Endocrine disrupters and child health

Possible developmental early effects of endocrine disrupters on child health
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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 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).
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 Müllarian 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 scrotum 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 scrotum (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 (T₃) and thyroxine (T₄). 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. T₄ is converted to the active hormone T₃ 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

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Sex</th>
<th>Observation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylstilbesterol (DES)</td>
<td>Male</td>
<td>Increased risk of hypospadias</td>
<td>Brouwers et al., 2006; Klip et al., 2002</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Tendency towards smaller testes</td>
<td>Bibbo et al., 1977; Gill et al., 1977, Ross et al., 1983,</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Increased prevalence of cryptorchidism</td>
<td>Palmer et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Capsular induration of testis</td>
<td>Bibbo et al., 1977; Gill et al., 1977</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Severe sperm abnormalities</td>
<td>Bibbo et al., 1977; Gill et al., 1977</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Epididymal cysts</td>
<td>Bibbo et al., 1977; Gill et al., 1977; Palmer et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Infection/inflammation of testis</td>
<td>Palmer et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Increased risk of breast cancer</td>
<td>Palmer et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Vaginal adenosis</td>
<td>Bibbo et al., 1977; Sherman et al., 1974</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Oligomenorrhea</td>
<td>Bibbo et al., 1977</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Increased risk of clear cell adenocarcinoma of the vagina and cervix</td>
<td>Herbst et al., 1971; Herbst et al., 1979; Verloop et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Increased frequency of preterm delivery, first-trimester spontaneous abortion, second-trimester pregnancy loss and ectopic pregnancy</td>
<td>Kaufman et al., 2000</td>
</tr>
<tr>
<td>Phthalate esters (DBP, DMP,BBP,DEHP, DEP, DOP)</td>
<td>Male</td>
<td>Associated with anogenital index</td>
<td>Swan et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Positive correlation with increased serum LH/testosterone ratio</td>
<td>Main et al., 2006a</td>
</tr>
<tr>
<td>Flame retardants (Polybrominated diphenyl ethers)</td>
<td>Male</td>
<td>Associated with cryptorchidism</td>
<td>Main et al., 2007</td>
</tr>
<tr>
<td>Phytoestrogens</td>
<td>Male</td>
<td>Associated with hypospadias</td>
<td>North et al., 2000</td>
</tr>
<tr>
<td>Dioxins</td>
<td>Female</td>
<td>Increased probability of female births</td>
<td>Mocarelli et al., 1996; Mocarelli et al., 2000</td>
</tr>
<tr>
<td>Polychlorinated biphenyls (PCBs)</td>
<td>Male</td>
<td>Higher percentage of oligospermia, abnormal morphology and reduced sperm capacity of binding and penetration to hamster oocyte</td>
<td>Hsu et al., 2003</td>
</tr>
</tbody>
</table>
### TABLE 2 Effects of endocrine disrupters observed in the reproductive system of animals

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Sex</th>
<th>Observation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylstilbestrol</td>
<td>Male</td>
<td>Sterility</td>
<td>McLachlan, 1977</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epididymal cysts</td>
<td>McLachlan, 1977</td>
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<tr>
<td></td>
<td></td>
<td>Cryptorchidism</td>
<td>McLachlan, 1977</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction in testis weight</td>
<td>Fisher et al., 1999; Lewis et al., 2003; McKinnell et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Testicular lesions</td>
<td>McLachlan, 1977</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inflammatory disease of the accessory sex glands</td>
<td>McLachlan, 1977</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction in the number of spermatogonia with multinucleate cells in lumina of testis</td>
<td>McLachlan, 1977</td>
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<tr>
<td></td>
<td></td>
<td>Nodular enlargements of the seminal vesicles and/or prostate</td>
<td>McLachlan, 1977</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distension and overgrowth of the rete testis</td>
<td>Fisher et al., 1999; McKinnell et al., 2001; Rivas et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distension and reduction in epithelial height of the efferent ducts</td>
<td>Fisher et al., 1999; McKinnell et al., 2001; Rivas et al., 2002</td>
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<tr>
<td></td>
<td></td>
<td>Underdevelopment of the epididymal duct epithelium</td>
<td>McKinnell et al., 2001; Rivas et al., 2002</td>
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<td></td>
<td></td>
<td>Reduction in epithelial height in the vas deferens</td>
<td>McKinnell et al., 2001; Rivas et al., 2002</td>
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<td></td>
<td></td>
<td>Convolution of the extra-epididymal vas</td>
<td>McKinnell et al., 2001; Rivas et al., 2002</td>
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<tr>
<td></td>
<td></td>
<td>Decreased testosterone levels</td>
<td>Rivas et al., 2002; Yamamoto et al., 2003</td>
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<tr>
<td></td>
<td></td>
<td>Increased gonadotrophin levels</td>
<td>Yamamoto et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased AR expression in testis, epithelium of the rete testis, caput and cauda epididymis and vas deferens</td>
<td>McKinnell et al., 2001</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Decrease in reproductive capacity</td>
<td>McLachlan, 1977</td>
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<tr>
<td></td>
<td></td>
<td>Impaired ovarian function</td>
<td>McLachlan, 1977</td>
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<td></td>
<td></td>
<td>Increased uterus weight</td>
<td>Lewis et al., 2003</td>
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<td>Squamous metaplasia in the oviducts, uterus and cervix</td>
<td>McLachlan, 1977</td>
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<td></td>
<td></td>
<td>Increased the size of sexually dimorphic nucleus of the preoptic area</td>
<td>Faber and Hughes, 1991; Lewis et al., 2003</td>
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<tr>
<td></td>
<td></td>
<td>Cystic hyperplasia of the endometrium and uterine adenocarcinoma</td>
<td>McLachlan, 1977</td>
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<td></td>
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<td>Epidermoid tumors of the cervix and vagina</td>
<td>McLachlan, 1977</td>
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<td></td>
<td></td>
<td>Glandular elements and cellular atypia in the vaginal epithelium</td>
<td>McLachlan, 1977</td>
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<tr>
<td>Compound</td>
<td>Gender</td>
<td>Effect</td>
<td>Reference(s)</td>
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<td>----------------------------------</td>
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<tr>
<td>Diethylstilbestrol (DES)</td>
<td>Female</td>
<td>Advanced development of primary and secondary follicles in the ovary</td>
<td>Yamamoto et al., 2003</td>
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<td></td>
<td></td>
<td>Decreased pituitary responsiveness to GnRH</td>
<td>Faber and Hughes, 1991</td>
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<td></td>
<td></td>
<td>Increased pubertal FSH levels</td>
<td>Yamamoto et al., 2003</td>
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<td>Tributyltin</td>
<td>Male</td>
<td>Increased anogenital distance</td>
<td>Adeeko et al., 2003</td>
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<td></td>
<td></td>
<td>Reduced the number of Sertoli cells and gonocytes in fetal testis</td>
<td>Kishta et al., 2007</td>
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<tr>
<td></td>
<td>Female</td>
<td>Reduced the number of germ cells in fetal ovaries</td>
<td>Kishta et al., 2007</td>
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<td></td>
<td></td>
<td>Increased post-implantation loss</td>
<td>Adeeko et al., 2003</td>
</tr>
<tr>
<td>Phytosterogens (Genistein, Daidzein)</td>
<td>Male</td>
<td>Decreased pituitary responsiveness to GnRH</td>
<td>Faber and Hughes, 1991</td>
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<td></td>
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<td>Increased the size of sexually dimorphic nucleus of the preoptic area</td>
<td>Faber and Hughes, 1991; Lewis et al., 2003</td>
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<td></td>
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<td>Increased the weight of uterus</td>
<td>Lewis et al., 2003</td>
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<td></td>
<td></td>
<td>Decreased the weight of ovaries</td>
<td>Awoniyi et al., 1998</td>
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<td>Reduced serum estradiol levels</td>
<td>Awoniyi et al., 1998</td>
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<td></td>
<td>Reduced serum progesterone levels</td>
<td>Awoniyi et al., 1998; Lewis et al., 2003</td>
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<td>Female</td>
<td>Irregular estrus cycle</td>
<td>Nagao et al., 2001</td>
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<td>Histopathological changes in the ovaries and uterus</td>
<td>Nagao et al., 2001</td>
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<td></td>
<td>Induced permanent estrus</td>
<td>Lewis et al., 2003</td>
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<td></td>
<td></td>
<td>Decreased the age of vaginal opening</td>
<td>Lewis et al., 2003</td>
</tr>
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<td>Alkyl phenol ethoxylates (p-tert-octylphenol, p-nonylphenol)</td>
<td>Male</td>
<td>Increased testis weight</td>
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<td>de Jager et al., 1996; Pocock et al., 2002</td>
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<td>de Jager et al., 1999</td>
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<td>Reduction in epithelial height of the efferent ducts</td>
<td>Fisher et al., 1999</td>
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<td>Harazono and Ema, 2001</td>
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<td>Irregular estrus cycle</td>
<td>Katsuda et al., 2000; Pocock et al., 2002</td>
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<td>Increased sexual motivation towards a female teaser</td>
<td>Pocock et al., 2002</td>
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<td>Decreased the weight of ovaries</td>
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<td>Increased the size of sexually dimorphic nucleus of the preoptic area</td>
<td>Herath et al., 2001</td>
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<td>Decreased the age of vaginal opening</td>
<td>Katsuda et al., 2000</td>
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<td>Persistent estrus</td>
<td>Katsuda et al., 2000</td>
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<td>Increased relative uterine weight</td>
<td>Katsuda et al., 2000</td>
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<td>Decreased serum gonadotrophin levels</td>
<td>Katsuda et al., 2000</td>
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<td>Decreased serum progesterone levels</td>
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<td>Increased serum inhibin levels</td>
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<td>Phthalate esters (DEHP, BBP, DINP, DBP)</td>
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<td>Nipple retention</td>
<td>Barlow et al., 2004; Borch et al., 2004; Gray et al., 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000</td>
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<td>Decreased testis weight</td>
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<td>Lesion of the rete testis</td>
<td>Barlow et al., 2004</td>
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<td>Hemorrhagic testis</td>
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<td>Cleft phallus and hypospadias</td>
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<td>Multinucleated gonocytes</td>
<td>Gray et al., 2000; Parks et al., 2000</td>
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<td>Agenesis of the seminal vesicles and coagulating glands</td>
<td>Gray et al., 2000; Mylchreest et al., 2000</td>
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<td>Agenesis of bulbourethral glands</td>
<td>Gray et al., 2000</td>
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<td>Agenesis of ventral prostate</td>
<td>Barlow et al., 2004; Gray et al., 2000</td>
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<td>Agenesis of gubernacular cords</td>
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<td>Barlow et al., 2004; Gray et al., 1999b; Mylchreest et al., 1999; Mylchreest et al., 2000</td>
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<td>Histopathological changes of testis</td>
<td>Barlow et al., 2004; Mylchreest et al., 1999; Mylchreest et al., 2000; Parks et al., 2000</td>
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| Phthalate esters (DEHP, BBP, DINP, DBP) | - 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 | - Uterine abnormalities: Gray et al., 1999b  
- Reduced fertility: Gray et al., 1999b  
- 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 | |
| Chlorinated pesticides (DDE) | - 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 | - 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 | - 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

- Decreased daily sperm production
- Dose-related tendencies to decrease plasma testosterone and DHT

#### Female

- Delayed puberty
- Clef phallus
- Vaginal thread
- Reduced ovarian weight
- Enhanced incidences of constant estrus
- Cystic endometrial hyperplasia
- Decreased fertility rate
- Reduced fecundity

### Polychlorinated Biphenyls (PCBs; PBC 77, 118, 126, 132, 169)

#### Male

- Reduced accessory sex organ weights
- Decreased testis weight
- Increased epididymis weight
- Reduced anogenital distance
- Delay in onset of spermatogenesis, preputial separation and sex accessory growth
- Decreased sperm number and total motile sperm count
- Increased daily sperm production
- Decreased serum testosterone levels
- Increased the number of abnormal sperm
- Altered sex behavior

#### Female

- Vaginal thread
- Mild hypospadias
- Delayed the timing of vaginal opening

### Dicarboximide Fungicides

#### Male

- Hypospadias with cleft phallus
- Reduced anogenital distance
- Decreased testis weight
- Cryptorchidism

#### Female

- Gray et al., 1994; Gray et al., 1999a; Gray et al., 1999b; Hellwig et al., 2000; Ostby et al., 1999

- 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

- Elzeinova et al., 2008; Hellwig et al., 2000

- Gray et al., 1994; Hellwig et al., 2000; Ostby et al., 1999
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<td>Dicarboximide Fungicides</td>
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<td>(Vinclozolin, Procymidone)</td>
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<td>Increased the number of apoptotic germ cells in testis</td>
<td>Cowin et al., 2010</td>
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<td>Nipple retention</td>
<td>Gray et al., 1994; Gray et al., 1999a; Hellwig et al., 2000; Ostby et al., 1999</td>
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<td>Reduced accessory sex organ weights</td>
<td>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</td>
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<td>Glandular atrophy and chronic inflammation of prostate</td>
<td>Cowin et al., 2010; Gray et al., 1999b; Hellwig et al., 2000; Ostby et al., 1999</td>
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<td>Reduced secretion and chronic inflammation of seminal vesicles</td>
<td>Hellwig et al., 2000</td>
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<td>Epididymal granulomas</td>
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<td>Chronic inflammation of epididymis</td>
<td>Hellwig et al., 2000</td>
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<td>Agenesis of prostate</td>
<td>Gray et al., 1994</td>
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<td>Spermatogenic granuloma</td>
<td>Hellwig et al., 2000</td>
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<td>Decreased sperm number and daily sperm production</td>
<td>Elzeinova et al., 2008; Gray et al., 1994; Gray et al., 1999a</td>
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<td>Increased sperm head abnormalities</td>
<td>Elzeinova et al., 2008</td>
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<td>Reduced elongated spermatid content per testis</td>
<td>Cowin et al., 2010</td>
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<td>Low ejaculated sperm count</td>
<td>Gray et al., 1999a</td>
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<td>Abnormal morphology of seminiferous tubules</td>
<td>Elzeinova et al., 2008; Gray et al., 1994</td>
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<td>Decreased fertility</td>
<td>Gray et al., 1994</td>
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<td>Reduction of erections during the ex copula penile reflex test</td>
<td>Colbert et al., 2005</td>
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<td>Increase in seminal emissions during the ex copula penile reflex tests</td>
<td>Colbert et al., 2005</td>
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<td>Decreased serum testosterone levels</td>
<td>Gray et al., 1994</td>
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<td>Hericides (Linuron)</td>
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<td>Nipple retention</td>
<td>Gray et al., 1999b</td>
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<td>Delayed preputial separation</td>
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<td>Reduced spermatid number</td>
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<td>Epispadias</td>
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<td>Testicular and epididymal malformations</td>
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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 T4 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 T3 after exposure to PFOS (Seacat et al., 2003).

Perinatal exposure to PFOS also reduced serum levels of T4, 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).

Testes descend to the scrotum 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

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

*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 Pettersson 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 1/10th 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-
ii. Mechanisms

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

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 Ins3 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., \textit{Hoxa10}, \textit{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 \textit{FGFR1} and downstream signaling molecules \textit{SOS1} and \textit{RAF1} (Hadziselimovic et al., 2010) that need to be tested in larger populations. A recent study did not find any mutations in \textit{FGFR1} and heterozygous \textit{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-\textit{INSL3} interaction.

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

![Figure 4. Clinical classification of location of the urethral meatus in hypospadias.](image)
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

<table>
<thead>
<tr>
<th>Country</th>
<th>Reference</th>
<th>Study type</th>
<th>Rate of hypospadias</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S., Rochester Minnesota, St. Mary’s Hospital</td>
<td>(Harris and Steinberg, 1954)</td>
<td>Prospective study (n=4474)</td>
<td>0.70% (BW&gt;2500g), 0.76% of all live-born boys</td>
</tr>
<tr>
<td>U.S., ante partum clinic of the Sloane Hospital, New York City</td>
<td>(McIntosh et al., 1954)</td>
<td>prospective study on pregnant women and infants (n=2793 live-born males)</td>
<td>0.54% of live-born boys</td>
</tr>
<tr>
<td>U.S., Collaborative perinatal project</td>
<td>(Myrianthopoulos and Chung, 1974)</td>
<td>prospective study (n=53394 consecutive single births (boys and girls))</td>
<td>0.80% of single-born boys (76% of cases detected at birth)</td>
</tr>
<tr>
<td>Korea, 38 hospitals</td>
<td>(Choi et al., 1989)</td>
<td>prospective study (n=7990)</td>
<td>0.21% of newborn boys</td>
</tr>
<tr>
<td>Southern Jordan</td>
<td>(al-Abbadi and Smadi, 2000)</td>
<td>Clinical study of 1748 boys (aged 6 to 12 years)</td>
<td>0.74% of boys</td>
</tr>
<tr>
<td>Finland, Turku, Turku University Hospital</td>
<td>(Virtanen et al., 2001)</td>
<td>Prospective cohort study (n=1505) Total hospital cohort (n=5798)</td>
<td>0.02% of live-born boys 0.33% of live-born boys</td>
</tr>
<tr>
<td>Netherlands, Rotterdam</td>
<td>(Pierik et al., 2002)</td>
<td>Prospective study (n=7292)</td>
<td>0.73% of newborn boys</td>
</tr>
<tr>
<td>Denmark, Copenhagen, Rigshospitalet</td>
<td>(Boisen et al., 2005)</td>
<td>Prospective cohort study (n=1072)</td>
<td>1.03% of live-born boys (at 3 years: 4.64% of boys (including also milder cases detected when physiological phimosis dissolved))</td>
</tr>
<tr>
<td>Bulgaria, 5 regions</td>
<td>(Kumanov et al., 2007)</td>
<td>Cross-sectional clinical study (n=6200 boys aged 0 to 19 years)</td>
<td>0.02% of boys</td>
</tr>
</tbody>
</table>

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 MAML1 (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). $MAML1$ 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 hypospadias (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 (Akssglaede et al., 2009b; Juul et al., 2006). As in the US studies, age at menarche advanced only slightly (Akssglaede 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

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Sex</th>
<th>Observation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated pesticides (DDT and DDE)</td>
<td>Male</td>
<td>No association with pubertal development</td>
<td>Gladen et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Younger age at menarche</td>
<td>Vasiliu et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Precocious puberty</td>
<td>Krstevska-Konstantinova et al., 2001</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>No association with breast stage or pubic hair development</td>
<td>Wolff et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>No association with pubertal development</td>
<td>Gladen et al., 2000</td>
</tr>
<tr>
<td>Dioxins</td>
<td>Male</td>
<td>No association with sexual maturation</td>
<td>Den Hond et al., 2002</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Later onset of breast development</td>
<td>Leijs et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>No association with the onset of menarche</td>
<td>Warner et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Lower stage of breast development</td>
<td>Den Hond et al., 2002</td>
</tr>
<tr>
<td>Polychlorinated biphenyls (PCBs)</td>
<td>Female</td>
<td>Slowed breast development</td>
<td>Staessen et al., 2001</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>No association with menarche or pubertal stages</td>
<td>Den Hond et al., 2002; Vasiliu et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>No association with breast stage or pubic hair development</td>
<td>Wolff et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>No association with pubertal development</td>
<td>Gladen et al., 2000</td>
</tr>
<tr>
<td>Polybrominated biphenyls (PBBs)</td>
<td>Female</td>
<td>Earlier age at menarche and pubic hair development</td>
<td>Blanck et al., 2000</td>
</tr>
<tr>
<td>Bisphenol-A</td>
<td>Female</td>
<td>No association with breast stage or pubic hair development</td>
<td>Wolff et al., 2008</td>
</tr>
<tr>
<td>Lead</td>
<td>Female</td>
<td>Delayed breast and pubic hair development</td>
<td>Selevan et al., 2003</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Delayed menarche and pubic hair development</td>
<td>Wu et al., 2003</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Inversely associated with inhibin B levels</td>
<td>Gollenberg et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Delayed breast development, pubic hair growth and age of attainment of menarche</td>
<td>Naicker et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Delayed onset of puberty on the basis of testicular volume of &gt; 3 ml, genitalia staging and pubic hair staging</td>
<td>Williams et al., 2010</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Female</td>
<td>High levels of both cadmium and lead is inversely associated with inhibin B levels</td>
<td>Gollenberg et al., 2010</td>
</tr>
</tbody>
</table>
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 (Staesen 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 phthalates, 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 inconsistencies 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 communities, 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 (Leijts et al., 2008). Total dioxin-like activity in serum was assessed 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 al., 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 T4 and T3. 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.
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 bioavailability 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**

Bioavailability 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 \([^{125}\text{I}]\text{T}_3\) 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 \(\text{T}_3\) (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 \(\text{T}_3\) by activation of \(\text{T}_4\) and inactivation of \(\text{T}_4\) and \(\text{T}_3\). 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 T3 and T4 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 FT4 and total T4 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 T3 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 T3 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 (T3 and FT4) (Alvarez-Pedrerol et al., 2007), 7-10 years (FT3) (Osius et al., 1999), and 10-15 years (T4 and FT4) (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).
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**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...
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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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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.