International Journal of Andrology
international journal of andrology ISSN 0105-6263

REVIEW ARTICLE

Hypothesis: exposure to endocrine-disrupting chemicals may interfere with timing of puberty

A. Mouritsen, L. Aksglaede, K. Sørensen, S. Sloth Mogensen, H. Leffers, K. M. Main, H. Frederiksen, A.-M. Andersson, N. E. Skakkebaek and A. Juul

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

Keywords:
adrenarche, endocrine-disrupting chemicals, pubarche, pubertal timing, puberty, sex differences, thelarche

Correspondence:
Annette Mouritsen, University Department of Growth and Reproduction, Rigshospitalet, Blegdamsvej 9, Section 5064, Copenhagen DK-2100, Denmark. E-mail: akm@dadlnet.dk

Received 30 October 2009; revised 29 December 2009; accepted 11 January 2010

doi:10.1111/j.1365-2605.2010.01051.x

Summary

A recent decline in onset of puberty – especially among girls – has been observed, first in the US in the mid-1990s and now also in Europe. The development of breast tissue in girls occurs at a much younger age and the incidence of precocious puberty (PP) is increasing. Genetic factors and increasing prevalence of adiposity may contribute, but environmental factors are also likely to be involved. In particular, the widespread presence of endocrine-disrupting chemicals (EDCs) is suspected to contribute to the trend of earlier pubertal onset. The factors regulating the physiological onset of normal puberty are poorly understood. This hampers investigation of the possible role of environmental influences. There are many types of EDCs. One chemical may have more than one mode of action and the effects may depend on dose and duration of the exposure, as well as the developmental stage of the exposed individual. There may also be a wide range of genetic susceptibility to EDCs. Human exposure scenarios are complex and our knowledge about effects of mixtures of EDCs is limited. Importantly, the consequences of an exposure may not be apparent at the actual time of exposure, but may manifest later in life. Most known EDCs have oestrogenic and/or anti-androgenic actions and only few have androgenic or anti-oestrogenic effects. Thus, it appears plausible that they interfere with normal onset of puberty. The age at menarche has only declined by a few months whereas the age at breast development has declined by 1 year; thus, the time span from initiation of breast development to menarche has increased. This may indicate an oestrogen-like effect without concomitant central activation of the hypothalamic–pituitary axis. The effects may differ between boys and girls, as there are sex differences in age at onset of puberty, hormonal profiles and prevalence of precocius puberty.

Introduction

The earliest physical sign of female puberty is most often the development of a palpable breast bud (thelarche), although the development of pubic hair (pubarche) may sometimes be the first physical sign of puberty in both genders. An increase in testicular volume is most often the first sign of puberty in boys. Age at onset of puberty depends on multiple genetic and environmental factors including psychosocial and socio-economic conditions, nutrition and ethnicity (Parent *et al.*, 2003). The general improvement of living conditions was most likely the major reason for the decline of age at onset of puberty over a period of 100 years until the middle of the twentieth century. From that time point onwards, age at onset of puberty appeared to be stable in most countries (Parent *et al.*, 2003). However, during the last 15 years trends have been noted of a new decline in the US and in Europe (Mul *et al.*, 2001; Euling *et al.*, 2008; Aksglaede *et al.*, 2009), which is not associated by any major change in socio-economic conditions. In addition, several clinics have reported an increase in the number of children referred and treated for precocious puberty (PP; Teilmann *et al.*, 2006; Mul *et al.*, 2002). The factors triggering the physiological onset of puberty are poorly understood (Tena-Sempere, 2009), which hampers investigations into

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the causes of premature maturation. In a search for causes for the significant trends in timing of puberty, we and others have suspected that exposures to endocrine-disrupting chemicals (EDCs) ubiquitously present in food and our environment may play a role. Theoretically, such hormones or substances with hormone-disrupting properties may interfere with pubertal development by actions at different levels, including the neuroendocrine signals, the hypothalamic–pituitary axis, the gonads and peripheral target organs such as breast, hair follicles and genitals.

Activation of the hypothalamic–pituitary–gonadal (HPG) axis at onset of puberty is initiated by changes in hypothalamic expression of several neurotransmitters. An increased expression of kisspeptin in hypothalamic neurons directly activates gonadotropin-releasing hormone (GnRH) neurons through a specific receptor (GPR54/Kiss1R; Tena-Sempere, 2010). It is likely that the activation of the pubertal onset is exerted by functionally interconnected regulatory neurons (Ojeda *et al.*, 2006). In rodents, the predominant locations of kisspeptin neurons in the hypothalamus are at the arcuate (ARC) nucleus and the anteroventral periventricular (AVPV) nucleus. The hypothalamic neurons respond in a contrary manner to regulatory actions of sex steroids; with some inhibition of kisspeptin expression at the ARC, but stimulation at the APVP (Tena-Sempere, 2010).

The HPG axis is transiently activated postnatally with maximum serum hormone levels at approximately 3 months of age. These levels decline to low or undetectable levels when a child is half-a-year old. The biological role of this first activation of the HPG axis remains unknown, but may reflect rebound activation after suppression of the foetal pituitary and hypothalamus by maternal and placental oestrogens during pregnancy. During childhood, the HPG axis is sensitive to negative feedback of oestrogen suggesting that very low levels of oestrogen (or other factors/hormones with oestrogenic activity) are capable of suppressing gonadotropin secretion until puberty where this restraint on the HPG axis is removed. The HPG axis is then reactivated and marks the onset of puberty. The factors responsible for this reactivation are not known, although peripheral factors, for example, leptin, are thought to play a role.

In girls, the increased levels of gonadotropins result in ovarian secretion of androgens from follicle theca cells and oestradiol from granulosa cells. The ovarian oestradiol (E_2) production initiates breast development (thelarche), uterine/endometrial growth and differentiation and epiphyseal maturation.

In boys, the gonadotropins stimulate production of testosterone from the Leydig cells in the testes and proliferation of seminiferous tubules, resulting in testicular enlargement.

The aims of this article were to: (i) review the evidence that age at onset of puberty has declined concurrently with an increase in PP; (ii) review the evidence that exposure to EDCs may contribute to this trend and (iii) also comment on a possible sex difference with regard to the decline in onset of puberty.

Change in timing of puberty and increased frequency of PP

During the past decade, studies from the US [Pediatric Research in Office Settings (PROS) and National Health and Nutrition Examination Survey III (NHANES III)] and Europe have reported earlier on the data related to breast development in girls (Herman-Giddens *et al.*, 1997; Sun *et al.*, 2002; Wu *et al.*, 2002; Chumlea *et al.*, 2003; Castellino *et al.*, 2005; Semiz *et al.*, 2008; Aksglaede *et al.*, 2009), as compared with historical data (Reynolds & Wines, 1948; Foster *et al.*, 1977; Lee, 1980; Nicolson & Hanley, 2000; Juul *et al.*, 2006; Euling *et al.*, 2008). The NHANES III and the PROS studies both reported a lower average age at entering Tanner breast stage 2 (B2) and Tanner pubic hair stage 2 (PH2) than earlier investigations (Table 1). Age at B2 in PROS had declined by approximately 0.6–1.0 year compared to earlier American studies (Reynolds & Wines, 1948; Nicolson & Hanley, 2000; Marshall & Tanner, 1969; African-American girls entering B2 earlier than the white American girls; Herman-Giddens *et al.*, 1997). Thus, the study clearly suggested an earlier age at puberty onset in American girls in the 1980s and 1990s as compared with the 1930s and 1940s. However, age at menarche occurred at the same time (12.9 years of age in PROS) or only 0.3 years earlier (12.6 years of age in NHANES III) as compared with the previous studies. Thus, the time span from breast development to menarche seems to have increased. These findings could reflect that the observed breast development and appearance of pubic hair at an earlier age might have been induced by exogenous factors, which might have influenced the typical sequence of pubertal events. In fact, the earlier onset of thelarche seen among contemporary girls does not seem to be associated with activation of the pituitary–gonadal axis, as seen in the early onset of thelarche in Danish girls, which was not associated to increased leuteinizing hormone (LH) or follicle-stimulating hormone (FSH) levels (Aksglaede *et al.*, 2009).

The NHANES III study has been criticized for assessing breast development by visual inspection rather than palpation. This methodology has an inherent risk of misclassification resulting from fat deposits around the mammary gland, which may be interpreted as mammary tissue (Irwin, 2005). The reported decline in age at

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Table 1 Pubertal development data (average ages) from the US

	Girls (age in years)									Boys (age in years)					
	B2			PH2			Menarche			G2			PH2		
Study	W	B	M	W	B	M	W	B	M	W	B	M	W	B	M
1948 (Reynolds & Wines, 1948)	10.8			11.0			12.9			11.5			12.2		
1953 (Nicolson & Hanley)	10.6			11.6			12.8			11.8					
NHES, 1963–1970 (MacMahon B., 1973)							12.8	12.5							
1969–1974 (Lee, 1980)	11.2			11.9			13.3			11.9			12.3		
Bogalusa, 1973–1974 (Foster *et al.*, 1977)	10.4	10.2		10.9	10.1		12.7	12.8		11.8	11.2		12.5	11.7	
PROS, 1992–1993 (Herman-Giddens *et al.*, 1997)	10.0	8.9		10.5	8.8		12.9	12.2							
NHANES III, 1988–1994 (Sun *et al.*, 2002)	10.4	9.5	9.8	10.6	9.4					10.0	9.2	10.3	12.0	11.2	12.3
NHANES III, 1988–1994 (Wu *et al.*, 2002)	10.3	9.5	9.7	10.6	9.5	10.3	12.6	12.2	12.2						
1995 (Biro *et al.*, 1995)										–			12.8		
NHANES III, 1988–1994 (Herman-Giddens *et al.*, 2001)										10.1	9.5	10.4	12.0	11.2	12.3

NHES, National Health Examination Survey; PROS, Pediatric Research in Office Settings; NHANES III, National Health and Nutrition Examination Survey III; B2, breast stage 2; G2, genital stage 2; PH2, pubic hair stage 2; W, white girls/boys; B, black girls/boys; M, Mexican American boys/girls.

attaining breast development has therefore been subject to much debate. However, the PROS study included palpation and visual assessment in 39% of the participants (Kaplowitz & Oberfield, 1999), and comparisons of findings for those participants indicated no evidence of biased staging when visual assessment alone was performed. Based on these results, an expert panel composed of researchers and clinicians from the US and Europe concluded that the available data for girls were sufficient to suggest a secular trend towards earlier onset of breast development (Euling *et al.*, 2008).

Recent European data support the US findings of a decline in age at pubertal onset (Table 2). An average age at B2 of 10.3 years and an average age at PH2 at 10.4 years were reported in a study of 1638 Italian girls (Castellino *et al.*, 2005). In a study of 1562 Turkish girls, an average age at B2 of 10.2 years, an average age at PH2 at 10.6 years and an average age of menarche at 12.4 years were reported (Semiz *et al.*, 2008). In a recent Danish study, where breast evaluation was supported by palpation, a decline in the age at breast development by 1 year was reported in girls examined during the years 2006–2008 as compared with girls examined in the years 1991–1993 (Juul *et al.*, 2006; Aksglaede *et al.*, 2009). An average age at B2 was 9.88 years and 13.1 years at menarche. Interestingly, the earlier age at developing breast tissue was not correlated to an earlier age of increasing level of gonadotropins (Aksglaede *et al.*, 2009). Other Danish researchers have previously reported earlier menarche among girls during recent decades. A study of age

Table 2 Pubertal development data (average ages) from Europe

	Girls (age in years)			Boys (age in years)		
Study	B2	PH2	Menarche	G2	PH2	TV > 3 mL
The Netherlands, 1965 (Mul *et al.*, 2001)	11.0	11.4[a]	13.4	11.0	11.8[a]	12.0
Denmark (Andersen, 1968)	10.6	11.6			12.8	
United Kingdom, 1969 (Marshall & Tanner, 1969, 1970)	11.2	11.7	13.5	11.6		
Switzerland, 1954–1980 (Largo & Prader, 1983a,b)	10.9	10.4	13.4	11.2	12.2	
Sweden, 1980 (Lindgren, 1996)	10.8	11.2		11.6	12.7	
Denmark, 1991–1993 (Juul *et al.*, 2006)	10.9	11.3	13.4	11.8	11.9	11.9
The Netherlands, 1997 (Fredriks *et al.*, 2000; Mul *et al.*, 2001)	10.7	11.0	13.2	11.5	11.7	11.5
Italy (De Simone *et al.*, 2004)				11.2	11.5	
Italy (Castellino *et al.*, 2005)	10.3	10.4		11.1	11.3	11.2
Turkey (Semiz *et al.*, 2008)	10.2	10.6	12.4			
Denmark, 2006–2008 (Aksglaede *et al.*, 2009)	9.9	11.1	13.1			
Denmark, 2006–2008 (Sorensen *et al.* 2010)				11.6	12.4	11.6

[a]Data extracted from figures.

B2, breast stage 2; G2, genital stage 2; PH2, pubic hair stage 2; TV, testis volume.

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at menarche in the same region of Denmark during 1965–1966 and 1982–1983 revealed a decrease from 13.4 to 13.0 years (Helm & Helm, 1984), although a subsequent study in 1996 from that region demonstrated a halt in the secular trend towards earlier menarche (Helm & Grolund, 1998). In line with the US and European studies, a recently conducted Chinese study reported a decline in the age of breast development. The median age for onset of breast development was 9.2 years, which was the youngest age for breast development ever reported for China (Ma *et al.*, 2009).

Taken together, the American, European and Asian investigations suggest that breast development in girls occurs at a much younger age than the same a few decades ago, irrespective of race. A recent decline in age at menarche has also been reported, but this phenomenon has not been as consistent as the trend in onset of breast development (Herman-Giddens *et al.,* 1997; Sun *et al.*, 2002; Castellino *et al.*, 2005; Semiz *et al.*, 2008; Aksglaede *et al.*, 2009).

Based on available studies in boys, an expert panel recently concluded that US data were inconclusive to suggest a similar secular trend towards earlier puberty in boys (Euling *et al.*, 2008). The European observations on puberty among boys are summarized in Table 2. The pubertal onset, defined as average age at entering Tanners genital stage 2 (G2), occurs at an age between 11.1 and 11.8 years in European boys, which is later than the reported 10.0 years of age in the US (Largo & Prader, 1983b; Lindgren, 1996; Mul *et al.*, 2001; Sun *et al.*, 2002; De Simone *et al.*, 2004; Castellino *et al.,* 2005; Juul *et al.*, 2006; Euling *et al.*, 2008; Sorensen *et al.*, 2010). However, in spite of these data there is evidence that American pre-pubertal boys today are taller at younger ages than previously, suggesting earlier maturity (Herman-Giddens, 2006). As pre-pubertal height and body mass index (BMI) are positively correlated, the observation of taller pre-pubertal boys may reflect a higher BMI than in previous generations, or changes in body composition towards a higher fat mass.

A retrospective study of age at voice break – a late, but characteristic event of male puberty – reported a decrease in age at voice break in 463 Danish choir boys over a recent 10-year period (1994–2003; Juul *et al.*, 2007). This trend was significantly associated with increasing BMI during pre-puberty stage (Juul *et al.*, 2007). In an Italian study of 1858 boys, an average age of 11.1 years at G2, an average age of 11.3 years at PH2 and an average age of 11.2 years at testicular volume (TV) ≥ 4 mL were reported (Castellino *et al.*, 2005). The age at entering G2 declined from 11.8 to 11.6 years and the decline was associated with an increase in BMI (Sorensen *et al.*, 2010). Altogether, these results indicate that the onset of puberty in boys may also be declining, although the trend is not as clear as for girls. This sex difference in trends in timing of puberty is interesting and suggests that the endocrine systems of boys and girls may respond differently to the same exogenous factors (see next).

Precocious puberty

The trend of earlier puberty in the general population is also reflected by an increased incidence of PP (Teilmann *et al.*, 2005). Traditionally, puberty is considered precocious if secondary sex characteristics occur before the age of 8 years in girls and 9 years in boys (Marshall & Tanner, 1969, 1970). Central PP predominantly occurs in girls. The male/female ratio is approximately 1 : 10–20 (Bridges *et al.*, 1994; Speiser *et al.*, 1994; Chemaitilly *et al.*, 2001). Only 10–20% of girls with PP have an organic aetiology (including central nervous system lesions or congenital adrenal hyperplasia; Bridges *et al.*, 1994). However, in most cases, no underlying aetiology of PP can be found. A particularly high frequency of PP was found among foreign adopted girls (Proos *et al.*, 1991; Krstevska-Konstantinova *et al.*, 2001; Teilmann *et al.*, 2009) and to a lesser extent, also among immigrant children (Teilmann *et al.*, 2002). Both adopted and immigrated children experience profound changes in lifestyle. Thus, it is likely that environmental factors must be involved in the aetiology of these cases. A higher level of *p,p′*-dichloro-diphenyl-ethylene (DDE), which is a metabolite of organochlorine pesticide dichloro-diphenyl-trichloroethane (DDT) has been reported in immigrated and adopted children with PP and hypothesized to be a causative agent (Krstevska-Konstantinova *et al.,* 2001).

Delayed puberty

It is common clinical knowledge that boys are more likely than girls to present with delayed puberty. In such cases, a positive family history can often be established (Sedlmeyer & Palmert, 2002; Wehkalampi *et al.*, 2008).

Other causative factors of delayed puberty include malnutrition such as eating disorders, increased energy expenditure as in excessive sports, malabsorption and chronic/recurrent inflammation or infection, chronic anaemia and direct effect on the gonadal axis by radiation or chemotherapy. However, in several cases, no causative factor could be diagnosed. Interestingly, some chemical agents have been associated with delayed puberty (Den Hond *et al.*, 2002) as mentioned in Table 4.

Sex differences

Puberty of girls and boys pose several interesting differences. Girls enter puberty 1–2 years earlier than boys, PP is much more common in girls than in boys (Fechner,

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2002) and delayed puberty is more often seen in boys (Wehkalampi *et al.,* 2008). Theoretically, some of these differences could be resulting from different actions of environmental factors, including EDCs on the two genders. One possible explanation comes from the fact that the combined sum of effects of EDCs in our food and environment seem to be oestrogenic and anti-androgenic. Girls have higher pre-pubertal oestradiol levels than boys and therefore additional environmentally derived oestrogen may have more pronounced effect at the hormone-sensitive organs than in boys.

Evidence for the hypothesis that EDCs may contribute to the timing of puberty

Multiple genetic and environmental factors influence the timing of puberty (Parent *et al.,* 2003). The high correlations of age at menarche within families, and between monozygotic twins as compared with dizygotic twins suggest a strong genetic influence on pubertal timing (heritability factor 50–70%; Kirk *et al.,* 2001; Towne *et al*., 2005; Anderson *et al*., 2007; van den Berg & Boomsma, 2007). No single puberty gene exists as evidenced by large genome-wide association studies (GWAS). However, it is possible that genes, by environment interactions, regulate the timing of puberty and that we have to look for genetic variation in genes not involved in the classical HPA axis. Such genes could involve susceptibility genes of importance for clearance and degradation of hormones as well as of chemicals with hormone-like activity. In a recent study, a correlation between pubertal timing and the genetic variation in the gene *LIN28B*, a potent and specific regulator of microRNA processing, was observed (Ong *et al.,* 2009a).

Many human studies have shown a positive relation between pre-pubertal BMI and onset of late pubertal markers such as peak height velocity or menarche (Hauspie *et al*., 1997). These studies suggest that pubertal timing in both genders may be influenced by body composition (Adair & Gordon-Larsen, 2001; He & Karlberg, 2001; Freedman *et al*., 2002; Anderson *et al*., 2003; Juul *et al*., 2007). Decreased insulin sensitivity as a result of low physical activity and changed dietary habits could also be involved in the observed secular trends (Sorensen *et al*., 2009). In a study of low birth weight (LBW) in girls, the effect of insulin as a major co-determinant of the pubertal tempo and pubertal height gain was also observed. LBW girls who were treated with insulin-sensitizing therapy (metformin) had a leaner body composition, prolongation of the time span from breast development to menarche and prolongation of pubertal growth (Ibanez *et al.,* 2006). However, genetics and obesity alone cannot explain the secular trends, although they may per se increase the sensitivity towards early puberty in concert with other factors, such as EDCs.

Theoretically, hormones or substances with hormone-disrupting capability may interfere with pubertal development by actions at different levels, including the neuroendocrine hypothalamic–pituitary axis, the gonads and peripheral target organs such as breast, hair follicles and genitals. In the brain, EDCs may act by stimulation of oestrogen-sensitive nuclei including hypothalamic neurons thereby releasing kisspeptin and promoting a maturation of the hypothalamus causing earlier onset of puberty or even PP. However, other compounds could act by gonadotropin inhibition through negative feedback. It is also possible that EDCs have direct effects on both body weight and the endocrine system of the HPG axis (Stahlhut *et al.,* 2007). Steroids from the adrenal glands also play a role for normal progression of puberty, including pubic hair development. Potentially, a dysfunction of the adrenal gland caused by EDCs may influence the oestrogenic hormonal milieu and thereby also influence pubertal development (Ulleras *et al.,* 2008).

A potential mechanism of EDC action at the HPG axis has been described in rats (Rasier *et al*., 2007). Animals were exposed to DDT or beta-oestradiol and GnRH pulsatile secretion was increased. Furthermore, an in vitro study showed amplification of the glutamate-evoked secretion of GnRH after exposure to DDT and E2 (Rasier *et al.,* 2008). Neonatal exposure to phyto-oestrogens, bisphenol A and oestradiol benzoate was correlated to early puberty in animals (Patisaul *et al*., 2009). Most evidence suggesting a role of EDCs on pubertal development stems from animal experiments and in vitro studies; for a review of the different modes of action mediated by EDCs relevant for human exposure (Table 3).

A large number of cross-sectional and longitudinal human cohort studies have evaluated the association between pubertal timing and prenatal or current exposure to different chemicals with suspected endocrine actions. Some investigations show effects of EDCs whereas others do not (Table 4). Nevertheless, several human studies support the hypothesis that exogenous compounds may have pronounced clinical effects, especially in pre-pubertal children with low or undetectable endogenous sex hormone levels. These include examples of outbreaks of early puberty in sub-populations in which exposure to exogenous hormones or hormone-like chemicals were strongly suspected. The literature includes the following examples.

- Gynaecomastia was observed in three pre-pubertal boys exposed indirectly through skin contact with their mothers who used strong oestrogen cream. Four months after the mothers discontinued the use of the topical oestrogen preparation, the gynaecomastia regressed and oestradiol levels returned to normal (Felner & White, 2000).

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Table 3 Examples of environmental endocrine-disrupting chemicals grouped according to their assumed actions (non-human studies)

Action	Compound
Oestrogenic	*Genistein, Daidzein* (Zand *et al.*, 2000; Thomsen *et al.*, 2006)
	PCB (Pereira & Rao, 2007)
	Bisphenol A (Doods & Lawson, 1936; Jorgensen *et al.*, 2000)
	Endosulphan (Jorgensen *et al.*, 2000)
	Zeranol (Leffers *et al.*, 2001)
	Brominated flame retardants (BFR; Meerts *et al.*, 2001; Legler, 2008)
	Diethylstilboestrol (McLachlan *et al.*, 2001)
	UV filters (Schlumpf *et al.*, 2001, 2008)
Anti-oestrogenic	*PCBs* (Cooke *et al.*, 2001)
	Prochloraz (Vinggaard *et al.*, 2002)
Androgenic	*Trenbolone* (Henricks *et al.*, 2001)
Anti-androgenic	*Vinclozolin* (Gray *et al.*, 1999a; Eustache *et al.*, 2009)
	DDE (Kelce *et al.*, 1995; Gray *et al.*, 1999b)
	Dioxin (Gray *et al.*, 1997)
	Phthalates (Wilson *et al.*, 2008)
	BFR (Stoker *et al.*, 2005)
Gestagenic	*Melengestrol acetate* (Patterson *et al.*, 1989)
Anti-thyroid	*Phthalates* (O'Connor *et al.*, 2002; Sugiyama *et al.*, 2005)
	PCB (Hallgren & Darnerud, 2002; Gu *et al.*, 2009)
	BFR (Hallgren & Darnerud, 2002;Legler, 2008)
	Dioxin (Butenhoff *et al.*, 2006; Zoeller, 2007)
	The effect of EDCs on thyroid function in general is summarized in Boas *et al.* (2006)
Aromatase-inhibiting	*Phyto-oestrogens* (except genistein; Almstrup *et al.*, 2002)
	Tributyltin (TBT; Oehlmann *et al.*, 2007)
	Prochloraz (Vinggaard *et al.*, 2002)
Interfering with steroid synthesis	*Prochloraz* (Vinggaard *et al.*, 2002)
	PFOA (Biegel *et al.*, 1995)
	Parabens (Taxvig *et al.*, 2008)
	Dibutylphthalate (Thompson *et al.*, 2004)
	DEHP (Gunnarsson *et al.*, 2008; Chauvigne *et al.*, 2009)

PFOA, perfluorooc-tanoic acid; DEHP, di(2-ethylhexyl) phthalate.

- A case of virilization was seen in a 2-year-old boy exposed through skin contact with his father, who used a testosterone cream. The virilization, except penis enlargement diminished after discontinued exposure (Yu *et al.*, 1999).
- An epidemic outburst of premature breast development and ovarian cysts in 2716 girls from Puerto Rico (1990–1995) was suspected to be associated with environmental factors, such as exposure to EDCs (Larriuz-Serrano *et al.*, 2001). Although studies did not find any EDCs explaining the outburst of premature thelarche, some correlations were observed with consumption of soy-based formula, and consumption of various meat products (Freni-Titulaer *et al.*, 1986)
- It was suspected that oestrogen exposure through poultry and beef from the school cafeteria could be the source of early breast development seen in North Italian children of both genders. Breast enlargement was not pronounced and disappeared within 8 months (Scaglioni *et al.*, 1978).
- Gynaecomastia in three pre-pubertal boys was suspected to be caused by oestrogenic and anti-androgenic activities of lavender and tea tree oils. The gynaecomastia resolved shortly after the discontinuance of the use of products containing these oils (Henley *et al.*, 2007).
- Premature thelarche was seen in four pre-pubertal girls after consumption of tea containing a phyto-oestrogen (*Foeniculum vulgare*). The thelarche resolved within 3–6 months after the consumption was stopped (Turkyilmaz *et al.*, 2008).
- High frequency of central precocious puberty (CPP) was observed in a region with high exposure to the oestrogenic mycotoxin zearalenone (Massart *et al.*, 2008).

In addition, few studies have observed a pubertal delay in association with exposure to certain endocrine disrupters. Delayed pubertal development was associated with higher Polychlorinated biphenyl (PCB) exposure in boys and delayed breast development with higher dioxin levels in girls (Den Hond, 2002). Delayed breast development and age at first ejaculation was seen after exposure to PCCD/F (Leijs *et al.*, 2008), and delayed sexual maturation in boys could be related to exposure to endosulphan (Saiyed *et al.*, 2003). Also, exposure to toxic metals like lead and mercury has been associated with delayed puberty (Selevan *et al.*, 2003; Wu *et al.*, 2003; Hauser *et al.*, 2008). This has been thought to reflect their general toxicity, but also endocrine-disrupting properties of these compounds have been suggested. Thus, some indicate effects of lead on the HPG axis (Dearth et al., 2002) and an in vitro study demonstrated effects of PCBs directly on GnRH gene expression indicating a hypothalamic level for endocrine disruption (Gore *et al.*, 2002). A novel mechanism for the EDCs could be a direct effect on GnRH neurons.

Discussion

A possible adverse role of EDCs for adult male (Toppari *et al.*, 1996) and female (Crain *et al.*, 2008) reproductive health has been suggested. This hypothesis has been and is still being investigated in numerous animal and human studies all over the world. It is a plausible hypothesis that these hormone-disrupting agents may also interfere with normal pubertal development (Buck Louis *et al.*, 2008). In particular, onset of puberty is a

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Table 4 Exogenous exposure for endocrine-disrupting chemicals and effect on puberty (human studies)

Compound	Study population	Study area	Methods	Main findings	References
Perinatal exposure					
Dioxin: PCCD/F	18 girls and 15 boys	Amsterdam/Zaandam area	Longitudinal follow-up, concentrations in breast milk	Delayed breast development and age at first ejaculation	(Leijs *et al.*, 2008)
PBBs	327 girls	Michigan food chain contamination	Prospective study, questionnaires, in utero exposure extrapolated from maternal serum levels at the time of the accident	Earlier age at menarche and earlier pubic hair stage in breastfed girls with in utero PBB exposure >7 ng/g serum	(Blanck *et al.*, 2000)
PCBs	196 boys	Faroese birth cohort	Prospective study, clinical and physical examination, concentrations in cord blood	No effect on pubertal stages or testicular volume	(Mol *et al.*, 2002)
PCBs/DDEs	151 girls	Michigan angler cohort of fish-eating mothers with serum DDE levels at the time of pregnancy up to 25 μg/L	Retrospective study, telephone interviews, in utero exposure calculated from maternal serum levels	Reduced age at menarche by 1 year associated with an increase in in utero DDE exposure of 15 μg/L	(Vasiliu *et al.*, 2004)
PCBs/DDEs	316 girls and 278 boys	North Carolina cohort with DDE concentrations up to 4 μg/g fat	Prospective study, mail questionnaires, concentrations in mother's milk and maternal serum	No association with pubertal stages	(Gladen *et al.*, 2000)
PCBs/PCDFs	55 boys	Yucheng	Prospective study, clinical and physical examination, maternal serum levels	Reduced penile length	(Guo *et al.*, 2004)
Pubertal exposure					
Bisphenol A	192 girls	New York, inner-city girls	Cross-sectional study, physical examination, urinary bisphenol A	No effect of bisphenol A	(Wolff *et al.*, 2008)
Zearalenone (Zeranol)	32 CPP 31 controls	Tuscany, Italy	Clinical examination, blood level	Higher levels of zearalenone in girls with PP from one area	(Massart *et al.*, 2008)
Oestrogens	213 boys and 110 girls; controls include: 1434 boys and 366 girls	Italian school	Clinical examination, blood oestrogens	Gynaecomastia in boys and girls	(Scaglioni *et al.*, 1978)
DDE	26 immigrant girls and 15 native Belgian girls	Precocious puberty patients (Belgium)	Patients' study interviews/physical examination, serum measurements	High levels of plasma DDE in immigrant girls as compared with Belgian native controls	(Krstevska-Konstantinova *et al.*, 2001)
DDE	138 girls	Mohawk nation girls aged 10–17 years	Menarche: yes/no	No association	(Denham *et al.*, 2005)
DDT/DDE	466 Chinese female textile workers	Shanghai	Recalled age at menarche	Higher DDT/DDE associated with earlier age at menarche	(Ouyang *et al.*, 2005)
Dioxin	282 girls exposed pre-pubertal	Seveso	Archived serum levels from time of the accident and extrapolated to age at menarche	No effect on age at menarche	(Warner *et al.*, 2004)

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Table 4 (*Continued*)

Compound	Study population	Study area	Methods	Main findings	References
Dioxin	80 boys and 120 girls	One rural and two urban villages in Belgium	Cross-sectional study, physical examination, pubertal serum levels	Retarded breast development associated with higher dioxin levels in girls	(Den Hond *et al.*, 2002)
Endosulphan	117 boys and 90 controls	Indian village with high levels of endosulphan used as pesticide	Cross-sectional study, physical examination, serum levels	Delayed sexual maturation (Tanner stages)	(Saiyed *et al.*, 2003)
PCBs	80 boys and 120 girls	One rural and two urban villages in Belgium	Cross-sectional study, physical examination, pubertal serum levels	Retarded pubertal development associated with higher PCB exposure in boys	(Den Hond *et al.*, 2002)
PCBs	192 girls	New York, inner-city girls	Cross-sectional study, physical examination, blood PCB	No effect of PCB	(Wolff *et al.*, 2008)
Phyto-oestrogens	192 girls	New York, inner-city girls	Cross-sectional study, physical examination, urinary phyto-oestrogen	Phyto-oestrogens associated with delayed breast development	(Wolff *et al.*, 2008)
Phyto-oestrogens	4 girls	Turkey	Physical examination, serum analysis	Use of *Foeniculum vulgare* associated with premature thelarche	(Turkyilmaz *et al.*, 2008)
Lavender and tea tree oil	3 boys		Physical examination, serum analysis	Gynaecomastia after use of lavender and tea tree oil	(Henley *et al.*, 2007)

complex process (Parent *et al.*, 2003) involving a close interplay between centres in the brain, including the hypothalamus, the pituitary gland, the gonads, the adrenals and peripheral steroid receptor target organs of the reproductive systems.

All endocrine organs depend on a delicate endogenous hormonal balance. From a theoretical point of view, this balance may be disturbed by external exposures to agents, which can interact with hormone receptors or interfere with hormone synthesis or metabolism. Notably, there are similarities among the chemical structures of the EDCs and the naturally occurring hormones as illustrated in Fig. 1. Some EDCs have oestrogenic and anti-androgenic properties whereas others may work as aromatase inhibitors (Almstrup *et al.*, 2002). Few EDCs act as androgens (Daxenberger *et al.*, 2001), but increased androgen levels may also result from aromatase-inhibiting effects. Oestrogenic substances may have different adverse health effects depending on the endogenous oestradiol levels of the exposed individual and the specific developmental window at which exposure occurs.

We are all exposed in utero via food, cosmetics, air and indoor climate to numerous agents with hormone-disrupting effects. These chemicals often occur in very low concentrations. However, human exposure is never isolated to one compound but linked to a mixture of many chemicals, which in combination may lead to clinical effects, the so-called cocktail effects (Kortenkamp, 2008). In fact, animal experiments have shown that in utero exposure to mixtures of 3–7 chemicals with anti-androgenic properties, at low doses which individually have no adverse effects, caused major impairment of *masculinization* and the occurrence of hypospadias in the male offspring (Gray *et al.*, 2006; Christiansen *et al.*, 2008).

The role of EDCs relating to reproductive health of humans and animals has received more attention from researchers in environmental sciences, whereas clinical endocrinologists have paid less attention to the concept. Nevertheless, evidence from paediatric practice suggest that administration of extremely small doses of pharmaceutical oestrogen to children can have significant effects on growth probably acting directly at the level of the growth plate (Ross *et al.*, 1983; Lampit *et al.*, 2002; Hochberg *et al.*, 2005).

Research challenges

Research into the possible role of EDCs for pubertal development in humans is challenging for several reasons.
1 We lack basic knowledge about the normal biological mechanisms that control the onset and progression of human puberty, although fascinating new data about the

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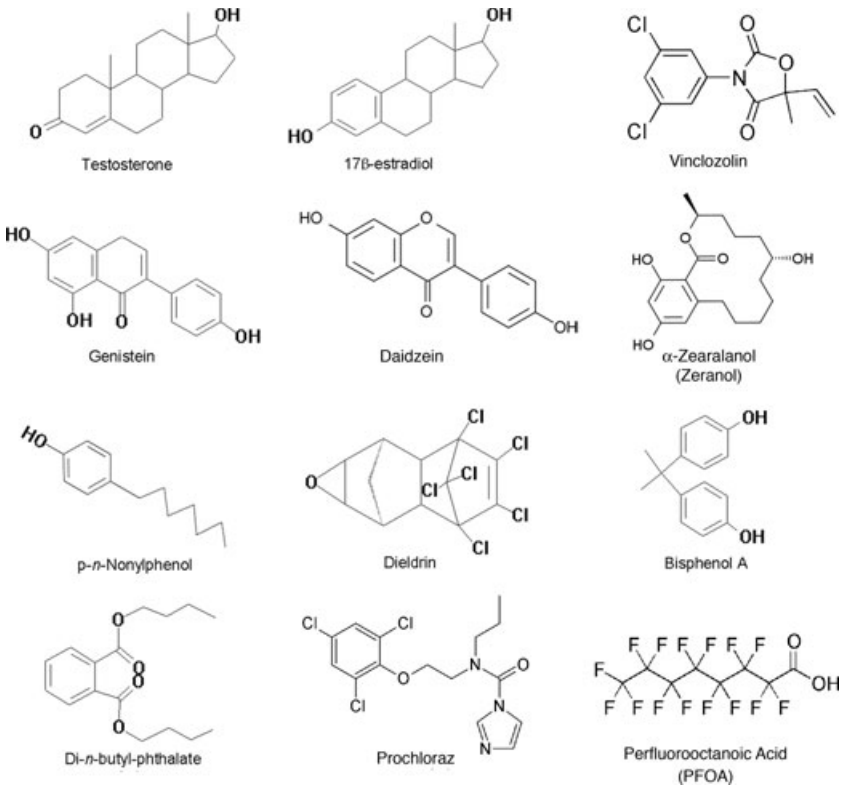

Figure 1 Chemical structure of endogenous steroids (testosterone and oestradiol) as compared to compounds with endocrine-disrupting properties. The compounds are: vinclozolin (androgen receptor antagonist); genistein and daidzein (phyto-oestrogens); α-zearalanol (mycoestrogen, also used as oestrogenic growth promotor in cattle in the US; trade name Zeranol); nonylphenol, dieldrin and bisphenol A (weak environmental oestrogens; dibutylphthalate (DBP, plasticizer); prochloraz (fungicide); and PFOA (surfactant).

role of hypothalamic factors are emerging (Navarro *et al.*, 2009).

2 There are many types of EDCs, some of which have more than one endocrine action, and may only cause effect if present in combination with other chemical compounds or life-style factors. Thus, many phyto-oestrogens are aromatase inhibitors at low concentration but oestrogenic at higher concentrations, resulting in a U-shaped dose–response curve (Almstrup *et al.*, 2002; vom Saal *et al.*, 2007).

3 Some EDCs may act as agonists and others as antagonists, and combinatory effects are difficult to predict.

4 Some EDCs are accumulating in fat tissue and thereby persistent in the body, whereas others are excreted within hours (Diamanti-Kandarakis *et al.*, 2009).

5 Moreover, there can be variable effects of the same EDC resulting from exposure level, the period and the duration of exposure, that is, whether exposure happens during a critical developmental window or not. For example, a prenatal exposure in one trimester might cause an adverse effect without causing any effect in the next trimester (Sharpe, 2006). Furthermore, the consequences of the exposure may not be apparent at the actual time of exposure but may manifest much later in life (Palmer *et al.*, 2009).

6 There may be a wide variation in genetic susceptibility to hormones (Spearow *et al.*, 1999).

7 An effect of exposure resulting in early puberty may be modified by nutritional status, growth and obesity, which may themselves cause early pubertal onset in girls (Ong *et al.*, 2009b).

8 Population studies of current exposures may be confounded by the fact that the previous exposures may have resulted in accumulation of toxic persistent pollutants in body fat. The concentrations of such chemicals may vary substantially between individuals and mask effects of current exposures (Krysiak-Baltyn *et al.*, 2010).

These recent challenges need to be addressed by controlled studies in collaboration between clinical scientists, biologists and epidemiologists.

Conclusion

A marked change in timing of puberty, specifically in girls, has recently been documented in some industrialized countries, and increasing numbers of children are

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admitted to paediatric clinics because of PP (Teilmann *et al.*, 2005). Early onset of puberty has major psychosocial and public health implications and is associated with increased long-term risks of diseases such as obesity, diabetes and cancer. It is therefore urgent to search for explanations for these trends to be able to take preventive measures. As neither genetic factors nor the rise in BMI in our populations can explain the earlier onset of puberty, the reasons for these developments must be sought for in environmental factors, including lifestyle. A hypothesis that EDCs contribute to earlier puberty and more cases of PP appears plausible from animal and in vitro studies as well as from data showing that EDCs are ubiquitously present in our food and environment. We are exposed to these compounds in utero and throughout postnatal life. Most of the EDCs have oestrogenic and/or anti-androgenic actions, whereas few or none have androgenic or anti-oestrogenic actions (Daxenberger *et al.*, 2001). We speculate that this oestrogenic/anti-androgenic overweight of the combined exposures may have strongest puberty-inducing effects in the female gender, where the endogenous pre-pubertal oestradiol levels are higher and effects of EDCs may therefore be more noticeable because they exceed a threshold level for effects.

We urge clinical endocrinologists and paediatricians to join the environmental researchers, already active in this research area for several years. There is strong evidence from clinical observations that administration of extremely small doses of pharmaceutical oestrogen can have significant effects (Hochberg *et al.*, 2005). It stands to reason that 'administration' of oestrogenic EDCs via contamination of our food and surroundings certainly cannot be without adverse effects.

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Panel discussion

Jean-Pierre Bourguignon (Liege, Belgium)

I am interested in the total duration of puberty, not just the timing of menarche but at what time does regular cycling begin and puberty ends? There may be a secular trend for the timing of regular cycling to be getting later and for the duration of puberty getting longer.

Anders Juul (Copenhagen, Denmark)

I am aware of the French core data suggesting that the age of regular menstrual cycling is delayed. Our study is cross-sectional and many of the post-menarchal girls are taking oral contraceptives so that age of natural regular cycling cannot be assessed. Our longitudinal puberty study is ongoing and this may be able to answer your questions when it has been completed.

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