

# Endocrine disruptors and child health



**Possible developmental  
early effects of endocrine  
disruptors on child health**



**World Health  
Organization**

---

# **Possible developmental early effects of endocrine disruptors on child health**



**World Health  
Organization**

---

## WHO Library Cataloguing-in-Publication Data

Possible developmental early effects of endocrine disrupters on child health.

1.Endocrine disruptors. 2.Disorders of sex development. 3.Sex differentiation. 4.Environmental exposure. 5.Child. I.  
World Health Organization.

ISBN 978 92 4 150376 1

(NLM classification: WK 102)

### © World Health Organization 2012

All rights reserved. Publications of the World Health Organization are available on the WHO web site ([www.who.int](http://www.who.int)) or can be purchased from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: [bookorders@who.int](mailto:bookorders@who.int)). Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to WHO Press through the WHO web site ([http://www.who.int/about/licensing/copyright\\_form/en/index.html](http://www.who.int/about/licensing/copyright_form/en/index.html)).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

This publication contains the collective views of an international group of experts and does not necessarily represent the decisions or the policies of the World Health Organization.

This document was initially planned by an international working group of experts convened by the World Health Organization and the United Nations Environment Programme in a series of meetings (December 2009 in Geneva, June 2010 in Geneva, November 2010 in Stockholm) and then developed by contributors from University of Turku (Finland) and Rigshospitalet (Denmark).

## **Contributors to this document**

Jorma Toppari, Departments of Physiology and Pediatrics, University of Turku, Turku, Finland (leader of the writing team)

Annika Adamsson, Departments of Physiology and Pediatrics, University of Turku, Turku, Finland

Malene Boas, University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark

Anders Juul, University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark

Katharina M. Main, University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark

Niels E. Skakkebaek, University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark

Helena E. Virtanen, Departments of Physiology and Pediatrics, University of Turku, Turku Finland

## **Reviewers**

Heli Bathija, Department of Reproductive Health and Research, World Health Organization, Geneva, Switzerland

Lizbeth López Carrillo, National Institute of Public Health, Mexico

## **Secretariat**

Nida Besbelli, Department of Public Health and Environment, World Health Organization, Geneva, Switzerland

Marie-Noel Bruné Drisse, Department of Public Health and Environment, World Health Organization, Geneva, Switzerland

Ruth A. Etzel, Department of Public Health and Environment, World Health Organization, Geneva, Switzerland

Agneta Sundén Byléhn, DTIE Chemicals Branch, United Nations Environment Programme, Geneva, Switzerland

Simona Surdu, Department of Public Health and Environment, World Health Organization, Geneva, Switzerland

## **International working group of experts contributing to the initial planning**

- Georg Becher, Norwegian Institute of Public Health, Oslo, Norway
- Åke Bergman, Stockholm University, Sweden
- Poul Bjerregaard, University of Southern Denmark, Denmark
- Riana Bornman, Pretoria Academic Hospital, South Africa
- Ingvar Brandt, Uppsala University, Sweden
- Jerry Heindel, National Institute of Environmental Health Sciences, US
- Taisen Iguchi, National Institutes of Natural Sciences, Okazaki, Japan
- Susan Jobling Eastwood, Brunel University, United Kingdom
- Karen Kidd, University of New Brunswick, Canada
- Andreas Kortenkamp, University of London, United Kingdom
- Derek Muir, Environment Canada, Canada
- Roseline Ochieng, Aga Khan University Hospital, Kenya
- Niels Erik Skakkebaek, University of Copenhagen, Denmark
- Hans-Christian Stolzenberg, Federal Environment Agency, Germany
- Jorma Toppari, University of Turku, Finland
- Thomas Zoeller, University of Massachusetts, US
- Tracey Woodruff, University of California San Francisco, US

The development and publication of this document was funded by the National Institute of Environmental Health Sciences through cooperative agreement 1 U01 ES02617 to the World Health Organization and its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS.



# Contents

|  |           |
|--|-----------|
| <b>1. Introduction</b>   | <b>1</b>  |
| a. Endocrine system  | 2         |
| b. Endocrine regulation of development   | 4         |
| i. gonadal hormones – sex differentiation  | 4         |
| ii. thyroid hormones – significance in brain development   | 5         |
| <br>   |           |
| <b>2. Endocrine disrupters (recognized on the basis of experimental work in vitro and in vivo)</b> | <b>7</b>  |
| a. Sex hormone disrupters  | 7         |
| b. Thyroid hormone disrupters  | 17        |
| <br>   |           |
| <b>3. Early effects, child health problems putatively associated with endocrine disruption</b>     | <b>21</b> |
| a. Cryptorchidism  | 21        |
| i. Epidemiology  | 21        |
| ii. Mechanisms   | 25        |
| iii. Endocrine disrupter association   | 26        |
| b. Hypospadias   | 28        |
| i. Epidemiology  | 28        |
| ii. Mechanisms   | 30        |
| iii. Endocrine disrupter association   | 32        |
| c. Timing of puberty   | 33        |
| i. Epidemiology  | 33        |
| ii. Mechanisms   | 35        |
| iii. Endocrine disrupter association   | 35        |
| d. Thyroid effects   | 40        |
| i. Epidemiology  | 40        |
| ii. Mechanisms   | 42        |
| iii. Endocrine disrupter association   | 45        |
| <br>   |           |
| <b>4. Data gaps and research needs</b>   | <b>48</b> |
| <br>   |           |
| <b>5. Summary</b>  | <b>50</b> |
| <br>   |           |
| <b>6. References</b>   | <b>52</b> |

## 1. Introduction

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

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

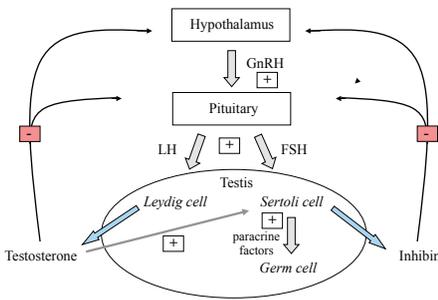
of adult men, are strongly associated with congenital disorders such as cryptorchidism and hypospadias. These disorders, together with testicular cancer and impaired semen quality, form the testicular dysgenesis syndrome (TDS) that by definition has a developmental origin. Epidemiological studies on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

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

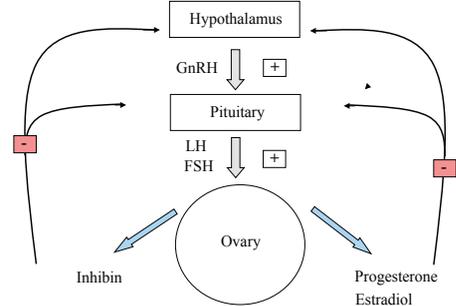
## **a. Endocrine system**

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

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

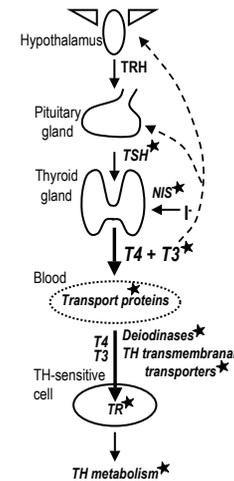


**Figure 1A.** Schematic representation of the hypothalamo-pituitary-testis axis. GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone.



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

in the brain controlling the GnRH neurons. In an analogous fashion, thyrotropin 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 thyroxine (Figure 2).



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

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

## **b. Endocrine regulation of development**

### ***i. Gonadal hormones – sex differentiation***

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

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

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

## **ii. Thyroid hormones – significance in brain development**

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

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

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

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

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

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

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

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

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

### **a. Sex hormone disrupters**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## **b. Thyroid hormone disrupters**

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

### **PCBs and dioxins**

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

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

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

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

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

## **Flame retardants**

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

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

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

## **Pesticides**

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

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

Exposures to DDT (Scollon, Carr and Cobb, 2004), HCB (Rozman *et al.*, 1986; van Raaij *et al.*, 1993a; van Raaij *et al.*, 1993b; Foster *et al.*, 1993; Alvarez *et al.*, 2005), and different mixtures of pesticides (den Besten *et al.*, 1993; Rawlings, Cook and Waldbillig, 1998) decrease serum levels of thyroid hormones in rats. Similarly NP decreases the level of T<sub>4</sub> in studies of salmon (McCormick *et al.*, 2005) and lambs (Beard *et al.*, 1999).

## **Perfluorinated chemicals**

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

Exposure to PFOS and PFOA decreased levels of T<sub>4</sub> after both short-term (Martin and Klaassen, 2007; Chang *et al.*, 2007) and long-term exposure (Yu, Liu and Jin, 2009). A study of monkeys showed reduction of T<sub>3</sub> after exposure to PFOS (Seacat *et al.*, 2003).

Perinatal exposure to PFOS also reduced serum levels of T<sub>4</sub>, both in pregnant dams (Thibodeaux *et al.*, 2003) and in the offspring (Lau *et al.*, 2003; Luebker *et al.*, 2005). Cross-over studies of rats exposed in utero or/ and in lactation document that both prenatal and postnatal exposure to PFOS may reduce thyroid hormone levels in the offspring (Yu *et al.*, 2009).

## **Phthalates**

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

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

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

## **Bisphenol A**

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

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

## **Ultraviolet filters**

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

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

#### a. Cryptorchidism

##### i. Epidemiology

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

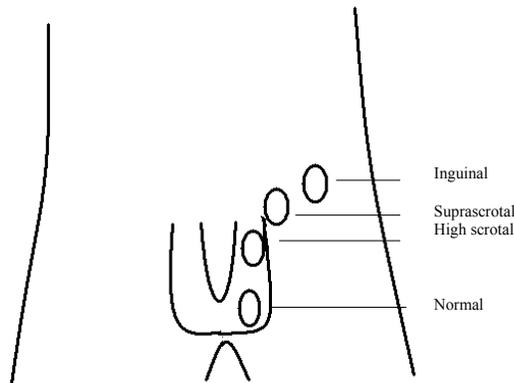


Figure 3. Clinical classification of testicular position in cryptorchidism (non-palpable testis excluded).

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

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

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

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

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

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

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

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

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

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

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

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

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

## **ii. Mechanisms**

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

Mutations in androgen receptor gene, steroidogenic enzymes needed for androgen production, or hypothalamo-pituitary regulators needed for testicular stimulation are all well characterized reasons for cryptorchidism, but occur very rarely (Virtanen *et al.*, 2007; Barthold, 2008). Chemicals that inhibit androgen production or action (anti-androgens) can directly disturb testicular descent, which has a robust experimental evidence. Mutations in *INSL3* and its receptor *RXFP2* have been reported in heterozygous form in cryptorchid boys (Ferlin *et al.*, 2003; Foresta *et al.*, 2008). However, these may be polymorphisms rather than mutations, because they were found as frequently in normal population as in cryptorchid subjects (El Houate *et al.*, 2008; Nuti *et al.*, 2008). No mutations either in *INSL3* or in *RXFP2* were found in Finnish patients (Koskimies *et al.*, 2000; Roh *et al.*, 2003). However, down-regulation of these genes might contribute to maldescent of the testes. Estrogens can down-regulate *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., *Hoxa10*, *Hoxa11* (Hsieh-Li *et al.*, 1995; Rijli *et al.*, 1995; Satokata, Benson and Maas, 1995; Overbeek *et al.*, 2001; Daftary and Taylor, 2006), but there is little evidence for their role in humans. Cryptorchidism can also be found as a part of several syndromes, many of which have an identified genetic reason (Virtanen *et al.*, 2007). However, a great majority of cryptorchidism occurs as a single disorder. Genome-wide association analyses and transcriptome analyses may bring new candidate genes, such as *FGFR1* and downstream signaling molecules *SOS1* and *RAF1* (Hadziselimovic *et al.*, 2010) that need to be tested in larger populations. A recent study did not find any mutations in *FGFR1* and heterozygous *GnRHR* mutations were found in similar frequency as in a group of controls (Laitinen *et al.*, submitted). The genes may also be the targets of adverse environmental effects as exemplified by estrogen-*INSL3* interaction.

### **iii. Endocrine disrupter association**

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

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

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

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

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

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

## b. Hypospadias

### i. Epidemiology

In hypospadias the urethra has failed to fuse normally on the ventral side of the penis and opens 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 (Källén *et al.*, 1986). If the urethra opens to the glans or corona (sulcus), it is called distal, and this mild form of hypospadias often does not necessitate any treatment. Therefore it is often

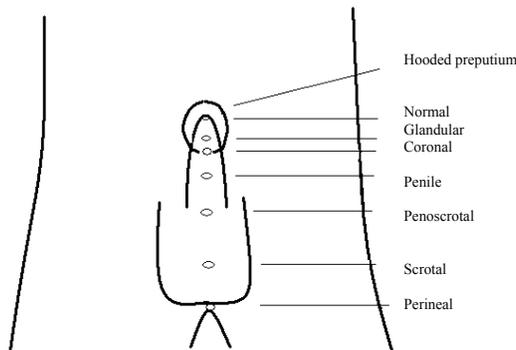


Figure 4. Clinical classification of location of the urethral meatus in hypospadias.

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

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

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

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

| Country  | Reference                         | Study type   | Rate of hypospadias  |
|--|-----------------------------------|--|--|
| U.S., Rochester Minnesota, St. Mary's Hospital                 | (Harris and Steinberg, 1954)      | Prospective study (n=4474)   | 0.70% (BW>2500g), 0.76% of all live-born boys  |
| U.S., ante partum clinic of the Sloane Hospital, New York City | (McIntosh <i>et al.</i> , 1954)   | prospective study on pregnant women and infants (n=2793 live-born males) | 0.54% of live-born boys  |
| U.S., Collaborative perinatal project                          | (Myrianthopoulos and Chung, 1974) | prospective study (n=53394 consecutive single births (boys and girls))   | 0.80% of single-born boys (76% of cases detected at birth)   |
| Korea, 38 hospitals  | (Choi <i>et al.</i> , 1989)       | prospective study (n=7990)   | 0.21% of newborn boys  |
| Southern Jordan  | (al-Abbadi and Smadi, 2000)       | Clinical study of 1748 boys (aged 6 to 12 years)                         | 0.74% of boys  |
| Finland, Turku, Turku University Hospital                      | (Virtanen <i>et al.</i> , 2001)   | Prospective cohort study (n=1505)<br>Total hospital cohort (n=5798)      | 0.27% of live-born boys<br>0.33% of live-born boys   |
| Netherlands, Rotterdam   | (Pierik <i>et al.</i> , 2002)     | Prospective study (n=7292)   | 0.73% of newborn boys  |
| Denmark, Copenhagen, Rigshospitalet                            | (Boisen <i>et al.</i> , 2005)     | Prospective cohort study (n=1072)  | 1.03% of live-born boys (at 3 years: 4.64% of boys (including also milder cases detected when physiological phimosis dissolved)) |
| Bulgaria, 5 regions  | (Kumanov <i>et al.</i> , 2007)    | Cross-sectional clinical study (n=6200 boys aged 0 to 19 years)          | 0.29% of boys  |

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

## ii. Mechanisms

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

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

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

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

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

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

### **iii. Endocrine disrupter association**

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

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

and paternal occupational exposure was not statistically significant (PRR of 1.19, CI= 1.00-1.41) (Rocheleau, Romitti and Dennis, 2009). Vegetarian diets of mothers were associated with an increased risk for hypospadias in the ALSPAC study (North and Golding, 2000), and a somewhat similar finding showed a decreased risk for mothers having fish or meat in their diet during pregnancy (Akre *et al.*, 2008). Subfertility and the use of assisted reproductive techniques are risk factors for hypospadias (Sweet *et al.*, 1974; Czeizel, 1985; Wennerholm *et al.*, 2000; Klemetti *et al.*, 2005; Källén *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; Aksglæde *et al.*, 2009b; Semiz *et al.*, 2008; Castellino *et al.*, 2005), as compared to previous data (Foster *et al.*, 1977; Lee 1980; Juul *et al.*, 2006; Euling *et al.*, 2008; Reynolds and Wines 1948; Nicolson and Hanley 1953). The American PROS and NHANES III studies both showed approximately 0.6-1.2 years advancement in entering breast stage 2 in the 1980s and 1990s compared to earlier data from the 1930s and 1940s (Herman-Giddens *et al.*, 1997), and the most recent study confirmed this development in the 2000s (Biro *et al.*, 2010). However, there was no change in age at menarche (12.9 years in PROS) or only small advancement (0.3 years) (12.6 years in NHANES III) compared to

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

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

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

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

## **ii. Mechanisms**

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

## **iii. Endocrine disrupter association**

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

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

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

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

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

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

### **Polychlorinated biphenyls (PCBs)**

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

Great Lakes area, Michigan in USA, or in 196 boys from the Faroe Islands (Mol *et al.*, 2002). High PCB levels in boys were correlated with late first ejaculation among 14 Dutch boys in a longitudinal cohort study, but no other pubertal sign was associated with PCB concentration (Leijds *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 (Leijds *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 (Leijs *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.

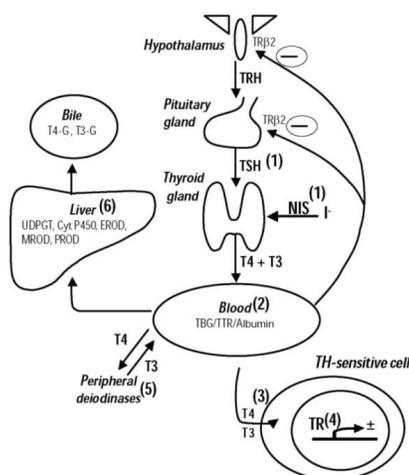
Estimating effects on levels of circulating thyroid hormones is dependent on well-defined population-based reference ranges, which are, however, quite large compared to intra-individual variations in thyroid hormone levels (Feldt-Rasmussen *et al.*, 1980). Thus, minor changes in thyroid hormone levels 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  $T_4$  and  $T_3$ . All of these hormone levels subsequently decrease, leaving evaluation of TSH and thyroid hormone levels highly dependent on exact age and individual factors. Thus, evaluation of especially TSH, but also thyroid hormone levels, in pregnancy, the neonatal period and early childhood for use in statistical associations with exposure to levels of environmental chemicals should allow for age as a critical confounder. In particular, TSH measured in cord blood may not be appropriate as a stable marker of thyroid function.

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

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



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

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

## ii. Mechanisms

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

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

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

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

## Transport proteins

In serum, the hormones  $T_3$  and  $T_4$  are transported to the tissues bound to transport proteins. Thyroxine binding globulin (TBG) is the most important thyroid hormone transport protein in humans, but albumin and transthyretin (TTR) also play a role. Competitive binding of environmental chemicals to thyroid hormone transport proteins may result in increased 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 [<sup>125</sup>I]T<sub>3</sub> uptake in red blood cells from bullfrog tadpoles (Shimada and Yamauchi, 2004).

## The thyroid hormone receptor

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

In experimental studies, BPA, and hydroxylated PCBs acted as antagonists to T<sub>3</sub> (Moriyama *et al.*, 2002; Sun *et al.*, 2009; Kitamura *et al.*, 2005a; Arulmozhiraja *et al.*, 2005; Iwasaki *et al.*, 2002). Similarly, the derivatives TBBPA and TCBPA competed for binding to the receptor (Kitamura *et al.*, 2005b; Jagnytsch *et al.*, 2006; Fini *et al.*, 2007; Hofmann, Schomburg and Kohrle, 2009). A possible pathway of interference with TR is regulation of TR-genes. Studies indicated that BPA, Dicyclohexyl phthalate (DCHP), BBP and PCP inhibit the expression of the TR beta gene (Seiwa *et al.*, 2004; Sugiyama *et al.*, 2005).

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

## Neural growth

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

## Metabolism of circulating thyroid hormones

Peripheral iodothyronine deiodinases are controlling the conversion of thyroid hormones in different organs and are thus essential in the regulation of levels of the biologically active T<sub>3</sub> by activation of T<sub>4</sub> and inactivation of T<sub>4</sub> and T<sub>3</sub>. 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 (Schoor *et al.*, 1998c; Schoor *et al.*, 1998b; Schoor *et al.*, 1998a).

### **iii. Endocrine disrupter association**

#### **PCBs**

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

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

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

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

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

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

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

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

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

## Flame retardants

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

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

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

### **Perfluorinated chemicals**

Recently, a substudy of the NHANES study in the US found that women with high levels of PFOA and men with high levels of PFOS were more likely to report current treated thyroid disease (Melzer *et al.*, 2010). A large study of 506 employees in a PFC manufacturer company showed negative associations between PFOA and FT<sub>4</sub> (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<sub>4</sub> and  $T_3$  have been reported in adult men (Meeker, Calafat and Hauser, 2007b), but studies of smaller populations did not find any relationships, probably due to lack of statistical power (Janjua *et al.*, 2007; Rais-Bahrami *et al.*, 2004).

## **Pesticides**

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

## **BPA, UV-filters**

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

## **4. Data gaps and research needs**

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

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

environmental measurements. Development of good biomarkers would be useful for health surveys. The prime targets of endocrine disruptors 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 disruptors need to be tested both in experimental animals and in human studies. Exposome data and new 'omics' data on genome, epigenome, metabolome etc. should be integrated for versatile analysis of exposure – outcome relationship. Environmental monitoring and follow-up of reproductive development and health, frequency of congenital hypothyroidism and other endocrine endpoints should be made systematic. Cancer registries in many countries are reliable especially for testicular germ cell cancers, but malformation registries give data on hypospadias that cannot be compared between countries and data on cryptorchidism are largely missing. There are no international or even national systems that would give information on semen quality in general population, although in some countries follow-up studies have been performed. All these data would be needed to follow up the trends that might alert us to environmental problems. Puberty is an important transition period from childhood to adulthood when endocrine systems mature to a terminally differentiated state. This process and its regulation remain poorly understood, and translational studies extending from molecular mechanisms of neuronal control in the brain to epidemiological studies on timing of puberty and environmental effects on it are needed. The ultimate goal is to recognize any adverse effects of environmental factors, which would give the opportunity to develop preventive measures to avoid future health problems. Child health is the basis of adult health and these two should not be separated in a larger context. The European Science Foundation recently published a science policy briefing on male reproductive health, its impacts in relation to general wellbeing and low European fertility rates (ESF Science Policy Briefing

40, September 2010; [www.esf.org](http://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 disruptors), 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 is 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.

## 6. References

- Abdelouahab N et al (2008). Gender differences in the effects of organochlorines, mercury, and lead on thyroid hormone levels in lakeside communities of Quebec (Canada). *Environmental Research*, 107:380-392.
- Abdelouahab N et al (2009). Thyroid Disruption by Low-Dose BDE-47 in Prenatally Exposed Lambs. *Neonatology*, 96:120-124.
- Acerini CL et al (2009). The descriptive epidemiology of congenital and acquired cryptorchidism in a UK infant cohort. *Archives of Disease in Childhood*, 94:868-872.
- Adeeko A et al (2003). Effects of *in utero* tributyltin chloride exposure in the rat on pregnancy outcome. *Toxicological Sciences*, 74:407-415.
- Aho M et al (2000). Is the incidence of hypospadias increasing? Analysis of Finnish hospital discharge data 1970-1994. *Environmental Health Perspectives*, 108:463-465.
- Akre O et al (2008). Maternal and gestational risk factors for hypospadias. *Environmental Health Perspectives*, 116:1071-1076.
- Akre O et al (1999). Risk factor patterns for cryptorchidism and hypospadias. *Epidemiology*, 10:364-369.
- Aksglæde L et al (2009a). Age at puberty and the emerging obesity epidemic. *PLoS One* 24:e8450.
- Aksglæde L et al (2008). Forty years trends in timing of pubertal growth spurt in 157,000 Danish school children. *PLoS One*, 3:e2728.
- Aksglæde et al (2009b). Recent decline in age at breast development: the Copenhagen Puberty Study. *Pediatrics*, 123:e932-e939.
- al-Abbadi K, Smadi SA (2000). Genital abnormalities and groin hernias in elementary-school children in Aqaba: an epidemiological study. *Eastern Mediterranean Health Journal*, 6:293-298.
- Alvarez L et al (2005). The role of type I and type II 5' deiodinases on hexachlorobenzene-induced alteration of the hormonal thyroid status. *Toxicology*, 207:349-362.
- Alvarez-Pedrerol M et al (2007). Effects of PCBs, p,p'-DDT, p,p'-DDE, HCB and {beta}-HCH on thyroid function in preschooler children. *Occupational and Environmental Medicine*, 65:452-457
- Andersson AM et al (1998). Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. *Journal of Clinical Endocrinology and Metabolism*, 83:675-681.

Andrade AJ et al (2006). A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): reproductive effects on adult male offspring rats. *Toxicology*, 228:85-97.

Arulmozhiraja S et al (2005). Structural requirements for the interaction of 91 hydroxylated polychlorinated biphenyls with estrogen and thyroid hormone receptors. *Toxicological Sciences*, 84:49-62.

Aschim EL et al (2004a). Risk factors for hypospadias in Norwegian boys - association with testicular dysgenesis syndrome? *International Journal of Andrology*, 27:213-221.

Aschim EL et al (2004b). Linkage between cryptorchidism, hypospadias, and GGN repeat length in the androgen receptor gene. *Journal of Clinical Endocrinology and Metabolism*, 89:5105-5109.

Auso E et al (2004). A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocortico-genesis alters neuronal migration. *Endocrinology*, 145:4037-4047.

Awoniyi CA et al (1998). Reproductive sequelae in female rats after in utero and neonatal exposure to the phytoestrogen genistein. *Fertility and Sterility*, 70:440-447.

Bansal R et al (2005). Maternal thyroid hormone increases HES expression in the fetal rat brain: an effect mimicked by exposure to a mixture of polychlorinated biphenyls (PCBs). *Developmental Brain Research*, 156:13-22.

Barlow NJ, McIntyre BS, Foster PM (2004). Male reproductive tract lesions at 6, 12, and 18 months of age following in utero exposure to di(n-butyl) phthalate. *Toxicologic Pathology*, 32:79-90.

Barthold JS (2008). Undescended testis: current theories of etiology. *Current Opinion in Urology*, 18:395-400.

Bashamboo A et al (2010). Human male infertility associated with mutations in NR5A1 encoding steroidogenic factor 1. *The American Journal of Human Genetics*, 87:505-512.

Bay K et al (2007). Insulin-like factor 3 levels in cord blood and serum from children: effects of age, postnatal hypothalamic-pituitary-gonadal axis activation, and cryptorchidism. *Journal of Clinical Endocrinology and Metabolism*, 92:4020-4027.

Beard AP et al (1999). Reproductive and endocrine function in rams exposed to the organochlorine pesticides lindane and pentachlorophenol from conception. *Journal of Reproduction and Fertility*, 115:303-314.

Beleza-Meireles A et al (2006). Polymorphisms of estrogen receptor beta gene are associated with hypospadias. *Journal of Endocrinological Investigation*, 29:5-10.

- Beleza-Meireles A et al (2007). FGFR2, FGF8, FGF10 and BMP7 as candidate genes for hypospadias. *European Journal of Human Genetics*, 15:405-410.
- Benoff S et al (2008). Link between low-dose environmentally relevant cadmium exposures and asthenozoospermia in a rat model. *Fertility and Sterility*, 89:e73-9.
- Berbel P et al (2009). Delayed neurobehavioral development in children born to pregnant women with mild hypothyroxinemia during the first month of gestation: the importance of early iodine supplementation. *Thyroid*, 19:511-519.
- Berkowitz GS et al (1993). Prevalence and natural history of cryptorchidism. *Pediatrics*, 92:44-49.
- Berkowitz GS et al (1995). Maternal and neonatal risk factors for cryptorchidism. *Epidemiology*, 6:127-131.
- Betts KS (2002). Rapidly rising PBDE levels in North America. *Environmental Science and Technology*, 36:50A-52A.
- Bibbo M et al (1977). Follow-up study of male and female offspring of DES-exposed mothers. *Obstetrics and Gynecology*, 49:1-8.
- Biro FM et al (2010). Pubertal assessment method and baseline characteristics in a mixed longitudinal study of girls. *Pediatrics*, 126:e583-590.
- Blanck HM et al. (2000). Age at menarche and tanner stage in girls exposed *in utero* and postnatally to polybrominated biphenyl. *Epidemiology*, 11:641-647.
- Boisen KA et al (2005). Hypospadias in a cohort of 1072 Danish newborn boys: prevalence and relationship to placental weight, anthropometrical measurements at birth, and reproductive hormone levels at three months of age. *Journal of Clinical Endocrinology and Metabolism*, 90:4041-4046.
- Boisen KA et al (2004). Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet*, 17:1264-1269.
- Borch J et al (2004). Steroidogenesis in fetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal and adult male rats. *Reproductive Toxicology*, 18:53-61.
- Breous E, Wenzel A, Loos U (2005). The promoter of the human sodium/iodide symporter responds to certain phthalate plasticisers. *Molecular and Cellular Endocrinology*, 244:75-78.
- Brouwers MM et al (2006). Hypospadias: a transgenerational effect of diethylstilbestrol? *Human Reproduction*, 21:666-669.
- Brouwers MM et al (2009). Hypospadias: risk factor patterns and different phenotypes. *British Journal of Urology International*, 105:254-262.

- Buermann B et al (1961). Incidence of undescended testis in the newborn. *Acta Chirurgica Scandinavica Supplement*, 283:289-293.
- Calafat AM et al (2008). Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. *Environmental Health Perspectives*, 116:39-44.
- Calzolari E et al (1986). Aetiological factors in hypospadias. *Journal of Medical Genetics*, 23:333-337.
- Canavese F et al (2009). Sperm count of young men surgically treated for cryptorchidism in the first and second year of life: fertility is better in children treated at a younger age. *European Journal of Pediatric Surgery*, 19:388-391.
- Carmichael SL et al (2003). Hypospadias in California: trends and descriptive epidemiology. *Epidemiology*, 14:701-706.
- Castellino N et al (2005). Puberty onset in Northern Italy: a random sample of 3597 Italian children. *Journal of Endocrinological Investigation*, 28:589-594.
- Cesh LS et al (2010). Polyhalogenated aromatic hydrocarbons and metabolites: Relation to circulating thyroid hormone and retinol in nestling bald eagles (*Haliaeetus leucocephalus*). *Environmental Toxicology and Chemistry*, 29:1301-1310.
- Chang SC et al (2007). Negative bias from analog methods used in the analysis of free thyroxine in rat serum containing perfluorooctanesulfonate (PFOS). *Toxicology*, 234:21-33.
- Chevrier J et al (2007). Associations between prenatal exposure to polychlorinated biphenyls and neonatal thyroid-stimulating hormone levels in a Mexican-American population, Salinas Valley, California. *Environmental Health Perspectives*, 115:1490-1496.
- Chevrier J et al (2008). Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. *American Journal of Epidemiology*, 168:298-310.
- Chevrier J et al (2010). Polybrominated diphenyl ether (PBDE) flame retardants and thyroid hormone during pregnancy. *Environmental Health Perspectives*, 118:1444-1449.
- Chiba I et al (2001). Negative correlation between plasma thyroid hormone levels and chlorinated hydrocarbon levels accumulated in seals from the coast of Hokkaido, Japan. *Environmental Toxicology and Chemistry*, 20:1092-1097.
- Choi H et al (1989). A survey of externally recognizable genitourinary anomalies in Korean newborns. Korean Urological Association. *Journal of Korean Medical Science*, 4:13-21.
- Chumlea WC et al (2003). Age at menarche and racial comparisons in US girls. *Pediatrics*, 111: 110-113.

Clarnette TD, Hutson JM (1997). Is the ascending testis actually 'stationary'? Normal elongation of the spermatic cord is prevented by a fibrous remnant of the processus vaginalis. *Pediatric Surgery International*, 12:155-157.

Colbert NK et al (2005). Perinatal exposure to low levels of the environmental antiandrogen vinclozolin alters sex-differentiated social play and sexual behaviors in the rat. *Environmental Health Perspectives*, 113:700-707.

Colon I et al (2000). Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. *Environmental Health Perspectives*, 108:895-900.

Comas AP (1982). Precocious sexula development in Puerto Rico. *Lancet*, 1:1299-1300.

Cowin PA et al (2010). Vinclozolin exposure in utero induces postpubertal prostatitis and reduces sperm production via a reversible hormone-regulated mechanism. *Endocrinology*, 151:783-792.

Cox MJ, Coplen DE, Austin PF (2008). The incidence of disorders of sexual differentiation and chromosomal abnormalities of cryptorchidism and hypospadias stratified by meatal location. *Journal of Urology*, 180: 2649-2652.

Crofton KM et al (2000). PCBs, thyroid hormones, and ototoxicity in rats: cross-fostering experiments demonstrate the impact of postnatal lactation exposure. *Toxicological Sciences*, 57:131-140.

Crofton KM et al (2005). Thyroid-hormone-disrupting chemicals: evidence for dose-dependent additivity or synergism. *Environmental Health Perspectives*, 113:1549-1554.

Czeizel A (1985). Increasing trends in congenital malformations of male external genitalia. *Lancet*, 1:462-463.

Czeizel A, Toth J, Erodi E (1979). Aetiological studies of hypospadias in Hungary. *Human Heredity*, 29:166-171.

Daftary GS, Taylor HS (2006). Endocrine regulation of HOX genes. *Endocrine Reviews*, 27:331-355.

Dallaire R et al (2008). Effects of prenatal exposure to organochlorines on thyroid hormone status in newborns from two remote coastal regions in Quebec, Canada. *Environmental Research*, 108:387-392.

Dallaire R et al (2009). Thyroid hormone levels of pregnant inuit women and their infants exposed to environmental contaminants. *Environmental Health Perspectives*, 117:1014-1020.

Damgaard IN et al (2007). Cryptorchidism and maternal alcohol consumption during pregnancy. *Environmental Health Perspectives*, 115:272-277.

Damgaard IN et al (2008). Risk factors for congenital cryptorchidism in a prospective birth cohort study. *PLoS One*, 3:e3051.

Damgaard IN et al (2006). Persistent pesticides in human breast milk and cryptorchidism. *Environmental Health Perspectives*, 114:1133-1138.

Darnerud PO et al (1996). Binding of a 3,3', 4,4'-tetrachlorobiphenyl (CB-77) metabolite to fetal transthyretin and effects on fetal thyroid hormone levels in mice. *Toxicology*, 106:105-114.

Darnerud PO et al (2001). Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. *Environmental Health Perspectives*, 109(1):49-68.

Darnerud PO et al (2010). POP levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from Uppsala, Sweden. *Environment International*, 36:180-187.

de Jager C, Bornman MS, Oosthuizen JM (1999). The effect of p-nonylphenol on the fertility potential of male rats after gestational, lactational and direct exposure. *Andrologia*, 31:107-113.

Dearth RK et al (2002). Effects of lead (Pb) exposure during gestation and lactation on female pubertal development in the rat. *Reproductive Toxicology*, 16:343-352.

Den Besten C et al (1993). The role of oxidative metabolism in hexachlorobenzene-induced porphyria and thyroid hormone homeostasis: a comparison with pentachlorobenzene in a 13-week feeding study. *Toxicology and Applied Pharmacology*, 119:181-194.

Den Hond E et al (2002). Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. *Environmental Health Perspectives*, 110:771-776.

Dieckmann KP, Pichlmeier U (2004). Clinical epidemiology of testicular germ cell tumors. *World Journal of Urology*, 22:2-14.

Dolk H et al (2004). Toward the effective surveillance of hypospadias. *Environmental Health Perspectives*, 112:398-402.

Donahue DA, Dougherty EJ, Meserve LA (2004). Influence of a combination of two tetrachlorobiphenyl congeners (PCB 47; PCB 77) on thyroid status, choline acetyltransferase (ChAT) activity, and short- and long-term memory in 30-day-old Sprague-Dawley rats. *Toxicology*, 203:99-107.

Eardley I, Saw KC, Whitaker RH (1994). Surgical outcome of orchidopexy. II. Trapped and ascending testes. *British Journal of Urology*, 73:204-206.

El Houate B et al (2008). No association between T222P/LGR8 mutation and cryptorchidism in the Moroccan population. *Hormone Research*, 70:236-239.

Elzeinova F et al (2008). Effect of low dose of vinclozolin on reproductive tract development and sperm parameters in CD1 outbred mice. *Reproductive Toxicology*, 26:231-238.

Emmen JM et al (2000). Involvement of insulin-like factor 3 (Insl3) in diethylstilbestrol-induced cryptorchidism. *Endocrinology*, 141:846-849.

Euling SY et al (2008). Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. *Pediatrics*, 121 (3):S172-S191.

Faber KA., Hughes CL Jr (1991). The effect of neonatal exposure to diethylstilbestrol, genistein, and zearalenone on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat. *Biology of Reproduction*, 45:649-653.

Fara GM et al (1979). Epidemic of breast enlargement in an Italian school. *Lancet*, 2:295-297.

Faqi AS et al (1998). Effects on developmental landmarks and reproductive capability of 3,3',4,4'-tetrachlorobiphenyl and 3,3',4,4',5-pentachlorobiphenyl in offspring of rats exposed during pregnancy. *Human and Experimental Toxicology*, 17:365-372.

Fatourech V (2009). Subclinical hypothyroidism: an update for primary care physicians. *Mayo Clinic Proceedings*, 84:65-71.

Feldt-Rasmussen U et al (1980). Long-term variability in serum thyroglobulin and thyroid related hormones in healthy subjects. *Acta Endocrinologica (Copenhagen)*, 95:328-334.

Ferlin A et al (2003). The INSL3-LGR8/GREAT ligand-receptor pair in human cryptorchidism. *Journal of Clinical Endocrinology and Metabolism*, 88:4273-4279.

Fernie KJ et al (2005). Exposure to polybrominated diphenyl ethers (PBDEs): changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (*Falco sparverius*). *Toxicological Sciences*, 88:375-383.

Fini JB et al (2007). An in vivo multiwell-based fluorescent screen for monitoring vertebrate thyroid hormone disruption. *Environmental Science and Technology*, 41:5908-5914.

Fisch H et al (2009). Hypospadias rates in new york state are not increasing. *Journal of Urology*, 181:2291-2294.

Fisher JS et al (1999). Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood. *Environmental Health Perspectives*, 107:397-405.

Fisher JS et al (2003). Human 'testicular dysgenesis syndrome': a possible model using in-utero exposure of the rat to dibutyl phthalate. *Human Reproduction*, 18:1383-1394.

Foote RH (1999). Cadmium affects testes and semen of rabbits exposed before and after puberty. *Reproductive Toxicology*, 13:269-277.

Foresta C et al (2008). Role of hormones, genes, and environment in human cryptorchidism. *Endocrine Reviews*, 29:560-580.

Foster TA et al (1977) Anthropometric and maturation measurements of children, ages 5 to 14 years, in a biracial community--the Bogalusa Heart Study. *American Journal of Clinical Nutrition*, 30:582-591.

Foster WG et al (1993). Body distribution and endocrine toxicity of hexachlorobenzene (HCB) in the female rat. *Journal of Applied Toxicology*, 13:79-83.

Fowles JR et al (1994). Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice. *Toxicology*, 86:49-61.

Frisen L et al (2003). A novel duplication in the HOXA13 gene in a family with atypical hand-foot-genital syndrome. *Journal of Medical Genetics*, 40:e49.

Fukami M et al (2006). CXorf6 is a causative gene for hypospadias. *Nature Genetics*, 38:1369-1371.

Fukami M et al (2008). Mastermind-like domain-containing 1 (MAMLD1 or CXorf6) transactivates the Hes3 promoter, augments testosterone production, and contains the SF1 target sequence. *Journal of Biological Chemistry*, 283:5525-5532.

Gauger KJ et al (2004). Polychlorinated biphenyls (PCBs) exert thyroid hormone-like effects in the fetal rat brain but do not bind to thyroid hormone receptors. *Environmental Health Perspectives*, 112:516-523.

Ghirri P et al (2002). Incidence at birth and natural history of cryptorchidism: a study of 10,730 consecutive male infants. *Journal of Endocrinological Investigation*, 25:709-715.

Gill WB, Schumacher GF, Bibbo M (1977). Pathological semen and anatomical abnormalities of the genital tract in human male subjects exposed to diethylstilbestrol *in utero*. *Journal of Urology*, 117:477-480.

Gladen BC, Ragan NB, Rogan WJ (2000). Pubertal growth and development, and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. *Journal of Pediatrics*, 136:490-496.

Gollenberg AL et al (2010). Association between Lead and Cadmium and Reproductive Hormones in Peripubertal U.S. Girls. *Environmental Health Perspectives*, 118:1782-1787.

Gray LE Jr, Ostby JS, Kelce WR (1994). Developmental effects of an environmental antiandrogen: the fungicide vinclozolin alters sex differentiation of the male rat. *Toxicology and Applied Pharmacology*, 129:46-52.

Gray LE Jr et al (1995). Exposure to TCDD during development permanently alters reproductive function in male Long Evans rats and hamsters: reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. *Toxicology and Applied Pharmacology*, 131:108-118.

Gray LE Jr, Ostby JS (1995). *In utero* 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. *Toxicology and Applied Pharmacology*, 133:285-294.

Gray LE Jr et al (1999a). Environmental antiandrogens: low doses of the fungicide vinclozolin alter sexual differentiation of the male rat. *Toxicology and Industrial Health*, 15:48-64.

Gray LE Jr et al (1999b). Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolate, p,p'-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. *Toxicology and Industrial Health*, 15:94-118.

Gray LE Jr et al (2000). Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicological Sciences*, 58:350-365.

Gray LE Jr et al (2006). Adverse effects of environmental antiandrogens and androgens on reproductive development in mammals. *International Journal of Andrology*, 29:96-104; discussion 105-108.

Group JRHCS (1992). Cryptorchidism: a prospective study of 7500 consecutive male births, 1984-8. John Radcliffe Hospital Cryptorchidism Study Group. *Archives of Disease in Childhood*, 67:892-899.

Guillette LJ Jr et al (1994). Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environmental Health Perspectives*, 102:680-688.

Guo YL et al (2004). Yucheng: Health effects of prenatal exposure to polychlorinated biphenyls and dibenzofurans. *International Archives of Occupational and Environmental Health*, 77:153-158.

Hack WW et al (2003a). Previous testicular position in boys referred for an undescended testis: further explanation of the late orchidopexy enigma? *British Journal of Urology International*, 92:293-296.

Hack WW et al (2003b). Natural course of acquired undescended testis in boys. *British Journal of Surgery*, 90:728-731.

Hack WW et al (2007a). Prevalence of acquired undescended testis in 6-year, 9-year and 13-year-old Dutch schoolboys. *Archives of Disease in Childhood*, 92:17-20.

Hack WW et al (2007b). Reduction in the number of orchidopexies for cryptorchidism after recognition of acquired undescended testis and implementation of expectative policy. *Acta Paediatrica*, 96:915-918.

Haddow JE et al (1999). Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *New England Journal of Medicine*, 341:549-555.

Hadziselimovic F, Herzog B (2001). The importance of both an early orchidopexy and germ cell maturation for fertility. *Lancet*, 358:1156-1157.

Hadziselimovic NO et al (2010). Decreased expression of FGFR1, SOS1, RAF1 genes in cryptorchidism. *International Journal of Urology*, 84:353-361.

Hadziselimovic F et al (2007). Infertility in cryptorchidism is linked to the stage of germ cell development at orchidopexy. *Hormone Research*, 68:46-52.

Hagmar L et al (2001a). Plasma levels of persistent organohalogenes and hormone levels in adult male humans. *Archives of Environmental Health*, 56:138-143.

Hagmar L et al (2001b). Plasma concentrations of persistent organochlorines in relation to thyrotropin and thyroid hormone levels in women. *International Archives of Occupational and Environmental Health*, 74:184-188.

Hallgren S, Darnerud PO (2002). Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats-testing interactions and mechanisms for thyroid hormone effects. *Toxicology*, 177:227-243.

Hallgren S et al (2001). Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. *Archives of Toxicology*, 75:200-208.

Haraguchi R et al (2001). Unique functions of Sonic hedgehog signaling during external genitalia development. *Development*, 128:4241-4250.

Harazono A, Ema M (2001). Effects of 4-tert-octylphenol on initiation and maintenance of pregnancy following oral administration during early pregnancy in rats. *Toxicology Letters*, 119:79-84.

- Harris LE, Steinberg AG (1954). Abnormalities observed during the first six days of life in 8,716 live-born infants. *Pediatrics*, 14:314-326.
- Hauser R et al (2008). Association of blood lead levels with onset of puberty in Russian boys. *Environmental Health Perspectives*, 116:976-980.
- Hellwig J et al (2000). Pre- and postnatal oral toxicity of vinclozolin in Wistar and Long-Evans rats. *Regulatory Toxicology and Pharmacology*, 32:42-50.
- Hemminki E, Merilainen J, Teperi J (1993). Reporting of malformations in routine health registers. *Teratology*, 48:227-231.
- Henley DV et al (2007). Prepubertal gynecomastia linked to lavender and tea tree oils. *New England Journal of Medicine*, 356:479-485.
- Herath CB et al (2001). Exposure of neonatal female rats to p-tert-octylphenol disrupts afternoon surges of luteinizing hormone, follicle-stimulating hormone and prolactin secretion, and interferes with sexual receptive behavior in adulthood. *Biology of Reproduction*, 64:1216-1224.
- Herbst AL, Ulfelder H, Poskanzer DC (1971). Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *New England Journal of Medicine*, 284:878-881.
- Herbst AL et al (1979). An analysis of 346 cases of clear cell adenocarcinoma of the vagina and cervix with emphasis on recurrence and survival. *Gynecologic Oncology*, 7:111-122.
- Herbstman JB et al (2008). Birth delivery mode modifies the associations between prenatal polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) and neonatal thyroid hormone levels. *Environmental Health Perspectives*, 116:1376-1382.
- Herman-Giddens ME et al (1997). Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatrics*, 99:505-512.
- Hjertkvist M, Damber JE, Bergh A (1989). Cryptorchidism: a registry based study in Sweden on some factors of possible aetiological importance. *Journal of Epidemiology and Community Health*, 43:324-329.
- Hofmann PJ, Schomburg L, Kohrle J (2009). Interference of endocrine disrupters with thyroid hormone receptor-dependent transactivation. *Toxicological Sciences*, 110:125-37.
- Hosie S et al (2000). Is there a correlation between organochlorine compounds and undescended testes? *European Journal of Pediatric Surgery*, 10:304-309.

Howarth JA et al (2001). Effects on male rats of di-(2-ethylhexyl) phthalate and di-n-hexylphthalate administered alone or in combination. *Toxicology Letters*, 121:35-43.

Hsieh JT, Huang TS (1985). A study on cryptorchidism. *Taiwan Yi Xue Hui Za Zhi*, 84:953-959.

Hsieh-Li HM et al (1995). Hoxa 11 structure, extensive antisense transcription, and function in male and female fertility. *Development*, 121:1373-1385.

Hsu PC et al (2003). Sperm changes in men exposed to polychlorinated biphenyls and dibenzofurans. *Journal of the American Medical Association*, 289:2943-2944.

Hsu PC et al (2007). Exposure *in utero* to 2,2',3,3',4,6'-hexachlorobiphenyl (PCB 132) impairs sperm function and alters testicular apoptosis-related gene expression in rat offspring. *Toxicology and Applied Pharmacology*, 221:68-75.

Huang PC et al (2007). Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. *Human Reproduction*, 22:2715-2722.

Ishihara A et al (2003). The effect of endocrine disrupting chemicals on thyroid hormone binding to Japanese quail transthyretin and thyroid hormone receptor. *General and Comparable Endocrinology*, 134:36-43.

Ishitobi H, Watanabe C (2005). Effects of low-dose perinatal cadmium exposure on tissue zinc and copper concentrations in neonatal mice and on the reproductive development of female offspring. *Toxicology Letters*, 159:38-46.

Iwasaki T et al (2002). Polychlorinated biphenyls suppress thyroid hormone-induced transactivation. *Biochemical and Biophysical Research Communications*, 299:384-388.

Jacobsen PR et al (2010). Combined exposure to endocrine disrupting pesticides impairs parturition, causes pup mortality and affects sexual differentiation in rats. *International Journal of Andrology*, 33:434-442.

Jacobsen R et al (2006). Trends in testicular cancer incidence in the Nordic countries, focusing on the recent decrease in Denmark. *International Journal of Andrology*, 29:199-204.

Jagnytsh O et al (2006). Effects of tetrabromobisphenol A on larval development and thyroid hormone-regulated biomarkers of the amphibian *Xenopus laevis*. *Environmental Research*, 101:340-348.

Janjua NR et al (2007). Systemic uptake of diethyl phthalate, dibutyl phthalate, and butyl paraben following whole-body topical application and reproductive and thyroid hormone levels in humans. *Environmental Science and Technology*, 41:5564-5570.

- Jarry H et al (2004). Multi-organic endocrine disrupting activity of the UV screen benzophenone 2 (BP2) in ovariectomized adult rats after 5 days treatment. *Toxicology*, 205:87-93.
- Jensen MS, Bonde JP, Olsen J (2007). Prenatal alcohol exposure and cryptorchidism. *Acta Paediatrica*, 96:1681-1685.
- Johnson KJ et al (2008). The orl rat with inherited cryptorchidism has increased susceptibility to the testicular effects of *in utero* dibutyl phthalate exposure. *Toxicological Sciences*, 105:360-367.
- Johnson MD et al (2003). Cadmium mimics the *in vivo* effects of estrogen in the uterus and mammary gland. *Nature Medicine*, 9:1081-1084.
- Jones ME et al (1998). Prenatal risk factors for cryptorchidism: a record linkage study. *Paediatric and Perinatal Epidemiology*, 12:383-396.
- Jørgensen N et al (2001). Regional differences in semen quality in Europe. *Human Reproduction*, 16:1012-1019.
- Jørgensen N et al (2002). East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. *Human Reproduction*, 17:2199-2208.
- Juul A et al (2006). Pubertal development in Danish children: comparison of recent European and US data. *International Journal of Andrology*, 29:247-255.
- Kalfa N, Philibert P, Sultan C. (2008). Is hypospadias a genetic, endocrine or environmental disease, or still an unexplained malformation? *International Journal of Andrology*, 32:187-197.
- Kaplowitz PB, Oberfield SE (1999). Reexamination of the age limit for defining when puberty is precocious in girls in the United States: implications for evaluation and treatment. Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society. *Pediatrics*, 104:936-941.
- Katsuda S et al (2000). Irreversible effects of neonatal exposure to p-tert-octylphenol on the reproductive tract in female rats. *Toxicology and Applied Pharmacology*, 165:217-226.
- Kaufman RH et al (2000). Continued follow-up of pregnancy outcomes in diethylstilbestrol-exposed offspring. *Obstetrics and Gynecology*, 96:483-489.
- Kelce WR et al (1995). Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. *Nature*, 375:581-585.
- Kim TH et al (2009). Effects of gestational exposure to decabromodiphenyl ether on reproductive parameters, thyroid hormone levels, and neuronal development in Sprague-Dawley rats offspring. *Journal of Toxicology and Environmental Health, A* 72:1296-1303.

- Kimmel CA et al (1980). Chronic low-level lead toxicity in the rat. I. Maternal toxicity and perinatal effects. *Toxicology and Applied Pharmacology*, 56:28-41.
- Kimura-Kuroda J, Nagata I, Kuroda Y (2005). Hydroxylated metabolites of polychlorinated biphenyls inhibit thyroid-hormone-dependent extension of cerebellar Purkinje cell dendrites. *Developmental Brain Research*, 154:259-263.
- Kirkegaard M et al (2011). Alterations in thyroid hormone status in Greenland sledge dogs exposed to whale blubber contaminated with organohalogen compounds. *Ecotoxicology and Environmental Safety*, 74:157-63.
- Kishta O et al (2007). *In utero* exposure to tributyltin chloride differentially alters male and female fetal gonad morphology and gene expression profiles in the Sprague-Dawley rat. *Reproductive Toxicology*, 23:1-11.
- Kitamura S et al (2005a). Thyroid hormone-like and estrogenic activity of hydroxylated PCBs in cell culture. *Toxicology*, 208:377-387.
- Kitamura S et al (2005b). Anti-thyroid hormonal activity of tetrabromobisphenol A, a flame retardant, and related compounds: Affinity to the mammalian thyroid hormone receptor, and effect on tadpole metamorphosis. *Life Sciences*, 76:1589-1601.
- Klammer H et al (2007). Effects of a 5-day treatment with the UV-filter octyl-methoxycinnamate (OMC) on the function of the hypothalamo-pituitary-thyroid function in rats. *Toxicology*, 238:192-199.
- Klemetti R et al (2005). Children born after assisted fertilization have an increased rate of major congenital anomalies. *Fertility and Sterility*, 84:1300-1307.
- Klip H et al (2002). Hypospadias in sons of women exposed to diethylstilbestrol *in utero*: a cohort study. *Lancet*, 359:1102-1107.
- Kodavanti PR et al (2010). Developmental exposure to a commercial PBDE mixture, DE-71: neurobehavioral, hormonal, and reproductive effects. *Toxicological Sciences*, 116:297-312.
- Koopman-Esseboom C et al (1994). Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. *Pediatric Research*, 36:468-473.
- Kortenkamp A, Faust M. (2010). Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment. *International Journal of Andrology*, 33:463-474.
- Koskimies P et al (2000). A common polymorphism in the human relaxin-like factor (RLF) gene: no relationship with cryptorchidism. *Pediatric Research*, 47:538-541.

- Kristensen P et al (1997). Birth defects among offspring of Norwegian farmers, 1967-1991. *Epidemiology*, 8:537-544.
- Krstevska-Konstantinova M (2001). Sexual precocity after immigration from developing countries to Belgium: Evidence of previous exposure to organochlorine pesticides. *Human Reproduction*, 16:1020-1026.
- Krysiak-Baltyn K et al (2010). Country-specific chemical signatures of persistent environmental compounds in breast milk. *International Journal of Andrology*, 33:270-278.
- Kudo Y, Yamauchi K (2005). In vitro and in vivo analysis of the thyroid disrupting activities of phenolic and phenol compounds in *Xenopus laevis*. *Toxicological Sciences*, 84:29-37.
- Kumanov P et al (2007). Prevalence of the hypospadias among Bulgarian boys--a prospective study. *European Journal of Pediatrics*, 166:987-988.
- Kuriyama SN, Chahoud I (2004). In utero exposure to low-dose 2,3',4,4',5-pentachlorobiphenyl (PCB 118) impairs male fertility and alters neurobehavior in rat offspring. *Toxicology*, 202:185-197.
- Kuriyama SN et al (2007). Developmental exposure to low-dose PBDE-99: Tissue distribution and thyroid hormone levels. *Toxicology*, 242:80-90.
- Källén B et al (1986). A joint international study on the epidemiology of hypospadias. *Acta Paediatrica Scandinavica*, Suppl 324:1-52.
- Källén B et al (2005). In vitro fertilization (IVF) in Sweden: risk for congenital malformations after different IVF methods. *Birth Defects Research. Part A: Clinical and Molecular Teratology*, 73:162-169.
- Lamah M et al (2001). The ascending testis: is late orchidopexy due to failure of screening or late ascent? *Pediatric Surgery International*, 17:421-423.
- Lans MC et al (1994). Different competition of thyroxine binding to transthyretin and thyroxine-binding globulin by hydroxy-PCBs, PCDDs and PCDFs. *European Journal of Pharmacology*, 270:129-136.
- Lau C et al (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. *Toxicological Sciences*, 74:382-392.
- Lavado-Autric R et al (2003). Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. *Journal of Clinical Investigation*, 111:1073-1082.
- Lee B et al (2006). Manganese acts centrally to activate reproductive hormone secretion and pubertal development in male rats. *Reproductive Toxicology*, 22:580-585.

- Lee PA (1980). Normal ages of pubertal events among American males and females. *Journal of Adolescent Health Care*, 1:26-29.
- Lee PA, Coughlin MT (2001). Fertility after bilateral cryptorchidism. Evaluation by paternity, hormone, and semen data. *Hormone Research*, 55:28-32.
- Lee E et al (2010). Evaluation of liver and thyroid toxicity in Sprague-Dawley rats after exposure to polybrominated diphenyl ether BDE-209. *Journal of Toxicological Sciences*, 35:535-545.
- Leijds MM et al (2008). Delayed initiation of breast development in girls with higher prenatal dioxin exposure; a longitudinal cohort study. *Chemosphere*, 73:999-1004.
- Lewis RW et al. (2003). The effects of the phytoestrogen genistein on the postnatal development of the rat. *Toxicological Sciences*, 71:74-83.
- Lilienthal H et al (2006). Effects of developmental exposure to 2,2 ,4,4 ,5-pentabromodiphenyl ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behavior in rats. *Environmental Health Perspectives*, 114:194-201.
- Liu B et al (2006). Activating transcription factor 3 is estrogen-responsive in utero and upregulated during sexual differentiation. *Hormone Research*, 65:217-222.
- Liu B et al (2005). Activating transcription factor 3 is up-regulated in patients with hypospadias. *Pediatric Research*, 58:1280-1283.
- Loaiza-Perez AI et al (1999) Hexachlorobenzene, a dioxin-type compound, increases malic enzyme gene transcription through a mechanism involving the thyroid hormone response element. *Endocrinology*, 140: 4142-4151.
- Longnecker MP et al (2000). Polychlorinated biphenyl (PCB) exposure in relation to thyroid hormone levels in neonates. *Epidemiology*, 11:249-254.
- Longnecker MP et al (2002). Maternal serum level of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene and risk of cryptorchidism, hypospadias, and polythelia among male offspring. *American Journal of Epidemiology*, 155:313-322.
- Lopez-Espinosa MJ et al (2010). Prenatal exposure to organochlorine compounds and neonatal thyroid stimulating hormone levels. *Journal of Exposure Science and Environmental Epidemiology*, 20:579-88
- Luebker DJ et al (2005). Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharmacokinetic parameters. *Toxicology*, 215:149-169.

- Lund L et al (2009). Prevalence of hypospadias in Danish boys: a longitudinal study, 1977-2005. *European Urology*, 55:1022-1026.
- Mably TA, Moore RW, Peterson RE (1992a). *In utero* and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 1. Effects on androgenic status. *Toxicology and Applied Pharmacology*, 114:97-107.
- Mably TA et al (1992b). *In utero* and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 3. Effects on spermatogenesis and reproductive capability. *Toxicology and Applied Pharmacology*, 114:118-126.
- Main KM et al (2007). Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. *Environmental Health Perspectives*, 115:1519-1526.
- Main KM et al (2006a). Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. *Environmental Health Perspectives*, 114:270-276.
- Main KM et al (2006b). Larger testes and higher inhibin B levels in Finnish than in Danish newborn boys. *Journal of Clinical Endocrinology and Metabolism*, 91:2732-2737.
- Martin, L, Klaassen CD (2010). Differential effects of polychlorinated biphenyl congeners on serum thyroid hormone levels in rats. *Toxicological Sciences*, 117:36-44.
- Martin MT et al (2007). Toxicogenomic study of triazole fungicides and perfluoroalkyl acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity. *Toxicological Sciences*, 97:595-613.
- Massart F et al (2005). High incidence of central precocious puberty in a bounded geographic area of Northwest Tuscany: An estrogen disrupter epidemic? *Gynecological Endocrinology*, 20:92-98.
- Matsuura N et al (2001). Effects of dioxins and polychlorinated biphenyls (PCBs) on thyroid function in infants born in Japan--the second report from research on environmental health. *Chemosphere*, 45:1167-1171.
- Mazdai A et al (2003). Polybrominated diphenyl ethers in maternal and fetal blood samples. *Environmental Health Perspectives*, 111:1249-1252.
- McCormick SD et al (2005). Endocrine disruption of parr-smolt transformation and seawater tolerance of Atlantic salmon by 4-nonylphenol and 17beta-estradiol. *General and Comparative Endocrinology*, 142:280-288.
- McGivern RF, Sokol RZ, and Berman NG (1991). Prenatal lead exposure in the rat during the third week of gestation: long-term behavioral, physiological, and anatomical effects associated with reproduction. *Toxicology and Applied Pharmacology*, 110:206-215.

McIntosh R et al (1954). The incidence of congenital malformations: a study of 5,964 pregnancies. *Pediatrics*, 14:505-522.

McKinnell C et al (2001). Suppression of androgen action and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol. *Journal of Andrology*, 22:323-338.

McLachlan JA (1977). Prenatal exposure to diethylstilbestrol in mice: toxicological studies. *Journal of Toxicology and Environmental Health*, 2:527-537.

McLachlan JA et al (2001). From malformations to molecular mechanisms in the male: three decades of research on endocrine disrupters. *Apmis*, 109:263-272.

McLachlan JA, Simpson E, Martin M (2006). Endocrine disrupters and female reproductive health. *Best Practice and Research: Clinical Endocrinology and Metabolism*, 20:63-75.

Meeker JD, Altshul L, Hauser R (2007a). Serum PCBs, p,p'-DDE and HCB predict thyroid hormone levels in men. *Environmental Research*, 104:296-304.

Meeker JD, Calafat AM, Hauser R (2007b). Di(2-ethylhexyl) phthalate metabolites may alter thyroid hormone levels in men. *Environmental Health Perspectives*, 115:1029-1034.

Meerts IA et al (2002). Placental transfer of a hydroxylated polychlorinated biphenyl and effects on fetal and maternal thyroid hormone homeostasis in the rat. *Toxicological Sciences*, 68:361-371.

Meerts IA et al (2004). Developmental exposure to 4-hydroxy-2,3,3',4',5-pentachlorobiphenyl (4-OH-CB107): long-term effects on brain development, behavior, and brain stem auditory evoked potentials in rats. *Toxicological Sciences*, 82:207-218.

Meerts IA et al (2000). Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicological Sciences*, 56:95-104.

Melzer D et al (2010). Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. *Environmental Health Perspectives*, 118:686-692.

Mital VK, Garg BK (1972). Undescended testicle. *Indian Journal of Pediatrics*, 39:171-174.

Mocarelli P et al (1996). Change in sex ratio with exposure to dioxin. *Lancet*, 348:409.

Mocarelli P et al (2000). Paternal concentrations of dioxin and sex ratio of offspring. *Lancet*, 355:1858-1863.

Mol NM et al (2002). Spermaturation and serum hormone concentrations at the age of puberty in boys prenatally exposed to polychlorinated biphenyls. *European Journal of Endocrinology*, 146:357-363.

Mongraw-Chaffin ML et al (2008). Maternal smoking, alcohol consumption, and caffeine consumption during pregnancy in relation to a son's risk of persistent cryptorchidism: a prospective study in the Child Health and Development Studies cohort, 1959-1967. *American Journal of Epidemiology*, 167:257-261.

Morgan EA et al (2003). Loss of Bmp7 and Fgf8 signaling in Hoxa13-mutant mice causes hypospadias. *Development*, 130:3095-3109.

Moriyama K et al (2002). Thyroid hormone action is disrupted by bisphenol A as an antagonist. *Journal of Clinical Endocrinology and Metabolism*, 87:5185-5190.

Mortlock DP, Innis JW (1997). Mutation of HOXA13 in hand-foot-genital syndrome. *Nature Genetics*, 15:179-180.

Mouriquand P, Mure P. Hypospadias. In: R. Gearhart, P. Mouriquand, eds. *Pediatric Urology*. WB Saunders Publishers, Philadelphia, 2001: 713-728.

Mul D et al (2001). Pubertal development in The Netherlands 1965-1997. *Pediatric Research*, 50:479-486.

Mylchreest E et al (1999). Disruption of androgen-regulated male reproductive development by di(n-butyl) phthalate during late gestation in rats is different from flutamide. *Toxicology and Applied Pharmacology*, 156:81-95.

Mylchreest E et al (2000). Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to Di(n-butyl) phthalate during late gestation. *Toxicological Sciences*, 55:143-151.

Mylchreest E et al (2002). Fetal testosterone insufficiency and abnormal proliferation of Leydig cells and gonocytes in rats exposed to di(n-butyl) phthalate. *Reproductive Toxicology*, 16:19-28.

Myriantopoulos NC, Chung CS (1974). Congenital malformations in singletons: epidemiologic survey. Report from the Collaborative Perinatal project. *Birth Defects Original Article Series*, 10:1-58.

Myrup C, Schnack TH, Wohlfahrt J (2007). Correction of cryptorchidism and testicular cancer. *New England Journal of Medicine*, 357:825-826.

Nagao T et al (2001). Reproductive effects in male and female rats of neonatal exposure to genistein. *Reproductive Toxicology*, 15:399-411.

Naicker N et al (2010). Lead exposure is associated with a delay in the onset of puberty in South African adolescent females: findings from the Birth to Twenty cohort. *Science of the Total Environment*, 408:4949-4954.

- Nassar N, Bower C, Barker A (2007). Increasing prevalence of hypospadias in Western Australia, 1980-2000. *Archives of Disease in Childhood*, 92:580-584.
- Nef S, Shipman T, Parada LF (2000). A molecular basis for estrogen-induced cryptorchidism. *Developmental Biology*, 224:354-361.
- Nicholson JL, Altman J (1972). The effects of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. I. Cell proliferation and differentiation. *Brain Research*, 44:13-23.
- Nicolson AB, Hanley C (1953). Indices of physiological maturity: derivation and interrelationships. *Child Development*, 24:3-38.
- Nieminen P et al (2002a). In vivo effects of bisphenol A on the polecat (*Mustela putorius*). *Journal of Toxicology and Environmental Health*, A65:933-945.
- Nieminen P et al (2002b). Bisphenol A affects endocrine physiology and biotransformation enzyme activities of the field vole (*Microtus agrestis*). *General and Comparative Endocrinology*, 126:183-189.
- Nishimura N et al (2002). Immunohistochemical localization of thyroid stimulating hormone induced by a low oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin in female Sprague-Dawley rats. *Toxicology*, 171:73-82.
- Nishimura N et al (2003). Rat thyroid hyperplasia induced by gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Endocrinology*, 144:2075-2083.
- North K, Golding J (2000). A maternal vegetarian diet in pregnancy is associated with hypospadias. The ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. *British Journal of Urology International*, 85:107-113.
- Nuti F et al (2008). The leucine-rich repeat-containing G protein-coupled receptor 8 gene T222P mutation does not cause cryptorchidism. *Journal of Clinical Endocrinology and Metabolism*, 93:1072-1076.
- O'Connor JC, Frame SR, Ladics GS (2002). Evaluation of a 15-day screening assay using intact male rats for identifying antiandrogens. *Toxicological Sciences*, 69:92-108.
- Ogata T, Laporte J, Fukami M (2009). MAMLD1 (CXorf6): a new gene involved in hypospadias. *Hormone Research*, 71:245-252.
- Ogata T, Wada Y, Fukami M (2008). MAMLD1 (CXorf6): a new gene for hypospadias. *Sexual Development*, 2:244-250.

- Ohsako Setal (2001). Maternal exposure to a low dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) suppressed the development of reproductive organs of male rats: dose-dependent increase of mRNA levels of 5alpha-reductase type 2 in contrast to decrease of androgen receptor in the pubertal ventral prostate. *Toxicological Sciences*, 60:132-143.
- Ohtake F et al (2003). Modulation of estrogen receptor signalling by association with the activated dioxin receptor. *Nature*, 423:545-550.
- Olsen GW, Zobel LR (2007). Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. *International Archives of Occupational and Environmental Health*, 81:231-246.
- Osius N et al (1999). Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. *Environmental Health Perspectives*, 107:843-849.
- Ostby J et al (1999). The fungicide procymidone alters sexual differentiation in the male rat by acting as an androgen-receptor antagonist in vivo and in vitro. *Toxicology and Industrial Health*, 15:80-93.
- Ouyang F et al (2005). Serum DDT, age at menarche, and abnormal menstrual cycle length. *Occupational and Environmental Medicine*, 62:878-884.
- Overbeek PA et al (2001). A transgenic insertion causing cryptorchidism in mice. *Genesis*, 30:26-35.
- Palmer JR et al (2006). Prenatal diethylstilbestrol exposure and risk of breast cancer. *Cancer Epidemiology Biomarkers and Prevention*, 15:1509-1514.
- Palmer JR et al (2009). Urogenital abnormalities in men exposed to diethylstilbestrol in utero: a cohort study. *Environmental Health*, 8:37.
- Pan L et al (2008). Exposure of juvenile rats to the phytoestrogen daidzein impairs erectile function in a dose-related manner in adulthood. *Journal of Andrology*, 29:55-62.
- Parent AS et al (2003). The timing of normal puberty and the age limits of sexual precocity: Variations around the world, secular trends and changes after migration. *Endocrine Reviews*, 24:668-693.
- Parks LG et al (2000). The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. *Toxicological Sciences*, 58:339-349.
- Pasquali R et al (2011). PCOS Forum: research in polycystic ovary syndrome today and tomorrow. *Clinical Endocrinology (Oxford)*, 74:424-433.
- Paulozzi LJ (1999). International trends in rates of hypospadias and cryptorchidism. *Environmental Health Perspectives*, 107:297-302.

- Paulozzi LJ, Erickson JD, Jackson RJ (1997). Hypospadias trends in two US surveillance systems. *Pediatrics*, 100:831-834.
- Perriton CL et al (2002). Sonic hedgehog signaling from the urethral epithelium controls external genital development. *Developmental Biology*, 247, 26-46.
- Persky V et al (2001). The effects of PCB exposure and fish consumption on endogenous hormones. *Environmental Health Perspectives*, 109:1275-1283.
- Peterson RE, Theobald HM, Kimmel GL (1993). Developmental and reproductive toxicity of dioxins and related compounds: cross-species comparisons. *Critical Reviews in Toxicology*, 23:283-335.
- Pettersson A et al (2007). Age at surgery for undescended testis and risk of testicular cancer. *New England Journal of Medicine*, 356:1835-1841.
- Pierik FH et al (2002). A high hypospadias rate in The Netherlands. *Human Reproduction*, 17:1112-1115.
- Pierik FH et al (2004). Maternal and paternal risk factors for cryptorchidism and hypospadias: a case-control study in newborn boys. *Environmental Health Perspectives*, 112:1570-1576.
- Pine M et al (2005). Manganese acts centrally to stimulate luteinizing hormone secretion: a potential influence on female pubertal development. *Toxicological Sciences*, 85:880-885.
- Pocock VJ et al (2002). Effects of perinatal octylphenol on ultrasound vocalization, behavior and reproductive physiology in rats. *Physiology and Behaviour*, 76:645-653.
- Poon R et al (1997). Subchronic oral toxicity of di-n-octyl phthalate and di(2-Ethylhexyl) phthalate in the rat. *Food and Chemical Toxicology*, 35:225-239.
- Pop VJ et al (2003). Maternal hypothyroxinaemia during early pregnancy and subsequent child development: a 3-year follow-up study. *Clinical Endocrinology (Oxford)*, 59:282-288.
- Porter MP et al (2005). Hypospadias in Washington State: maternal risk factors and prevalence trends. *Pediatrics*, 115:e495-499.
- Preiksa RT et al (2005). Higher than expected prevalence of congenital cryptorchidism in Lithuania: a study of 1204 boys at birth and 1 year follow-up. *Human Reproduction*, 20:1928-1932.
- Purkey HE et al (2004). Hydroxylated polychlorinated biphenyls selectively bind transthyretin in blood and inhibit amyloidogenesis: rationalizing rodent PCB toxicity. *Chemistry and Biology*, 11:1719-1728.
- Quigley CA et al (1995). Androgen receptor defects: historical, clinical, and molecular perspectives. *Endocrine Reviews*, 16:271-321.

- Rais-Bahrami K et al (2004). Follow-up study of adolescents exposed to di(2-ethylhexyl) phthalate (DEHP) as neonates on extracorporeal membrane oxygenation (ECMO) support. *Environmental Health Perspectives*, 112:1339-1340.
- Rajpert-De Meyts E (2006). Developmental model for the pathogenesis of testicular carcinoma in situ: genetic and environmental aspects. *Human Reproduction Update*, 12:303-323.
- Raman-Wilms L et al (1995). Fetal genital effects of first-trimester sex hormone exposure: a meta-analysis. *Obstetrics and Gynecology*, 85:141-149.
- Rasier G et al (2006). Female sexual maturation and reproduction after prepubertal exposure to estrogens and endocrine disrupting chemicals: A review of rodent and human data. *Molecular and Cellular Endocrinology*, 254-255:187-201.
- Rawlings NC, Cook SJ, Waldbillig D (1998). Effects of the pesticides carbofuran, chlorpyrifos, dimethoate, lindane, triallate, trifluralin, 2,4-D, and pentachlorophenol on the metabolic endocrine and reproductive endocrine system in ewes. *Journal of Toxicology and Environmental Health*, A54:21-36.
- Rey RA et al (2005). Low risk of impaired testicular Sertoli and Leydig cell functions in boys with isolated hypospadias. *Journal of Clinical Endocrinology and Metabolism*, 90:6035-6040.
- Rey RA, Grinspon RP (2011). Normal male sexual differentiation and aetiology of disorders of sex development. *Best Practice and Research: Clinical Endocrinology and Metabolism*, 25:221-238.
- Reynolds EL, Wines JV (1948). Individual differences in physical changes associated with adolescence in girls. *American Journal of Diseases of Children*, 75:329-350.
- Ribas-Fito N et al (2003). Organochlorine compounds and concentrations of thyroid stimulating hormone in newborns. *Occupational and Environmental Medicine*, 60:301-303.
- Rider CV et al (2010). Cumulative effects of in utero administration of mixtures of reproductive toxicants that disrupt common target tissues via diverse mechanisms of toxicity. *International Journal of Andrology*, 33:443-462.
- Rijli FM et al (1995). Cryptorchidism and homeotic transformations of spinal nerves and vertebrae in Hoxa-10 mutant mice. *Proceedings of the National Academy of Sciences of the United States of America*, 92:8185-8189.
- Ritzen EM et al (2007). Nordic consensus on treatment of undescended testes. *Acta Paediatrica*, 96:638-643.

Rivas A et al (2002). Induction of reproductive tract developmental abnormalities in the male rat by lowering androgen production or action in combination with a low dose of diethylstilbestrol: evidence for importance of the androgen-estrogen balance. *Endocrinology*, 143:4797-4808.

Rocheleau CM, Romitti PA, Dennis LK (2009). Pesticides and hypospadias: a meta-analysis. *Journal of Pediatric Urology*, 5:17-24.

Roh J et al (2003). Lack of LGR8 gene mutation in Finnish patients with a family history of cryptorchidism. *Reproductive BioMedicine Online*, 7:400-406.

Ronis MJ et al (1996). Reproductive toxicity and growth effects in rats exposed to lead at different periods during development. *Toxicology and Applied Pharmacology*, 136:361-371.

Ross RK et al (1983). Effect of *in utero* exposure to diethylstilbestrol on age at onset of puberty and on postpubertal hormone levels in boys. *Canadian Medical Association Journal*, 128:1197-1198.

Rozman K et al (1986). Reduced serum thyroid hormone levels in hexachlorobenzene-induced porphyria. *Toxicology Letters*, 30:71-78.

Saiyed H et al (2003). Effect of endosulfan on male reproductive development. *Environmental Health Perspectives*, 111:1958-1962.

Santini F et al (2003). In vitro assay of thyroid disruptors affecting TSH-stimulated adenylate cyclase activity. *Journal of Endocrinological Investigation*, 26:950-955.

Satokata I, Benson G, Maas R (1995). Sexually dimorphic sterility phenotypes in Hoxa10-deficient mice. *Nature*, 374:460-463.

Schell LM et al (2004). Thyroid function in relation to burden of PCBs, p,p'-DDE, HCB, mirex and lead among Akwesasne Mohawk youth: a preliminary study. *Environmental Toxicology and Pharmacology*, 18:91-99.

Schell LM et al (2008). Relationship of thyroid hormone levels to levels of polychlorinated biphenyls, lead, p,p'-DDE, and other toxicants in Akwesasne Mohawk youth. *Environmental Health Perspectives*, 116:806-813.

Schmutzler C et al (2007). The ultraviolet filter benzophenone 2 interferes with the thyroid hormone axis in rats and is a potent in vitro inhibitor of human recombinant thyroid peroxidase. *Endocrinology*, 148:2835-2844.

Schmutzler C et al (2004). Endocrine active compounds affect thyrotropin and thyroid hormone levels in serum as well as endpoints of thyroid hormone action in liver, heart and kidney. *Toxicology*, 205:95-102.

Schnack TH et al (2010a). Familial coaggregation of cryptorchidism, hypospadias, and testicular germ cell cancer: a nationwide cohort study. *Journal of the National Cancer Institute*, 102:187-192.

Schnack TH et al (2010b). Familial coaggregation of cryptorchidism and hypospadias. *Epidemiology*, 21:109-113.

Schuur AG et al (1998a). Inhibition of thyroid hormone sulfation by hydroxylated metabolites of polychlorinated biphenyls. *Chemico-Biological Interactions*, 109:293-297.

Schuur AG et al (1998b). In vitro inhibition of thyroid hormone sulfation by polychlorobiphenyls: isozyme specificity and inhibition kinetics. *Toxicological Sciences*, 45:188-194.

Schuur AG et al (1998c). In vitro inhibition of thyroid hormone sulfation by hydroxylated metabolites of halogenated aromatic hydrocarbons. *Chemical Research in Toxicology*, 11:1075-1081.

Scollon EJ, Carr JA, Cobb GP (2004). The effect of flight, fasting and p,p'-DDT on thyroid hormones and corticosterone in Gambel's white-crowned sparrow, *Zonotrichia leucophrys gambelli*. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 137:179-189.

Scorer CG (1964). The descent of the testis. *Archives of Disease in Childhood*, 39:605-609.

Scott HM, Mason JI, Sharpe RM (2009). Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds. *Endocrine Reviews*, 30:883-925.

Seacat AM et al (2003). Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats. *Toxicology*, 183:117-131.

Seidlova-Wuttke D et al (2006). Comparison of effects of estradiol with those of octylmethoxycinnamate and 4-methylbenzylidene camphor on fat tissue, lipids and pituitary hormones. *Toxicology and Applied Pharmacology*, 214:1-7.

Seiwa C et al (2004). Bisphenol A exerts thyroid-hormone-like effects on mouse oligodendrocyte precursor cells. *Neuroendocrinology*, 80:21-30.

Selevan SG et al (2003). Blood lead concentration and delayed puberty in girls. *New England Journal of Medicine*, 348:1527-1536.

Semiz S et al (2008). Pubertal development of Turkish children. *Journal of Pediatric Endocrinology and Metabolism*, 21:951-961.

Seo BW et al (1995). Effects of gestational and lactational exposure to coplanar polychlorinated biphenyl (PCB) congeners or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on thyroid hormone concentrations in weanling rats. *Toxicology Letters*, 78:253-262.

- Sharlin DS, Bansal R., Zoeller RT (2006). Polychlorinated biphenyls exert selective effects on cellular composition of white matter in a manner inconsistent with thyroid hormone insufficiency. *Endocrinology*, 147:846-858.
- Sharpe RM et al (2003). Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction*, 125:769-784.
- Sharpe RM, Skakkebaek NE (2008). Testicular dysgenesis syndrome: mechanistic insights and potential new downstream effects. *Fertility and Sterility*, 89:e33-38.
- Shen H et al (2006). Enantiomeric ratios as an indicator of exposure processes for persistent pollutants in human placentas. *Chemosphere*, 62:390-395.
- Shen O et al (2009). Comparison of in vitro hormone activities of selected phthalates using reporter gene assays. *Toxicology Letters*, 191:9-14.
- Sherman AI et al (1974). Cervical-vaginal adenosis after in utero exposure to synthetic estrogens. *Obstetrics and Gynecology*, 44:531-545.
- Shimada N, Yamauchi K (2004). Characteristics of 3,5,3'-triiodothyronine (T3)-uptake system of tadpole red blood cells: effect of endocrine-disrupting chemicals on cellular T3 response. *Journal of Endocrinology*, 183, 627-637.
- Simanainen U et al (2004). Pattern of male reproductive system effects after in utero and lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure in three differentially TCDD-sensitive rat lines. *Toxicological Sciences*, 80:101-108.
- Skaare JU et al (2001). Relationships between plasma levels of organochlorines, retinol and thyroid hormones from polar bears (*Ursus maritimus*) at Svalbard. *Journal of Toxicology and Environmental Health, A* 62:227-241.
- Skakkebaek NE, Rajpert-De Meyts E, Main KM (2001). Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Human Reproduction*, 16:972-978.
- Smith JA et al (1989). The relationship between cerebral palsy and cryptorchidism. *Journal of Pediatric Surgery*, 24:1303-1305.
- Song R et al (2008). Effects of fifteen PBDE metabolites, DE71, DE79 and TBBPA on steroidogenesis in the H295R cell line. *Chemosphere*, 71:1888-1894.
- Sormo EG et al (2005). Thyroid hormone status in gray seal (*Halichoerus grypus*) pups from the Baltic Sea and the Atlantic Ocean in relation to organochlorine pollutants. *Environmental Toxicology and Chemistry*, 24:610-616.
- Staessen JA et al (2001). Renal function, cytogenetic measurements and sexual development in adolescents in relation to environmental pollutants: A feasibility study of biomarkers. *Lancet*, 357:1660-1669.

Steuerwald U et al (2000). Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. *Journal of Pediatrics*, 136:599-605.

Stoker TE et al (2005). In vivo and in vitro anti-androgenic effects of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture. *Toxicology and Applied Pharmacology*, 207:78-88.

Stoker TE et al (2004). Assessment of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture, in the EDSP male and female pubertal protocols. *Toxicological Sciences*, 78:144-155.

Su PH et al (2010). Growth and thyroid function in children with *in utero* exposure to dioxin: a 5-year follow-up study. *Pediatric Research*, 67:205-210.

Sugiyama S et al (2005). Detection of thyroid system-disrupting chemicals using in vitro and in vivo screening assays in *Xenopus laevis*. *Toxicological Sciences*, 88:367-374.

Sun H et al (2009). Anti-thyroid hormone activity of bisphenol A, tetrabromobisphenol A and tetrachlorobisphenol A in an improved reporter gene assay. *Toxicology In Vitro*, 23:950-954.

Sun SS et al (2002). National estimates of the timing of sexual maturation and racial differences among US children. *Pediatrics*, 110:911-919.

Suzawa M, Ingraham HA (2008). The herbicide atrazine activates endocrine gene networks via non-steroidal NR5A nuclear receptors in fish and mammalian cells. *PLoS One*, 3:e2117.

Swan SH et al (2005). Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environmental Health Perspectives*, 113:1056-1061.

Sweet RA et al (1974). Study of the incidence of hypospadias in Rochester, Minnesota, 1940-1970, and a case-control comparison of possible etiologic factors. *Mayo Clinic Proceedings*, 49:52-58.

Takser L et al (2005). Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. *Environmental Health Perspectives*, 113:1039-1045.

Tam PP, Liu WK (1985). Gonadal development and fertility of mice treated prenatally with cadmium during the early organogenesis stages. *Teratology*, 32:453-462.

Tena-Sempere M (2010) Neuroendocrinology of puberty: recent milestones and new challenges. *Molecular and Cellular Endocrinology*, 324:1-2.

Thibodeaux JR et al (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations. *Toxicological Sciences*, 74:369-381.

Thong M, Lim C, Fatimah H (1998). Undescended testes: incidence in 1,002 consecutive male infants and outcome at 1 year of age. *Pediatric Surgery International*, 13:37-41.

Thorup J, Cortes D, Petersen BL (2006). The incidence of bilateral cryptorchidism is increased and the fertility potential is reduced in sons born to mothers who have smoked during pregnancy. *Journal of Urology*, 176:734-737.

Toman R, Massányi P, Uhrín V. (2002) Changes in the testis and epididymis of rabbits after an intraperitoneal and peroral administration of cadmium. *Trace Elements and Electrolytes*, 19:114-117.

Tonacchera M et al (2004). Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. *Thyroid*, 14:1012-1019.

Toppari J (2008). Environmental endocrine disrupters. *Sexual Development*, 2:260-267.

Toppari J, Kaleva M, Virtanen HE (2001). Trends in the incidence of cryptorchidism and hypospadias, and methodological limitations of registry-based data. *Human Reproduction Update*, 7:282-286.

Toppari J et al (1996). Male reproductive health and environmental xenoestrogens. *Environmental Health Perspectives*, 104(4):741-803.

Turyk ME, Anderson HA, Persky VW (2007). Relationships of thyroid hormones with polychlorinated biphenyls, dioxins, furans, and DDE in adults. *Environmental Health Perspectives*, 115:1197-1203.

Turyk ME et al (2008). Hormone disruption by PBDEs in adult male sport fish consumers. *Environmental Health Perspectives*, 116:1635-1641.

Ulbrich B, Stahlmann R (2004). Developmental toxicity of polychlorinated biphenyls (PCBs): a systematic review of experimental data. *Archives of Toxicology*, 78:252-268.

Van den Steen E et al (2010). Endocrine disrupting, haematological and biochemical effects of polybrominated diphenyl ethers in a terrestrial songbird, the European starling (*Sturnus vulgaris*). *Science of the Total Environment*, 408:6142-6147.

Van den Berg KJ, Zurcher C, Brouwer A (1988). Effects of 3,4,3',4'-tetrachlorobiphenyl on thyroid function and histology in marmoset monkeys. *Toxicology Letters*, 41:77-86.

van den Berg, KJ (1990). Interaction of chlorinated phenols with thyroxine binding sites of human transthyretin, albumin and thyroid binding globulin. *Chemico-Biological Interactions*, 76:63-75.

van der Plas SA et al (2001). Effects of subchronic exposure to complex mixtures of dioxin-like and non-dioxin-like polyhalogenated aromatic compounds on thyroid hormone and vitamin A levels in female Sprague-Dawley rats. *Toxicological Sciences*, 59:92-100.

van der Zanden et al (2010). Genetics of hypospadias: are single-nucleotide polymorphisms in SRD5A2, ESR1, ESR2, and ATF3 really associated with the malformation? *Journal of Clinical Endocrinology and Metabolism*, 95:2384-2390.

van der Zanden LF et al (2011). Common variants in DGKK are strongly associated with risk of hypospadias. *Nature Genetics*, 43:48-50.

van Raaij JA, Frijters CM, van den Berg KJ (1993a). Hexachlorobenzene-induced hypothyroidism. Involvement of different mechanisms by parent compound and metabolite. *Biochemical Pharmacology*, 46:1385-1391.

van Raaij JA et al (1993b). Increased glucuronidation of thyroid hormone in hexachlorobenzene-treated rats. *Biochemical Pharmacology*, 45:627-631.

Vasiliu O, Muttinemi J, Karmaus W (2004). In utero exposure to organochlorines and age at menarche. *Human Reproduction*, 19:1506-1512.

Verloop J et al (2010). Cancer risk in DES daughters. *Cancer Causes Control*, 21:999-1007.

Viluksela M et al (2004). Tissue-specific effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the activity of 5'-deiodinases I and II in rats. *Toxicology Letters*, 147:133-142.

Virtanen HE et al (2007). Cryptorchidism: classification, prevalence and long-term consequences. *Acta Paediatrica*, 96:611-616.

Virtanen HE et al (2001). The birth rate of hypospadias in the Turku area in Finland. *Apmis*, 109:96-100.

Virtanen HE et al (2006). Mild gestational diabetes as a risk factor for congenital cryptorchidism. *Journal of Clinical Endocrinology and Metabolism*, 91:4862-4865.

Wade MG et al (2002). Thyroid toxicity due to subchronic exposure to a complex mixture of 16 organochlorines, lead, and cadmium. *Toxicological Sciences*, 67:207-218.

Walsh,TJ et al (2007). Prepubertal orchiopexy for cryptorchidism may be associated with lower risk of testicular cancer. *Journal of Urology*, 178:1440-1446.

Wang MH, Baskin LS (2008). Endocrine disruptors, genital development, and hypospadias. *Journal of Andrology*, 29:499-505.

Wang SL et al (2005). *In utero* exposure to dioxins and polychlorinated biphenyls and its relations to thyroid function and growth hormone in newborns. *Environmental Health Perspectives*, 113:1645-1650.

Wang Y et al (2008). Analysis of five single nucleotide polymorphisms in the ESR1 gene in cryptorchidism. *Birth defects research. Part A, Clinical and molecular teratology*, 82:482-485.

Warner M et al (2004). Serum dioxin concentrations and age at menarche. *Environmental Health Perspectives*, 112:1289-1292.

Watanabe M et al (2007). Haplotype analysis of the estrogen receptor 1 gene in male genital and reproductive abnormalities. *Human Reproduction*, 22:1279-1284.

Weidner IS et al (1998). Cryptorchidism and hypospadias in sons of gardeners and farmers. *Environmental Health Perspectives*, 106:793-796.

Weidner IS et al (1999). Risk factors for cryptorchidism and hypospadias. *Journal of Urology*, 161:1606-1609.

Welsh M et al (2008). Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. *Journal of Clinical Investigation*, 118:1479-1490.

Wennerholm UB et al (2000). Incidence of congenital malformations in children born after ICSI. *Human Reproduction*, 15:944-948.

Wikström AM et al (2007). Immunoexpression of androgen receptor and nine markers of maturation in the testes of adolescent boys with Klinefelter syndrome: evidence for degeneration of germ cells at the onset of meiosis. *Journal of Clinical Endocrinology and Metabolism*, 92:714-719.

Wikström AM et al (2004). Klinefelter syndrome in adolescence: onset of puberty is associated with accelerated germ cell depletion. *Journal of Clinical Endocrinology and Metabolism*, 89:2263-2270.

Wilhelm M et al (2008). The Duisburg birth cohort study: influence of the prenatal exposure to PCDD/Fs and dioxin-like PCBs on thyroid hormone status in newborns and neurodevelopment of infants until the age of 24 months. *Mutation Research*, 659:83-92.

Williams PL et al (2010). Blood lead levels and delayed onset of puberty in a longitudinal study of Russian boys. *Pediatrics*, 125:e1088-96.

Willingham E, Baskin LS (2007). Candidate genes and their response to environmental agents in the etiology of hypospadias. *Nature Clinical Practice Urology*, 4:270-279.

Wilson VS et al (2008). Diverse mechanisms of anti-androgen action: impact on male rat reproductive tract development. *International Journal of Andrology*, 31:178-187.

Wohlfahrt-Veje C et al (2009). Acquired cryptorchidism is frequent in infancy and childhood. *International Journal of Andrology*, 32:423-428.

- Wolff MS et al (2008). Environmental exposures and puberty in inner-city girls. *Environmental Research*, 107:393-400.
- Wormke M et al (2003). The aryl hydrocarbon receptor mediates degradation of estrogen receptor alpha through activation of proteasomes. *Molecular and Cellular Biology*, 23:1843-1855.
- Wu T, Mendola P, Buck GM (2002). Ethnic differences in the presence of secondary sex characteristics and menarche among US girls: the Third National Health and Nutrition Examination Survey, 1988-1994. *Pediatrics*, 110:752-757.
- Wu T et al (2003). Blood lead levels and sexual maturation in U.S. girls: The Third National Health and Nutrition Examination Survey, 1988-1994. *Environmental Health Perspectives*, 111:737-741.
- Xu X et al (2007). Perinatal bisphenol A affects the behavior and SRC-1 expression of male pups but does not influence on the thyroid hormone receptors and its responsive gene. *Neuroscience Research*, 58:149-155.
- Yamamoto M et al (2003). Effects of maternal exposure to diethylstilbestrol on the development of the reproductive system and thyroid function in male and female rat offspring. *Journal of Toxicological Sciences*, 28:385-394.
- Yamauchi K et al (2003). Competitive interactions of chlorinated phenol compounds with 3,3',5-triiodothyronine binding to transthyretin: detection of possible thyroid-disrupting chemicals in environmental waste water. *Toxicology and Applied Pharmacology*, 187:110-117.
- Ye X et al (2008). Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: the Generation R study. *Environmental Research*, 108:260-267.
- Yoshida R et al (2005). Association of cryptorchidism with a specific haplotype of the estrogen receptor alpha gene: implication for the susceptibility to estrogenic environmental endocrine disruptors. *Journal of Clinical Endocrinology and Metabolism*, 90:4716-4721.
- You L et al (1998). Impaired male sexual development in perinatal Sprague-Dawley and Long-Evans hooded rats exposed in utero and lactationally to p,p'-DDE. *Toxicological Sciences*, 45:162-173.
- Yu WG, Liu W, Jin YH (2009). Effects of perfluorooctane sulfonate on rat thyroid hormone biosynthesis and metabolism. *Environmental Toxicology and Chemistry*, 28:990-996.
- Yu WG et al (2009). Prenatal and postnatal impact of perfluorooctane sulfonate (PFOS) on rat development: a cross-foster study on chemical burden and thyroid hormone system. *Environmental Science and Technology*, 43:8416-8422.

Yucel S et al (2004). Anatomical studies of the fibroblast growth factor-10 mutant, Sonic Hedge Hog mutant and androgen receptor mutant mouse genital tubercle. *Advances in Experimental Medicine and Biology*, 545:123-148.

Zhang S et al (2009). Reproductive and developmental toxicity of a pentabrominated diphenyl ether mixture, DE-71, to ranch mink (*Mustela vison*) and hazard assessment for wild mink in the Great Lakes region. *Toxicological Sciences*, 110:107-116.

Zhou LX et al (1995). Cytochrome P450 catalyzed covalent binding of methoxychlor to rat hepatic, microsomal iodothyronine 5'-monodeiodinase, type I: does exposure to methoxychlor disrupt thyroid hormone metabolism? *Archives of Biochemistry and Biophysics*, 322:390-394.

Zhou T et al (2001). Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicological Sciences*, 61:76-82.

Zhou T et al (2002). Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. *Toxicological Sciences*, 66:105-116.

Zoeller RT, Bansal R, Parris C (2005). Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. *Endocrinology*, 146:607-612.

Zoeller RT, Dowling AL, Vas AA (2000). Developmental exposure to polychlorinated biphenyls exerts thyroid hormone-like effects on the expression of RC3/neurogranin and myelin basic protein messenger ribonucleic acids in the developing rat brain. *Endocrinology*, 141:181-189.

## PUBLIC HEALTH AND ENVIRONMENT

The present document is a short summary of the current knowledge of the effects of endocrine disruptors 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 disruptors have been elucidated in experimental animals, but there is limited knowledge of the association of human disorders with exposure to endocrine disruptors. 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.

### **Public Health & Environment Department (PHE)**

Health Security & Environment Cluster (HSE)

World Health Organization (WHO)

Avenue Appia 20, CH-1211 Geneva 27, Switzerland

[www.who.int/phe/en/](http://www.who.int/phe/en/)

[www.who.int/ceh](http://www.who.int/ceh)

ISBN 978 92 4 150376 1

