on a national hospital discharge registry and cancer registry did not corroborate any effect of the age at treatment of cryptorchidism on the risk of testicular cancer (Myrup, Schnack and Wohlfahrt, 2007). The finding that the testis cancer risk was higher in the men that were operated on after puberty than before it (Pettersson *et al.*, 2007) may reflect the fact that this group included only those who did not have spontaneous descent of acquired cryptorchid testes in puberty, whereas the prepubertally-operated group included a large group of boys who would have had spontaneous descent in puberty (Hack *et al.*, 2003b; Hack *et al.*, 2007b). The differences between these groups may reflect the underlying pathology and explain the small difference in the risk observed in the study by Petterson et al. (Pettersson *et al.*, 2007). The absence of putative spermatogenic stem cells, type A spermatogonia, was linked to poor spermatogenic prognosis independent of timing of surgery (Hadziselimovic and Herzog, 2001; Hadziselimovic *et al.*, 2007). However, the distinction of different types of spermatogonia only on a morphological basis is difficult and immunohistochemical analysis may differ from conventional histologic assessment (Wikström *et al.*, 2004; Wikström *et al.*, 2007). Testicular biopsies are not recommended, unless there is a specific reason such as suspicion of malignancy (Ritzen et al., 2007).

Cryptorchidism is a well characterized risk factor for testicular cancer, and men with a history of cryptorchidism have a 4 to 6-fold higher risk

of testicular cancer than men without cryptorchidism (Dieckmann and Pichlmeier, 2004; Schnack *et al.*, 2010b). However, most of the men with a history of cryptorchidism never develop testicular cancer, and only about ten percent of men with testicular cancer have been cryptorchid. Furthermore, orchidopexy does not abolish the cancer risk. Thus, although cryptorchidism is a risk factor for testicular cancer, it does not seem to cause it. These two disorders most likely share aetiological factors. Against this background it is not surprising that a high incidence of cryptorchidism is accompanied by a high rate of testicular cancer, which is apparent e.g. in Denmark and Finland, which have high and low incidence rates, respectively (Boisen *et al.*, 2004) (Jacobsen *et al.*, 2006). This implies that any causal relationship of cryptorchidism with environmental effects can be considered a putative risk factor for testicular cancer.

Semen quality and fertility are also related to cryptorchidism (Lee and Coughlin, 2001; Virtanen *et al.*, 2007), and epidemiological findings reflect also this connection. For example men in Finland and Denmark also differ from each other in semen quality. Danish men have lower sperm counts than do Finns (Jørgensen *et al.*, 2001; Jørgensen *et al.*, 2002). Features that might predict such a difference can appear in early childhood, as seen in the Finnish-Danish cohort study of cryptorchidism, in which the testes were measured by ultrasound and reproductive hormones were analyzed at the age of three months (Boisen *et al.*, 2004; Main *et al.*, 2006b). Danish boys had smaller testes than Finnish boys and testicular growth was slower in Denmark than in Finland (Main *et al.*, 2006b). Similarly, inhibin B levels were lower in Danish boys than in Finnish boys and correlated closely to the testis volumes (Main *et al.*, 2006b). All these findings together suggest that cryptorchidism is also linked to semen quality.

Hypospadias is a disorder of penile development that is common, but the incidence is still only approximately $1/10^{th}$ of the cryptorchidism rate (Toppari *et al.*, 2001). Hypospadias is also linked to cryptorchidism and they occur together more often than expected by chance (Schnack *et al.*, 2010a). The prevalence of hypospadias varies between Denmark and Finland in a similar pattern as testicular cancer and cryptorchidism (Virtanen *et al.*, 2001; Boisen *et al.*, 2005). All these disorders and sperm production capacity of the testis are critically linked to androgen action and related hormonal regulation during development (Sharpe and Skakkebaek, 2008). One or more of the disorders may arise from maldevelopment of the testis, called testicular dysgenesis syndrome (TDS) (Skakkebaek, Rajpert-

De Meyts and Main, 2001). It is useful, therefore, to consider all these problems together in epidemiological and experimental studies.

ii. Mechanisms

Testes differentiate in the fetal gonadal ridge during early gestation (embryonic weeks 6-7) and become hormonally active soon after differentiation. The interstitial Leydig cells in the testis secrete testosterone and insulin-like peptide 3 (INSL3) that regulate testicular descent. INSL3 stimulates outgrowth of the gubernaculum that is attached to the testis and epididymis and anchors the gonad to the bottom of the pelvis close to the inner opening of the inguinal canal. When the fetus grows rapidly, the testes become separated from the kidneys and other organs that move upwards along the growing body. During late gestation the testes rapidly move through the inguinal canals to the scrota. This transinguinal descent is dependent on normal androgen action. In androgen insensitive persons and those with defects in androgen production, the gonads remain either in the bottom of abdomen or in the inguinal canals. The same is true in androgen deficient and androgen insensitive rats and mice. Thus, it is easy to hypothesize that anything that will perturb INSL3 and/or testosterone production or action can cause cryptorchidism.

Mutations in androgen receptor gene, steroidogenic enzymes needed for androgen production, or hypothalamo-pituitary regulators needed for testicular stimulation are all well characterized reasons for cryptorchidism, but occur very rarely (Virtanen *et al.*, 2007; Barthold, 2008). Chemicals that inhibit androgen production or action (anti-androgens) can directly disturb testicular descent, which has a robust experimental evidence. Mutations in *INSL3* and its receptor *RXFP2* have been reported in heterozygous form in cryptorchid boys (Ferlin *et al.*, 2003; Foresta *et al.*, 2008). However, these may be polymorphisms rather than mutations, because they were found as frequently in normal population as in cryptorchid subjects (El Houate *et al.*, 2008; Nuti *et al.*, 2008). No mutations either in *INSL3* or in *RXFP2* were found in Finnish patients (Koskimies *et al.*, 2000; Roh *et al.*, 2003). However, down-regulation of these genes might contribute to maldescent of the testes. Estrogens can down-regulate *Insl3* expression in mice, which may explain why estrogens can cause cryptorchidism (Emmen *et al.*, 2000; Nef, Shipman and Parada, 2000). Lower cord blood levels of INSL3 were found in boys with cryptorchidism persisting at 3 months compared to a group of control boys, suggesting that perturbed INSL3 production may have contributed to the disorder (Bay *et al.*, 2007).

There are several other genes that have been linked to cryptorchidism in experimental animals with knock-out techniques e.g., *Hoxa10*, *Hoxa11* (Hsieh-Li *et al.*, 1995; Rijli *et al.*, 1995; Satokata, Benson and Maas, 1995; Overbeek *et al.*, 2001; Daftary and Taylor, 2006), but there is little evidence for their role in humans. Cryptorchidism can also be found as a part of several syndromes, many of which have an identified genetic reason (Virtanen et al., 2007). However, a great majority of cryptorchidism occurs as a single disorder. Genome-wide association analyses and transcriptome analyses may bring new candidate genes, such as *FGFR1* and downstream signaling molecules *SOS1* and *RAF1* (Hadziselimovic et al., 2010) that need to be tested in larger populations. A recent study did not find any mutations in *FGFR1* and heterozygous *GnRHR* mutations were found in similar frequency as in a group of controls (Laitinen *et al.*, submitted). The genes may also be the targets of adverse environmental effects as exemplified by estrogen-*INSL3* interaction.

iii. Endocrine disrupter association

Risk factors for cryptorchidism that have been reported in several studies include low birth weight, being small for gestational age, prematurity and having other genital malformations (Hjertkvist, Damber and Bergh, 1989; Group 1992; Berkowitz *et al.,* 1993; Berkowitz *et al.,* 1995; Jones *et al.,* 1998; Thong, Lim and Fatimah, 1998; Akre *et al.,* 1999; Weidner *et al.,* 1999; Ghirri *et al.,* 2002; Boisen *et al.,* 2004; Preiksa *et al.,* 2005). The most robust evidence of increased risk is associated with intrauterine growth retardation and being small for gestational age. This was also evident in Finnish newborns (Boisen *et al.*, 2004). Prematurity is another risk factor, but many of the premature newborns have a spontaneous descent of the testes before the due date, reflecting normal physiology. Life style factors, such as mothers' smoking and alcohol consumption may also increase the risk, although the evidence is less clear. In a prospective, clinical cohort study, mothers' alcohol consumption was associated with a dose-dependent increase in the risk of cryptorchidism (Damgaard *et al.*, 2007), whereas in registry- and interview-based studies including persistent and severe cases, *i.e.* those who usually needed treatment, only early gestation binge drinking showed an association with a slightly increased risk (Jensen *et al.*, 2007; Mongraw-Chaffin *et al.*, 2008; Strandberg-Larsen *et al.*, 2009). Most studies have not shown any effect of mothers' smoking (Mongraw-Chaffin *et al.*, 2008; Damgaard *et al.*, 2008), whereas the use of nicotine patches was associated with an increased risk (Damgaard *et al.*, 2008).

However, in one study heavy smoking was associated with an increased risk of bilateral cryptorchidism (Thorup, Cortes and Petersen, 2006). Diet-treated gestational diabetes was also found to increase the risk, possibly by altering the hormone balance of the developing fetus (Virtanen *et al.*, 2006). Occupational risk factors include gardening and farming, putatively due to pesticide exposure (Weidner *et al.*, 1998), (Kristensen *et al.*, 1997).

Many pesticides have been recognized as endocrine disrupters, but there are not many studies linking direct exposure measurements and cryptorchidism. Studies using occupational and job matrix analyses as proxies for exposure have hinted at a possible association (Weidner *et al.*, 1998). Breast milk samples from mothers of cryptorchid boys had a higher total amount of chlorinated pesticides than those from mothers of boys without cryptorchidism (Damgaard *et al.*, 2006), and these originated from historical rather than recent exposures of the mothers as judged by enantiomeric analysis (Shen *et al.*, 2006). The levels of these chemicals are declining, but because of the persistence of the compounds they continue to add to the contaminant load of children in future generations. The associations for individual chemicals, such as DDT or DDE, are not apparent (Damgaard *et al.*, 2006; Longnecker *et al.*, 2002), emphasizing the need to integrate data and use bioinformatic tools to analyze complex data sets. Already rather simple principal component analyses can demonstrate distinct chemical signatures between different regions as exemplified by contrasts between Denmark and Finland (Krysiak-Baltyn *et al.*, 2010). However, some studies have identified differences in individual compounds, e.g., higher levels of heptachloroepoxide and hexachlorobenzene were found in fat samples of cryptorchid boys than in controls (Hosie *et al.*, 2000).

Polybrominated diphenyl ethers are used mainly as flame retardants and they are also rather persistent in nature. Some of the PBDEs are anti-androgenic (Stoker *et al.*, 2005). Mothers of cryptorchid boys had higher breast milk concentrations of these compounds than mothers of control boys (Main *et al.*, 2007). Environmental contamination with PBDEs is higher in the USA than in Europe, and many of these compounds have been banned after initial introduction (Darnerud *et al.*, 2001; Betts 2002; Main *et al.*, 2007).

Phthalate esters are ubiquitous environmental chemicals that are everywhere in the modern milieu. They are used in plastics as softeners, and they occur in packaging, tubing, surface materials, office and household

equipments. Humans are exposed mainly by food and drink, but also through skin and indoor air. Diethyl hexyl phthalate and dibutyl phthalate interfere with testosterone production and therefore have anti-androgenic effects in developing rodents (Scott, Mason and Sharpe, 2009). In humans, phthalate levels in mothers' urine have been associated with the anogenital index (defined as the anogenital distance (AGD) divided by the weight of the boy at examination) of their sons, suggesting also anti-androgenic effects (Swan *et al.*, 2005). Phthalate levels in breast milk were positively correlated with increased LH/testosterone ratios, compatible with an anti-androgenic effect forcing pituitary to exert a stronger stimulation to Leydig cells to maintain normal androgen levels (Main *et al.*, 2006a). Phthalate levels in mothers' breast milk were not directly associated with the risk of cryptorchidism in the offspring (Main *et al.*, 2006a). Different species and strains show varying susceptibility to the testicular effects of in utero phthalate exposure (Johnson *et al.*, 2008; Scott, Mason and Sharpe, 2009).

b. Hypospadias

i. Epidemiology

In hypospadias the urethra has failed to fuse normally on the ventral side of the penis and opens inappropiately to the end of the split (Figure 4). The meatus can locate anywhere between the glans and perineum depending on the severity of hypospadias (Källen *et al.*, 1986). If the urethra opens to the glans or corona (sulcus), it is called distal, and this mild form of hypospadias often does not necessitate any treatment. Therefore it is often

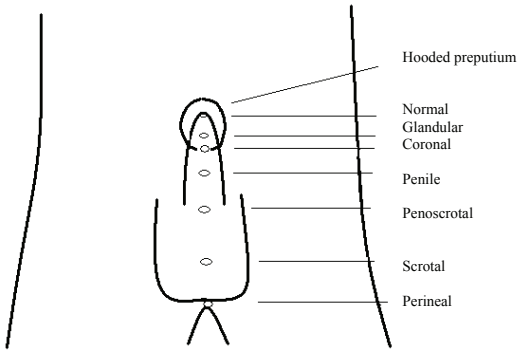


Figure 4. Clinical classification of location of the urethral meatus in hypospadias.

not registered at all and malformation registries vary in their practices of recording these defects. If the urethral meatus is located in the penile

shaft or penoscrotal area, the hypospadias is called proximal and these require surgical management. A third category of middle hypospadias also has been used to separate cases with penile shaft location of the urethral meatus from distal and proximal defects (Brouwers *et al.*, 2009). To make it even more complicated, the distinction between distal and proximal forms varies, because some studies include cases with mid shaft penile hypospadias in distal forms (Cox, Coplen and Austin, 2008). Therefore it is important to consider which types of hypospadias are included in epidemiological studies before comparing the results and making any conclusions. Physiological phimosis may hinder diagnosis of distal forms at birth, and these may become visible only later when the foreskin can be retracted behind the glans, as shown in Denmark where the birth rate of hypospadias was 1% and the cumulative incidence at 3 years was 4.6% (Boisen *et al.*, 2005).

Registry-based studies on the incidence of hypospadias tend to underestimate the true rate (Toppari *et al.*, 2001). The reasons include poor ascertainment in routine clinical work, under-reporting to the registry, and varying policies in recording distal cases. In many malformation registries distal hypospadias are not considered at all, although these are very common in population-based prospective clinical studies (Virtanen *et al.*, 2001; Pierik *et al.*, 2002; Boisen *et al.*, 2005). Several European studies have shown higher prevalence rates than previous estimates of 0.4 and 2.4 per 1000 total births (Dolk *et al.*, 2004). Despite the caveats in epidemiological analyses of hypospadias, there is ample evidence of increased rates in several regions of Australia, Europe, and the USA (Källen *et al.*, 1986; Paulozzi, 1999; Toppari *et al.*, 2001; Nassar *et al.*, 2007). Many malformation registries changed their approach to hypospadias to more active search in 1990s when it became evident that a large proportion of cases remained unregistered (Hemminki, Merilainen and Teperi, 1993), which may also explain many controversies in trend analyses (Aho *et al.*, 2000; Carmichael *et al.*, 2003; Dolk *et al.*, 2004; Porter *et al.*, 2005; Fisch *et al.*, 2009). An increasing trend in the 1970s and 1980s in the USA was reported on the basis of malformation registry data that showed an increase especially in proximal hypospadias (Paulozzi, Erickson and Jackson, 1997). Hospital discharge registries on operated cases of hypospadias reflect well the prevalence of proximal hypospadias, but they do not include the mild coronal and glanular forms that are not operated. In Denmark, the birth rate of hypospadias was estimated to be 0.52% according to hospital

Table 4. Rate of hypospadias in boys in prospective or cross-sectional clinical (non-register based) studies

Country	Reference	Study type	Rate of hypospadias
U.S., Rochester Minnesota, St. Mary's Hospital	(Harris and Steinberg, 1954)	Prospective study (n=4474)	0.70% (BW>2500g), 0.76% of all live-born boys
U.S., ante partum clinic of the Sloane Hospital, New York City	(McIntosh *et al.*, 1954)	prospective study on pregnant women and infants (n=2793 live-born males)	0.54% of live-born boys
U.S., Collaborative perinatal project	(Myrianthopoulos and Chung, 1974)	prospective study (n=53394 consecutive single births (boys and girls))	0.80% of single-born boys (76% of cases detected at birth)
Korea, 38 hospitals	(Choi *et al.*, 1989)	prospective study (n=7990)	0.21% of newborn boys
Southern Jordan	(al-Abbadi and Smadi, 2000)	Clinical study of 1748 boys (aged 6 to 12 years)	0.74% of boys
Finland,Turku, Turku University Hospital	(Virtanen *et al.*, 2001)	Prospective cohort study (n=1505) Total hospital cohort (n=5798)	0.27% of live-born boys 0.33% of live-born boys
Netherlands, Rotterdam	(Pierik *et al.*, 2002)	Prospective study (n=7292)	0.73% of newborn boys
Denmark, Copenhagen, Rigshospitalet	(Boisen *et al.*, 2005)	Prospective cohort study (n=1072)	1.03% of live-born boys (at 3 years: 4,64% of boys (including also milder cases detected when physiological phimosis dissolved))
Bulgaria, 5 regions	(Kumanov *et al.*, 2007)	Cross-sectional clinical study (n=6200 boys aged 0 to 19 years)	0.29% of boys

registries (Lund *et al.*, 2009), whereas the prospective cohort study showed the rate of 1.03% (Boisen *et al.*, 2005). Interestingly, in Finland the birth rate of hypospadias was only 0.3% in a parallel study to that of Boisen et al. (Virtanen *et al.*, 2001). Incidence data of hypospadias are presented in Table 4.

ii. Mechanisms

Androgens regulate male urogenital differentiation. Defects in androgen biosynthesis, metabolism or action can cause hypospadias. Genetic mutations leading to disorders of testicular differentiation, testosterone synthesis, conversion to dihydrotestosterone or androgen receptor action may result in hypospadias (Kalfa, Philibert and Sultan, 2008). Hypospadias is graded by the same Prader classification that is used for description of the severity of androgen insensitivity (Quigley *et al.*, 1995).

However, only about 20% of patients with isolated hypospadias have signs of testicular dysfunction or other endocrine abnormalities (Rey *et al.*, 2005). Environmental effects on androgen action influence penile development, as shown in experimental animals, in which anti-androgenic compounds typically cause hypospadias (Wilson *et al.*, 2008). The critical role of androgens in both penile development and testicular descent is another physiological link between cryptorchidism and hypospadias, and it provides justification for the search for environmental etiologies for both of these conditions.

The penis develops from the genital tubercle and several genes are known to be involved in this, but only a few have been associated with human hypospadias (Kalfa, Philibert and Sultan, 2008; Wang and Baskin, 2008). Homeobox genes, HOXA and HOXD genes contribute to the development of urogenital structures and loss of their function causes agenesis or malformations of the genitalia (Morgan *et al.*, 2003). HOXA13 mutations have been found in the human hand-foot-genital syndrome (Mortlock and Innis, 1997; Frisen *et al.*, 2003). Expression of fibroblast growth factor (FGF) 8 and bone morphogenetic protein 7 in the developing urethra depend on HOXA13, which also influences vascularisation and androgen receptor expression (Mouriquand and Mure, 2001). FGF 10 and FGF receptor 2 have also been linked to the risk of hypospadias in humans (Beleza-Meireles *et al.*, 2007). *Sonic Hedgehog* (*Shh*) has been shown to be crucial for normal genital development in the mouse models (Haraguchi *et al.*, 2001; Perriton *et al.*, 2002; Yucel *et al.*, 2004), but no human mutations have been reported. Activating transcription factor (ATF) 3 was suggested to be involved in the development of hypospadias, because its transcripts were elevated in the foreskin samples in 86 % of operated hypospadias patients, whereas only 13% of samples from circumcision patients had elevated levels (Liu *et al.*, 2005). ATF3 is influencing TGF-beta signalling and it is estrogen-responsive, which might give one explanation why estrogens increase the risk for hypospadias (Liu *et al.*, 2006; Willingham and Baskin, 2007). In addition to hand-foot-genital syndrome, hypospadias can be found in many other multi-organ syndromes, which suggests genetic causes. Genes that are identified may also be targets of endocrine disrupters that can disturb their regulation during critical developmental windows.

Mutations in *MAMLD1* (or *CXORF6*) cause hypospadias and testicular dysgenesis (Fukami *et al.*, 2006). The defect appears to cause disruption of

androgen production, because the gene affects hormone synthesis and has the *NR5/SF1* target sequence (Fukami *et al.*, 2008). Mutation in *NR5/SF1* cause testicular dysgenesis, too (Bashamboo *et al.*, 2010) and this gene may be an important target for endocrine disrupters (Suzawa and Ingraham, 2008). *MAMLD1* mutations are rare in patients with hypospadias, but this mutation can be a part of the cascade of events leading to this disorder (Ogata, Wada and Fukami, 2008; Ogata, Laporte and Fukami, 2009).

Genetic polymorphisms in androgen and estrogen receptors have been associated with the risk of TDS disorders including hypospadias (Aschim *et al.*, 2004b; Yoshida *et al.*, 2005; Beleza-Meireles *et al.*, 2006; Watanabe *et al.*, 2007). However, contradictory results have been published and the associations with the single nucleotide polymorphisms will need to be replicated in larger populations (van der Zanden *et al.*, 2010b ; Wang *et al.*, 2008). A genome-wide association study revealed a common variant of *DGKK*, encoding diacylglycerol kinase, to be linked to an increased risk of hypsopadias (van der Zanden *et al.*, 2011).

iii. Endocrine disrupter association

Cryptorchidism and hypospadias share risk factors, such as being small-for-gestational age (Akre *et al.*, 1999; Aschim *et al.*, 2004a; Pierik *et al.*, 2004; Akre *et al.*, 2008). Anti-androgens and estrogens can cause both conditions, as demonstrated in epidemiological studies that followed the children of women who used diethyl stilbestrol (DES) during pregnancy (for review see (Toppari *et al.*, 1996)). There is also evidence of second-generation effects of DES, because the sons of women exposed in utero have a higher prevalence of hypospadias than other men (Klip *et al.*, 2002; Brouwers *et al.*, 2006; Kalfa, Philibert and Sultan, 2008), suggesting epigenetic effects by DES. The adverse developmental effects of DES in humans are very similar to those described in animals (McLachlan *et al.*, 2001).

Epidemiological studies on hypospadias have used many different ways to assess exposures, including direct measurements in biological samples from mothers or children, environmental measurements, and job-exposure matrices. Pesticides have been high on the list of suspected chemicals because of their endocrine disrupting properties. A meta-analysis of 9 studies assessing the association of pesticide exposure with hypospadias found elevated but marginally significant risks associated with maternal occupational exposure [pooled risk ratio (PRR) of 1.36, CI = 1.04-1.77],

and paternal occupational exposure was not statistically significant (PRR of 1.19, CI= 1.00-1.41) (Rocheleau, Romitti and Dennis, 2009). Vegetarian diets of mothers were associated with an increased risk for hypospadias in the ALSPAC study (North and Golding, 2000), and a somewhat similar finding showed a decreased risk for mothers having fish or meat in their diet during pregnancy (Akre *et al.*, 2008). Subfertility and the use of assisted reproductive techniques are risk factors for hypospadias (Sweet *et al.*, 1974; Czeizel, 1985; Wennerholm *et al.*, 2000; Klemetti *et al.*, 2005; Källen *et al.*, 2005). The causes can be both genetic and epigenetic, including environmental effects. The role of pharmaceutical sex steroids other than DES is controversial. Use of progestins was associated with an increased risk of hypospadias (Czeizel, Toth and Erodi, 1979; Calzolari *et al.*, 1986), but a meta-analysis of fourteen studies showed no association between exposure to sex steroids (excluding DES) during the first trimester and external genital malformations (Raman-Wilms *et al.*, 1995).

c. Timing of puberty

i. Epidemiology

Age at menarche has been approximately 13 years for several decades, whereas 200 years ago it was around 17 years (Aksglæde *et al.*, 2008 and 2009a). Improved nutrition, health and better living conditions may have caused the decline of the age at menarche (Parent *et al.*, 2003). Now there appears to be a new downwards trend; breast development that normally occurs about two years before menarche appears much earlier than before.

Three American studies (PROS, NHANES III, BCERC) and studies from Europe report earlier breast development in girls (Biro *et al.*, 2010; Herman-Giddens *et al.*, 1997; Sun *et al.*, 2002; Wu, Mendola and Buck, 2002; Chumlea *et al.*, 2003; Aksglaede *et al.*, 2009b; Semiz *et al.*, 2008; Castellino *et al.*, 2005), as compared to previous data (Foster *et al.*, 1977; Lee 1980; Juul *et al.*, 2006; Euling *et al.*, 2008; Reynolds and Wines 1948; Nicolson and Hanley 1953). The American PROS and NHANES III studies both showed approximately 0.6-1.2 years advancement in entering breast stage 2 in the 1980s and 1990s compared to earlier data from the 1930s and 1940s (Herman-Giddens *et al.*, 1997), and the most recent study confirmed this development in the 2000s (Biro *et al.*, 2010). However, there was no change in age at menarche (12.9 years in PROS) or only small advancement (0.3 years) (12.6 years in NHANES III) compared to

the previous studies. The girls were assessed only by visual inspection in the NHANES III, which has been criticized because this may have caused some misclassification of some girls as having breast development when there was just fat around the mammary gland. In the PROS study, 39% of the girls were also palpated in addition to visual inspection to detect breast tissue (Kaplowitz and Oberfield 1999), which demonstrated only limited bias compared to visual assessment alone. An international expert panel concluded in 2003 that the available data for girls were sufficient to suggest a secular trend toward earlier onset of breast development among American girls (Euling *et al.*, 2008). At that time there were not yet studies supporting such a trend in age at breast development among European girls (Mul *et al*, 2001; Juul *et al*, 2006). However, recent European data support the US findings of a decline in age at pubertal onset. The age at B2 was 10.3 years in 1638 Italian girls (Castellino *et al.*, 2005), and 10.2 years in 1562 Turkish girls (Semiz *et al.*, 2008). In Denmark, two similar cohort studies in which breast development was judged by palpation of glandular breast tissue showed 12 months earlier age at B2 in 2006-8 (mean age at B2 was 9.9 years) than in 1991-93 (Aksglaede *et al.*, 2009b; Juul *et al.*, 2006). As in the US studies, age at menarche advanced only slightly (Aksglaede *et al.*, 2009b).

Several outbreaks of precocious puberty have been reported, e.g., in Puerto Rico and in Italy (Comas, 1982; Fara *et al.*, 1979). These have appeared to be peripheral, i.e. not central, precocious puberty, and the real causes remained elusive despite many exposure measurements. There are also some areas with a high incidence of central precocious puberty, e.g. in Northwest Tuscany (Massart *et al.*, 2005). Pollution from greenhouses and several small navy yards in that area were suspected to contribute to the problem, but no causal relationships have been demonstrated.

Adopted and immigrant children from developing countries have an increased susceptibility to central precocious puberty, which has been reported in several Western countries (for references see Parent *et al.*, 2003). The reason is not known, but endocrine disrupters may contribute (Krstevska-Konstantinova *et al.*, 2001). Relatively high levels of *p,p*'-DDE were found in 26 immigrant girls with precocious puberty in Belgium, whereas only two of 15 native Belgian patients had detectable serum DDE concentration (Krstevska-Konstantinova *et al.*, 2001), which lead to a hypothesis that early and temporary exposure to weakly estrogenic dichlorodiphenyltrichloroethane (DDT, parent compound to DDE) in

certain developing countries could stimulate hypothalamic and pituitary maturation at the same time that it inhibits the pituitary gonadotrophin secretion via a negative feedback that prevents manifestation of central maturation. After migration, the exposure dramatically decreases and the negative feedback disappears allowing the onset of puberty (Rasier *et al.*, 2006). The problem in this hypothesis is the long half life of DDT that makes the sudden decline in exposure unlikely. Experimental work on DDT, however, has shown its capability to influence GnRH activity (Rasier *et al.*, 2006).

ii. Mechanisms

Regulation of pubertal onset occurs at the central nervous system where several neuronal and humoral inputs act in the neuronal network controlling GnRH neurons. The puberty starts when these cells start to secrete GnRH in a pulsatile manner, which in turn activates pituitary gonadotropes to secrete gonadotropins FSH and LH that act on the gonads. After the testes and ovaries have started to secrete sex steroids, secondary sexual characteristics start to appear. Endocrine disrupters can interfere with pubertal onset on several levels. They may influence the neuronal network in the brain, GnRH neurons, the pituitary gland, the gonads, and they may exert direct peripheral effects as hormone agonists or antagonists or both, depending on the dose and background hormone levels. The same compound can have an agonistic effect when the endogenous hormone level is very low (childhood), whereas it can be an antagonist when the real hormone is available (adulthood). Kisspeptin and its receptor in GnRH neurons was found to be a central upstream signal triggering GnRH neuron activity, and therefore much interest has recently been focused on the regulation of Kisspeptin producing neurons as targets of endocrine disruption (Tena-Sempere, 2010).

iii. Endocrine disrupter association

Exposure of children to pharmaceuticals containing sex steroids or any other products with such endocrine activities cause typically peripheral precocious puberty, which has been described in many case reports. Estrogens stimulate breast development, whereas androgens cause growth of pubic hair and changes in skin (oily skin and hair, adult-type sweat odour). Ointments and salves containing estrogenic compounds have been linked to prepubertal gynecomastia (Henley *et al.*, 2007). If the

Table 5 Overview of epidemiological studies investigating the effects of endocrine disrupters on onset of human puberty

Contaminant	Sex	Observation	References
Chlorinated pesticides (DDT and DDE)	Male	No association with pubertal development	Gladen *et al.*, 2000
	Female	Younger age at menarche	Vasiliu *et al.*, 2004
		Precocious puberty	Krstevska-Konstantinova *et al.*, 2001
		No association with breast stage or pubic hair development	Wolff *et al.*, 2008
		No association with pubertal development	Gladen *et al.*, 2000
Dioxins	Male	No association with sexual maturation	Den Hond *et al.*, 2002
	Female	Later onset of breast development	Leijs *et al.*, 2008
		No association with the onset of menarche	Warner *et al.*, 2004
		Lower stage of breast development	Den Hond *et al.*, 2002
Polychlorinated biphenyls (PCBs)	Female	Slowed breast development	Staessen *et al.*, 2001
		No association with menarche or pubertal stages	Den Hond *et al.*, 2002; Vasiliu *et al.*, 2004
		No association with breast stage or pubic hair development	Wolff *et al.*, 2008
		No association with pubertal development	Gladen *et al.*, 2000
	Male	Late first ejaculation	Leijs *et al.*, 2008
		Reduced penile length	Guo *et al.*, 2004
		Slowed genital development	Den Hond *et al.*, 2002; Staessen et al. 2001
		No association with the development of puberty	Mol *et al.*, 2002
		No association with pubertal development	Gladen *et al.*, 2000
Polybrominated biphenyls (PBBs)	Female	Earlier age at menarche and pubic hair development	Blanck *et al.*, 2000
Bisphenol-A	Female	No association with breast stage or pubic hair development	Wolff *et al.*, 2008
Lead	Female	Delayed breast and pubic hair development	Selevan *et al.*, 2003
		Delayed menarche and pubic hair development	Wu *et al.*, 2003
		Inversely associated with inhibin B levels	Gollenberg *et al.*, 2010
		Delayed breast development, pubic hair growth and age of attainment of menarche	Naicker *et al.*, 2010
	Male	Delayed onset of puberty on the basis of testicular volume of > 3 ml, genitalia staging and pubic hair staging	Williams *et al.*, 2010
Cadmium	Female	High levels of both cadmium and lead is inversely associated with inhibin B levels	Gollenberg *et al.*, 2010

source of exposure can be recognized and eliminated, peripheral puberty does not advance and breast tissue disappears slowly. Peripheral puberty also may stimulate central puberty, which presents a complex problem. Table 5 summarizes epidemiological studies on the exposure-outcome relationships in pubertal development.

Timing of puberty among 151 daughters of fish-eating mothers and their controls was studied in the Michigan anglers' cohort in which exposure to DDT was measured (Vasiliu, Muttinemi and Karmaus, 2004). Early age at menarche was associated with fetal exposure to high levels of DDE. In contrast, in the North Carolina infant feeding study of 316 girls and 278 boys, pubertal timing was not significantly associated with exposure to DDE (Gladen, Ragan and Rogan, 2000). No association of DDE exposure and breast development was found in 9-year-old inner city girls in New York (Wolff *et al.*, 2008). Higher serum DDT levels were associated with earlier age at menarche in 466 Chinese textile workers, (Ouyang *et al.*, 2005).

High exposure to endosulfan was associated with later puberty in a study comparing 117 boys from a highly contaminated area to 90 matched control boys from an uncontaminated area (Saiyed *et al.*, 2003). It was suggested that the antisteroidogenic properties of endosulfan could have contributed to the effect.

Polychlorinated biphenyls (PCBs)

Epidemiological studies on exposure to PCBs in relation to the timing of puberty have yielded somewhat controversial results. In a Belgian study, a delay of puberty was found among boys in urban areas and in association with high serum PCB levels (PCB congeners 138, 153 and 180), whereas no association of PCB levels to pubertal timing was found among girls (Staessen et al., 2001; Den Hond *et al.*, 2002). The study included 120 girls and 80 boys, examined by trained physicians, from rural and urban areas. In the North Carolina infant feeding study, no association of PCB exposure to the self-reported timing of puberty (including age at menarche) was found among 316 girls and 278 boys, although there was a tendency to early maturation among the girls in the highest prenatal exposure group (Gladen, Ragan and Rogan, 2000). No association of PCB exposure with self-reported timing of puberty was found in 327 (Blanck *et al.*, 2000) or 151 girls (Vasiliu, Muttinemi and Karmaus, 2004) in studies from the

Great Lakes area, Michigan in USA, or in 196 boys from the Faroe Islands (Mol *et al.*, 2002). High PCB levels in boys were correlated with late first ejaculation among 14 Dutch boys in a longitudinal cohort study, but no other pubertal sign was associated with PCB concentration (Leijs *et al.*, 2008). In the Yucheng accident, 55 boys were exposed to high levels of PCB and polychlorinated dibenzofuran (PCDF) levels, and in the follow-up studies they had shorter penile length than the control boys at the same age, suggesting pubertal delay (Guo *et al.*, 2004). Among girls in the inner city of New York, PCB levels were associated with a smaller likelihood of having breast development among lean 9-year-old girls, whereas no associations were found with DDE, lead and bisphenol A concentrations (Wolff *et al.*, 2008). The girls with breast development in that study had lower levels of urinary biomarkers of phytoestrogens than control girls. In a small longitudinal cohort study in the Netherlands, no association was found between PCB and polybrominated diphenyl ether levels and pubertal development either in boys or girls (Leijs *et al.*, 2008). In summary, there are two studies suggesting a correlation with delayed puberty and two studies showing no effect of PCB exposure on the timing of puberty among boys, whereas there are no consistent associations found among girls.

Polybrominated biphenyls (PBBs)

An animal feeding accident in Michigan in the 1970s caused a secondary exposure to polybrominated biphenyls (PBBs) in thousands of people using the products from the farm. In the follow-up studies some years later, PBBs were measured in the serum of mothers. These measurements were then used to approximate perinatal exposure of their children. High exposure through breast feeding was associated with earlier pubic hair development and an earlier age at menarche among the girls, whereas breast development was not associated with exposure levels. This study was based on self-assessment of pubertal development, which might have caused more inaccuracy in detection of breast development than that of pubic hair appearance and age at menarche (Blanck *et al.*, 2000).

Phthalates

Children are ubiquitously exposed to phthalate compounds. Animal studies have shown clear endocrine disrupting properties of many phthtalates, but there are not many human studies on their possible effects on pubertal

development. The epidemic of early breast development in Puerto Rico was followed by many studies on putative endocrine disrupters, including phthalates (Colon *et al.*, 2000). Phthalates were linked to gynecomastia, because two thirds of 41 girls with early breast development and only 14 % of 35 controls had measurable phthalate levels in serum. However, the phthalate measurements were criticized for technical inconsistensies and the serum exposure profile raised a serious concern about possible sample contamination or technical problems, because the levels of unmetabolized diethyl hexyl phthalate were high as compared to other phthalates (McKee *et al.*, 2004).

Dioxins

Dioxins are a group of well-characterized endocrine disrupters, whose mechanisms of action are at least partly known: they act through aryl hydrocarbon receptors and thereby interact with other nuclear receptors (Wormke *et al.*, 2003). In July 1976 an explosion occurred in a chemical company in Medina, Italy. A toxic cloud with high concentrations of dioxins affected neighbouring communties, including the village of Seveso. After the Seveso accident, large amounts of dioxins were spread to the environment. In a retrospective analysis of the age of menarche and the level of exposure, no association was found, but uncertainty remained whether the timing of exposure was relevant for pubertal effects in these girls (Warner *et al.*, 2004). In the Yucheng (Taiwan) accident, children were exposed to both PCBs and PCDFs (furans) via contaminated rice oil. The exposed boys had signs of delayed puberty as described earlier (Guo *et al.*, 2004). In a small (n=18) cohort study in the Netherlands, later onset of breast development was correlated in girls with higher prenatal dioxin exposure (Leijs *et al.*, 2008). Total dioxin-like activity in serum was assessded by the Calux assay among the children from rural and two urban areas in Belgium (Staessen *et al.*, 2001; Den Hond *et al.*, 2002). Dioxin-like activities in children's serum were higher in urban areas than in the rural area. The age at menarche and pubic hair development showed no correlation with exposure, but slow breast development to the adult stage was associated with high dioxin activity (Den Hond *et al.*, 2002). Among boys there was no significant exposure-pubertal outcome relationship found. However, the testes of boys living in the urban areas were significantly smaller than those of the boys in the rural area (Den Hond *et al.*, 2002). Dioxins are known to have both estrogenic and anti-estrogenic effects, because dioxin-AhR-nuclear translocator complex

interacts with estrogen receptors (Ohtake *et a*l., 2003). These effects could have contributed to breast development in highly exposed girls.

Lead

Studies on the association of lead exposure with the timing of puberty have given the most consistent results of the epidemiological puberty studies. Lead exposure is associated with a delay in pubertal onset. High lead levels in blood were associated with a delayed age at menarche and delayed pubic hair development in two studies from the National Health and Nutrition Examination Survey in U.S. (NHANES III) (Selevan *et al.*, 2003; Wu *et al.*, 2003). In the study of 2186 girls, breast development was also delayed (Selevan *et al.*, 2003). Similar findings were reported from South Africa (Naicker *et al.*, 2010). In a cross-sectional study including 705 10-11 years old girls, blood lead levels were inversely correlated with inhibin B levels, suggesting a delay of the onset of puberty that is marked by increasing inhibin B levels (Gollenberg *et al.*, 2010). The correlation was even stronger when the urinary cadmium concentration was high (Gollenberg *et al.*, 2010). Lead exposure also is associated with delayed puberty and growth in boys. Even rather low lead levels in blood were associated with growth and pubertal development among boys in Central Russia (Hauser *et al.*, 2008).

d. Thyroid effects

i. Epidemiology

Hypothyroidism is the most frequent thyroid disease, the incidence of which is influenced by both sex and age (Fatourechi, 2009). Clinical hypothyroidism is a relatively frequent disease in fertile women, thus potentially affecting the fetus. Among children, the incidence of hypothyroidism is highest in adolescence. Furthermore, subclinical hypothyroidism is a condition probably affecting a considerable number of both children and adults, and that may be more relevant with respect to effects of endocrine disrupting chemicals.

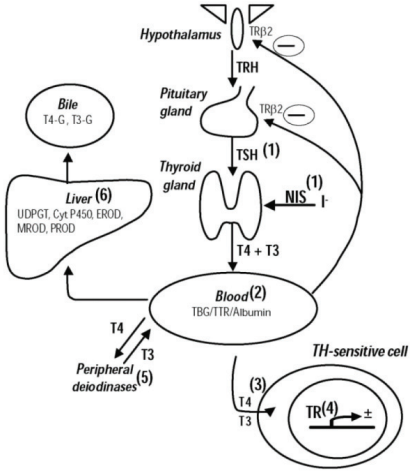
Estimating effects on levels of circulating thyroid hormones is dependent on well-defined population-based reference ranges, which are, however, quite large compared to intra-individual variations in thyroid hormone levels (Feldt-Rasmussen *et al.*, 1980). Thus, minor changes in thyroid hormone levels due to exposure to endocrine disrupting chemicals may not

be detected in small cross-sectional human studies, in which the expected inter-individual variations may camouflage real differences associated with exposure.

During different life stages levels of both TSH and thyroid hormone levels vary greatly. In pregnancy, endocrinological and physiological alterations, including an estrogen-induced increase in TBG, result in an additional stimulation of the maternal thyroid gland. Accordingly, total thyroid hormone levels increase, and free thyroid hormone levels decrease in the first half of pregnancy until a new steady-state is reached. In the neonate, TSH increases dramatically immediately after birth peaking at 30 minutes, followed by an increase in both T_4 and T_3. All of these hormone levels subsequently decrease, leaving evaluation of TSH and thyroid hormone levels highly dependent on exact age and individual factors. Thus, evaluation of especially TSH, but also thyroid hormone levels, in pregnancy, the neonatal period and early childhood for use in statistical associations with exposure to levels of environmental chemicals should allow for age as a critical confounder. In particular, TSH measured in cord blood may not be appropriate as a stable marker of thyroid function.

Thyroid hormone levels influence not only neurological development but also metabolic processes in the body, including elimination processes serving to eliminate endocrine disrupting chemicals from the body. Thus, persons with high TH-levels may have a better capacity to eliminate endocrine disrupting chemicals and thus lower levels of endocrine disrupting chemicals in biological samples. This may be misleading in the interpretation of research results as a high level of endocrine disrupting chemicals may be causally linked to the levels of thyroid hormones. However, these questions have not yet been addressed directly by experimental or human studies.

Effects on cognitive function resulting from exposure to thyroid-disrupting chemicals are extremely difficult to estimate. It is not yet clear which specific cognitive functions, or methods of testing, may be the most representative of thyroid function during development. Furthermore, as in the case of hypothyroidism, effects may be subclinical and require very thorough testing to detect.



Boas M., Feldt-Rasmussen U, Skkakebaek N., Main K. Toppari J. *European Journal of Endocrinology*, 2006, 154:599-611. © Society of the European Journal of Endocrinology (2006). Used with permission.

Figure 5. Possible mechanisms of action of environmental chemicals on the hypothalamic-pituitary-thyroid axis. (1) Synthesis of thyroid hormones (TH): interference with NIS, TPO or TSH receptor. (2) Transport proteins. (3) Cellular uptake mechanisms. (4) The TH receptor. (5) Iodothyronine deiodinases. (6) Metabolism of THs in the liver. TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone; NIS, sodium-iodide symporter; T4, thyroxine; T3, triiodothyronine; TPO, thyroid peroxidise. From: Boas M., Feldt-Rasmussen U, Skkakebaek N., Main K. Toppari J. European Journal of Endocrinology, 2006, 154:599-611. © Society of the European Journal of Endocrinology (2006). Used with permission.

ii. Mechanisms

The mechanisms involved in thyroid homeostasis are numerous and complex. Consequently, environmental chemicals can act at many levels in the thyroid system. (See Figure 5.)

Synthesis of thyroid hormones: interference with the sodium iodide symporter, thyroid peroxidase activity or TSH-receptor

The basic synthesis of thyroid hormones may be compromised by substances interfering with the processes in the thyroid gland, e.g. uptake of iodine and the function of thyroid peroxidase (TPO). Thus, both perchlorate and the phthalates DIDP, butyl benzyl phthalate (BBP) and Di-n-octylphthalate (DnOP) have been shown to interfere with the activity of the sodium iodide symporter (NIS) (Tonacchera *et al.*, 2004; Breous, Wenzel and Loos, 2005). Thyroid peroxidase (TPO) activity was in vitro inhibited by nonylphenol (NP), BPA and BP2 (Schmutzler *et al.*, 2004; Schmutzler *et al.*, 2007). The activity of the thyroid gland is stimulated by TSH and may thus be altered by environmental chemicals affecting the function of the TSH receptor. DDT and the PCB-mixture Aroclor

1254 interfered in vitro with post-receptor signalling by inhibition of the adenylate cyclase activity and cAMP production (Santini *et al.*, 2003).

Transport proteins

In serum, the hormones T_3 and T_4 are transported to the tissues bound to transport proteins. Thyroxine binding globulin (TBG) is the most important thyroid hormone transport protein in humans, but albumin and transthyretin (TTR) also play a role. Competitive binding of environmental chemicals to thyroid hormone transport proteins may result in increased bioavailibility of endogenous thyroid hormones. The investigation of this mechanism of action is restrained by interspecies differences, as TTR is the principal transport protein in rodents and TBG in humans. It is unlikely that enough T_4 could be displaced from TTR to be toxic in adult humans (Purkey *et al.*, 2004). However, TTR is the major thyroid hormone transport protein in the human brain, presumably playing an essential role in the determination of FT_4 levels in the extracellular compartment, which is independent of the T_4 homeostasis in the body. Furthermore, TTR may mediate the delivery of T_4 across the blood-brain barrier and the maternal to fetal transport through the placenta. Thus, environmental chemicals bound to TTR may be transported to the fetal compartment and fetal brain, and be able to decrease fetal brain T_4 levels (Ulbrich *et al.*, 2004).

In experimental studies, PCBs (Meerts et al., 2002; Purkey et al., 2004), flame retardants (Meerts et al., 2000), phenol compounds (Yamauchi et al., 2003; Kudo and Yamauchi, 2005) and phthalates (Ishihara et al., 2003) competitively bind to transthyretin (TTR). Metabolites and derivatives of PCBs, several brominated flame retardants and phenol compounds had remarkably stronger binding affinity than their parent compounds, indicating an important role for hydroxylation and halogenation in thyroid toxicity (Meerts et al., 2000). In contrast to the interference with TTR, no environmental chemicals have been demonstrated to compete with thyroid hormones for binding to TBG or albumin with significant strength (van den Berg, 1990; Lans et al., 1994).

Cellular uptake mechanisms

Bioavailibity of thyroid hormones to the nuclear thyroid hormone receptors may become compromised as TH are probably actively transported across the cell surface via membrane bound transporters.

Several environmental chemicals, including di-*n*-butyl phthalate (DBP) and *n*-butylbenzyl phthalate (BBP) inhibited [^{125}I]T_3 uptake in red blood cells from bullfrog tadpoles (Shimada and Yamauchi, 2004).

The thyroid hormone receptor

Environmental chemicals can change thyroid hormone-stimulated gene transcription, but it is still not clear through which mechanisms these changes are induced.

In experimental studies, BPA, and hydroxylated PCBs acted as antagonists to T_3 (Moriyama *et al.*, 2002; Sun *et al.*, 2009; Kitamura *et al.*, 2005a; Arulmozhiraja *et al.*, 2005; Iwasaki *et al.*, 2002). Similarly, the derivatives TBBPA and TCBPA competed for binding to the receptor (Kitamura *et al.*, 2005b; Jagnytsch *et al.*, 2006; Fini *et al.*, 2007; Hofmann, Schomburg and Kohrle, 2009). A possible pathway of interference with TR is regulation of TR-genes. Studies indicated that BPA, Dicyclohexyl phthalate (DCHP), BBP and PCP inhibit the expression of the TR beta gene (Seiwa *et al.*, 2004; Sugiyama *et al.*, 2005).

Environmental chemicals may also alter the expression of TH-responsive genes. PCB and HCB induced several TH-responsive genes (Gauger et al., 2004; Bansal *et al.*, 2005 Zoeller *et al.*, 2000; Loaiza-Perez *et al.*, 1999).

Neural growth

Oligodendrocyte development and myelination are under thyroid hormone control, as well as the extension of Purkinje cell dendrites, which is essential for normal neuronal circuit formation (synaptogenesis) and subsequent behavioral functions. PCBs, PBDE and BPA caused abnormal development of Purkinje cell dendrites, human neural progenitor cells or mouse oligodendrocytes (Sharlin, Bansal and Zoeller, 2006; Kimura-Kuroda, Nagata and Kuroda, 2005; Seiwa *et al* ., 2004).

Metabolism of circulating thyroid hormones

Peripheral iodothyronine deiodinases are controlling the conversion of thyroid hormones in different organs and are thus essential in the regulation of levels of the biologically active T_3 by activation of T_4 and inactivation of T_4 and T_3. In the liver, several enzymes are involved in the metabolism of thyroid hormones.

Type I 5'deiodinase (5'DI) in the liver was in vitro decreased by several environmental chemicals: octyl-methoxycinnamate (OMC), 4-methylbenzylidene-camphor (MBC) (Schmutzler *et al.*, 2004), methoxychlor (Zhou *et al.,* 1995), dioxins (Viluksela *et al.*, 2004) and a mixture of organochlorines, lead and cadmium (Wade *et al.,* 2002). Mechanistic studies indicated that PCBs, dioxins, PBDEs and PFOS may act through interference with hepatic glucuronidation (Nishimura *et al.*, 2002; Hallgren *et al.*, 2001; Yu, Liu and Jin, 2009; Nieminen *et al.*, 2002a) or sulfation (Schuur *et al.*, 1998c; Schuur *et al.*, 1998b; Schuur *et al.*, 1998a).

iii. Endocrine disrupter association

PCBs

Multiple studies of PCB exposure and effects have been carried out in human populations, several of which raise concern that environmental levels of PCBs may reduce peripheral thyroid hormone levels (Hagmar *et al.*, 2001b; Persky *et al.*, 2001; Abdelouahab *et al.*, 2008; Turyk, Anderson and Persky, 2007; Schell *et al.*, 2008). A few studies also demonstrated a positive correlation between PCB-exposure and TSH (Osius *et al.*, 1999; Schell *et al.*, 2008).

Alterations in fetal and infant thyroid homeostasis due to environmental exposures are of special concern, as it is well known that normal thyroid function is crucial for neurological development. In recent years, several studies have aimed at elucidating the potential toxic effects of environmental levels of PCBs on human thyroid function in developmentally-important age groups. Thus, environmental levels of PCBs are associated with reduced thyroid hormone levels and/or positive associations with TSH in pregnant women in several studies (Takser *et al.*, 2005; Chevrier *et al.*, 2008), but not in all (Wilhelm *et al.*, 2008). This indicates that maternal thyroid function, which is important for the neurological development in the fetus, may be altered by PCBs or other organochlorine compounds.

Studies of newborn babies and infants have been performed in different settings, but the results are not consistent. This may be due to difficulties in obtaining sufficiently large populations as well as obtaining blood samples for evaluation of thyroid hormone levels. Serum levels of especially thyroid-stimulating hormone, and to a lesser degree peripheral thyroid hormones, change dramatically over the first few days of life, influenced by various factors related to pregnancy, delivery and perinatal health (Herbstman *et al.*, 2008). An optimal evaluation of thyroid hormones in

the newborn infant therefore relies on the timing of blood samples.

In 1994 a study of 105 mother-infant pairs analysed associations between PCBs and dioxin-like toxicants in breast milk with thyroid hormones in maternal serum samples and infant serum samples obtained at two weeks and 3 months of age. PCB levels were significantly correlated with lower maternal T_3 and T_4 in late pregnancy and postpartum, with higher TSH in infants at two weeks and three months of age. Infants with high toxic equivalents levels had lower FT_4 and total T_4 at the age of two weeks (Koopman-Esseboom *et al.*, 1994).

Darnerud et al measured PCBs and dioxin in breast milk and thyroid hormones in infant blood samples from 150 mother-infant pairs. After adjustment for confounding factors, they found a negative correlation between PCBs and total T_3 at 3 weeks of age (Darnerud *et al.*, 2010). In a study of 98 mother-infant pairs in a polluted area, PCB levels in cord blood were positively correlated with TSH in 3 days old infants. Peripheral thyroid hormones were not analysed in this study (Ribas-Fito *et al.*, 2003).

Other studies of newborns have confirmed these associations (Herbstman et al., 2008; Chevrier *et al.*, 2007), but several other studies did not find any associations between PCB levels and levels of TSH and thyroid hormones in cord blood (Wilhelm *et al.*, 2008; Longnecker *et al.*, 2000; Dallaire *et al.*, 2008; Dallaire *et al.*, 2009; Wang *et al.*, 2005; Steuerwald *et al.*, 2000; Lopez-Espinosa *et al.*, 2009).

Focusing on long-term effects of perinatal exposure, Matsuura et al. found no associations between PCB levels in breast milk and thyroid hormone levels at the age of 1 year (Matsuura *et al.*, 2001). Similarly, Su et al. found no associations between dioxins/furans in placentas and TH at 2 years of age, but at 5 years T_3 levels were higher in highly exposed individuals (Su *et al.*, 2010).

In older children, several studies have found negative correlations between PCB levels in serum and thyroid hormone levels at the age of 4 years (T_3 and FT_4) (Alvarez-Pedrerol *et al.*, 2007), 7-10 years (FT_3) (Osius *et al.*, 1999), and 10-15 years (T_4 and FT_4) (Schell *et al.*, 2004).

Flame retardants

Few human studies exist regarding flame retardants and thyroid function. These compounds accumulate in animal fat, (in fish, for instance), therefore bio-accumulating through the food chain. However, recently a large

study of consumers of fish from the Great Lakes (US) reported negative associations between concentrations of PBDE congeners in serum and serum levels of T_3 and TSH, as well as a positive relation with T_4 (Turyk, Anderson and Persky, 2008). However, a previous study of men exposed through Baltic fish consumption showed negative associations between TSH and PBDE (Hagmar *et al.*, 2001a).

Recently, a study among 270 pregnant women (in gestational week 27) showed negative associations between serum levels of PBDEs and TSH (Chevrier *et al.*, 2010). In a small study of 12 mother-infant pairs, PBDE levels in pregnancy were not significantly associated with thyroid hormones in cord blood (Mazdai *et al.*, 2003). Thus, evidence on the effect of flame retardants on human thyroid function is very limited, and current results are conflicting.

Perfluorinated chemicals

Recently, a substudy of the NHANES study in the US found that women with high levels of PFOA and men with high levels of PFOS were more likely to report current treated thyroid disease (Melzer *et al.*, 2010). A large study of 506 employees in a PFC manufacturer company showed negative associations between PFOA and FT4 (Olsen and Zobel, 2007), but epidemiological human studies of effects of environmental PFC levels are lacking. These studies indicate that exposure to high levels of PFOS may interfere with human thyroid function. No studies among pregnant women or children have been identified.

Phthalates

One study examined the associations between urinary levels of phthalates in 76 pregnant women and thyroid function and found a significant negative association between DBP-levels and T_4 and free T_4 (Huang *et al.*, 2007). Likewise, negative associations between DEHP-exposure and FT_4 and T_3 have been reported in adult men (Meeker, Calafat and Hauser, 2007b), but studies of smaller populations did not find any relationships, probably due to lack of statistical power (Janjua *et al.*, 2007; Rais-Bahrami *et al.*, 2004).

Pesticides

Some human studies of HCB exposure have reported an inverse association with thyroid hormone levels (Meeker, Calafat and Hauser, 2007a; Schell *et al.*, 2010).

BPA, UV-filters

No studies of effects of BPA and ultraviolet filters on thyroid function in humans have been identified.

4. Data gaps and research needs

Recent trends in the frequency of reproductive problems and other endocrine disorders among children and adolescents are a matter of great concern and suggest that our modern environment can interfere with endocrine systems. Particularly noteworthy is that even adult reproductive disorders may have a fetal origin, although onset of the clinical problem may not be noted until the reproductive age has been reached. However, although these trends are established our understanding of their causes is quite poor. Animal experiments have clearly demonstrated that there are sensitive developmental periods when endocrine disruption causes permanent organizational changes that may appear as structural and functional anomalies much later. Mixture studies in animals have shown the dose-additive effects of chemicals acting on the common endocrine pathways. This challenges all our estimates of dose-response relationships when the fact is that we are exposed to a wide variety of chemicals at the same time. We should gain more knowledge on the endocrine disrupting properties and mechanisms of action of all those chemicals that have not yet been analyzed and to which we are potentially exposed. We need to know more about the influence of mixtures. These should be analyzed both experimentally in animals and in vitro, and by methods of systems biology combining data from different sources.

Human studies of endocrine disrupters are still largely missing, because either the exposure data are weak or the outcome data are vague. Thus, human studies with proper exposure data from a relevant exposure window and reliable ascertainment of the outcome are of vital importance. Long term cohort studies with standardized examination methods can give valuable information. It is important to harmonize both clinical and

environmental measurements. Development of good biomarkers would be useful for health surveys. The prime targets of endocrine disrupters are naturally endocrine systems, such as reproductive organs and their function. Since adult reproductive health depends on normal fetal and early childhood development, the focus on exposure measurements should be in these periods without forgetting about contemporary exposure. Outcome variables, such as genital abnormalities (cryptorchidism, hypospadias) should be diagnosed using defined criteria, and in adult studies e.g. semen analyses should be performed with good external quality control. Genetic susceptibility may vary and this should be taken into account in these analyses. This will require new genetic studies including genome-wide association analyses, deep sequencing and rigorous testing of candidate susceptibility genes. Genetic data need to be integrated with exposure data. New findings on epigenetic effects of endocrine disrupters need to be tested both in experimental animals and in human studies. Exposome data and new 'omics' data on genome, epigenome, metabolome etc. should be integrated for versatile analysis of exposure – outcome relationship. Environmental monitoring and follow-up of reproductive development and health, frequency of congenital hypothyroidism and other endocrine endpoints should be made systematic. Cancer registries in many countries are reliable especially for testicular germ cell cancers, but malformation registries give data on hypospadias that cannot be compared between countries and data on cryptorchidism are largely missing. There are no international or even national systems that would give information on semen quality in general population, although in some countries follow-up studies have been performed. All these data would be needed to follow up the trends that might alert us to environmental problems. Puberty is an important transition period from childhood to adulthood when endocrine systems mature to a terminally differentiated state. This process and its regulation remain poorly understood, and translational studies extending from molecular mechanisms of neuronal control in the brain to epidemiological studies on timing of puberty and environmental effects on it are needed. The ultimate goal is to recognize any adverse effects of environmental factors, which would give the opportunity to develop preventive measures to avoid future health problems. Child health is the basis of adult health and these two should not be separated in a larger context. The European Science Foundation recently published a science policy briefing on male reproductive health, its impacts in relation to general wellbeing and low European fertility rates (ESF Science Policy Briefing

40, September 2010; www.esf.org). Its conclusions and recommendations are also very valid for child health. International conventions, such as the Stockholm Convention, call for the ban of certain persistent organic pollutants (POPs) (including some endocrine disrupters), the list of which is updated as new evidence arises. The updated list is available at http://chm.pops.int/default.aspx

5. Summary

Several reproductive and other endocrine disorders have reached epidemic frequencies and birth rates are extremely low in many countries. The background for these trends is poorly understood. One of the main reasons for low birth rates in the increased use of contraception, but increased infertility might be partially attributed to environmental factors. Some of the disorders such as undescended testis and hypospadias often lead to early surgery of affected infants, who nevertheless have increased risk of infertility and testis cancer later in life. Fetal development is a critical period for all these disorders, also for testis cancer and some cases of infertility and it is likely that the same factors can lead to all of them, albeit not necessarily all at the same time. This quadrad (cryptorchidism, hypospadias, testis cancer and failure of spermatogenesis) has been called testicular dysgenesis syndrome (TDS). Exposure to antiandrogenic compounds at a critical developmental window leads to a TDS-like phenotype in the rat. These chemicals have additive effects, and adverse effects in mixture studies appear at chemical doses that are below no-adverse-effect levels for individual compounds. Therefore it is difficult to estimate, whether current safety margins for allowed daily intakes are adequate. In epidemiological studies, exposure to some endocrine disrupter groups, such as polybrominated flame retardants and chlorinated pesticides, has been associated with an increased risk of cryptorchidism. However, much more work is needed to expand the information on exposure-outcome relationships both for different chemicals and for different outcomes. Normal thyroid function is crucial for development, and any disruption of thyroid hormone action may have disastrous consequences in children's health. The first two years of life when the central nervous system is rapidly developing are the most critical period. It is therefore very important to recognize any endocrine disrupters that can interfere with thyroid function or thyroid hormone action. The most

subtle effects would appear only as a small decline in intellectual capacity. However, for society such changes would have far reaching ramifications. Similarly, subtle adverse effects on reproductive health can appear as a reduced sperm production capacity in the adulthood, which may have dramatic effects on a man's personal life if a couple is suffering from infertility. For a society it can be reflected in an increased demand for expensive assisted reproductive techniques and extremely low fertility rates, which are now seen in several parts of the industrialized world, including many European Countries and Asia. International and national efforts are needed to pursue multiple unresolved research questions. This necessitates intensive interdisciplinary and translational research targeting the developmental processes with all means that we have from chemistry and genetics to epidemiology and modern systems biology. Improving fetal and child health will influence the whole life of an individual and reflect the wellbeing and future of our society.

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PUBLIC HEALTH AND ENVIRONMENT

The present document is a short summary of the current knowledge of the effects of endocrine disrupters on child health. The main focus is on the congenital disorders, cryptorchidism and hypospadias, which have an endocrine connection, on thyroid hormone-related problems, and on puberty. There is ample evidence of endocrine disruption in wildlife, and the mechanisms of action of endocrine disrupters have been elucidated in experimental animals, but there is limited knowledge of the association of human disorders with exposure to endocrine disrupters. Accumulating data suggest that many adult diseases have fetal origins, but the causes have remained unexplained. Improving fetal and child health will influence the whole life of an individual and reflect the wellbeing and future of our society.

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