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ABSTRACT

Serono Symposia International convened an expert panel to review the impact of environmental influences on the regulation of pubertal onset and progression while identifying critical data gaps and future research priorities. An expert panel reviewed the literature on endocrine-disrupting chemicals, body size, and puberty. The panel concluded that available experimental animal and human data support a possible role of endocrine-disrupting chemicals and body size in relation to alterations in pubertal onset and progression in boys and girls. Critical data gaps prioritized for future research initiatives include (1) etiologic research that focus on environmentally relevant levels of endocrine-disrupting chemicals and body size in relation to normal puberty as well as its variants, (2) exposure assessment of relevant endocrine-disrupting chemicals during critical windows of human development, and (3) basic research to identify the primary signal(s) for the onset of gonadotropin-releasing hormone-dependent/central puberty and gonadotropin-releasing hormone-independent/peripheral puberty. Prospective studies of couples who are planning pregnancies or pregnant women are needed to capture the continuum of exposures at critical windows while assessing a spectrum of pubertal markers as outcomes. Coupled with comparative species studies, such research may provide insight regarding the causal ordering of events that underlie pubertal onset and progression and their role in the pathway of adult-onset disease.

In light of an increasing body of evidence supporting environmental influences on pubertal onset and progression in contemporary birth cohorts, an expert panel met during and after the Serono Symposia International’s “The Role of Environmental Factors on the Onset and Progression of Puberty” to identify research priorities for delineating the impact of environmental influences on children’s pubertal experiences. An expert review of available animal, clinical, and epidemiologic data was undertaken and synthesized for identifying critical data gaps that are relevant for prioritizing a multidisciplinary research agenda. For purposes of this article, the environment is defined as the sum of all external conditions that affect life, development, and survival of an organism (www.epa.gov/ocepeterm/eterms.html). By extension, an environmental factor is defined as a non-

The views expressed in this article are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency. Mention of trade names of commercial products does not constitute endorsement or recommendation for use. Drs Buck Louis, Gray, and Marcus contributed equally to this work.

Key Words
human puberty, puberty timing, breast development, menarche, pubic hair development, genital development, endocrine disruptors, body fat

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genetic factor; however, for purposes of brevity and consistent with the weight of evidence regarding environmental influences on puberty, this review focuses on endocrine-disrupting chemicals (EDCs) and body size in relation to puberty timing. An in-depth discussion of the literature cited in this article is beyond the scope of this article, but key study aspects are summarized in accompanying tables.

REGULATION OF NORMAL PUBERTY ONSET AND PROGRESSION IN HUMANS AND ANIMALS

Puberty is the period of transition from childhood to adolescence and is marked by the development of secondary sexual characteristics, accelerated growth, behavioral changes, and eventual attainment of reproductive capacity. Available approaches for the assessment of pubertal onset and progression have recently been reviewed, including the use of Tanner stages for female pubertal onset and progression have recently been re- viewed, including the use of Tanner stages for female pubertal onset and progression have recently been re- viewed, including the use of Tanner stages for female pubertal onset and progression have recently been re- viewed, including the use of Tanner stages for female pubertal onset and progression have recently been re- viewed, including the use of Tanner stages for female pubertal onset and progression have recently been re- reviewed, including the use of Tanner stages for female pubertal onset and progression have recently been re- viewed, including the use of Tanner stages for female pubertal onset and progression have recently been re- viewed, including the use of Tanner stages for female pubertal onset and progression have recently been re- viewed, including the use of Tanner stages for female pubertal onset and progression have recently been re- viewed, including the use of Tanner stages for female pubertal onset and progression have recently been reviewed, including the use of Tanner stages for female pubertal onset and progression have recently been reviewed, including the use of Tanner stages for female pubertal onset and progression have recently been reviewed, including the use of Tanner stages for female pubertal onset and progression have recently been reviewed. Menarche is also an important marker used for assessing puberty in girls. Biochemical markers for female puberty onset and progression include assaying elevations in reproductive hormones (eg, luteinizing hormone [LH], follicle-stimulating hormone [FSH], 17β-estradiol, inhibin A and B) before the onset of physical signs (eg, Tanner breast development stage 2). Other matura- tional markers, such as bone age determinations (radiograph of left wrist) and ultrasonographic assessment of ovarian and uterine volume, can be used.

Puberty changes occur as a consequence of the activation of the hypothalamic-pituitary-gonadal (HPG) axis and hypothalamic-pituitary-adrenal (HPA) axis. The HPG axis is under the control of both inhibitory and stimulatory mechanisms, as illustrated in Fig 1. In the human, the HPG is active in the midfetal, neonatal, and early infancy periods but becomes relatively quiescent during childhood. Puberty is marked by the reactivation of the HPG axis manifested by an increase in frequency and amplitude of gonadotropin releasing hormone (GnRH) pulses in the hypothalamus, leading to a rise in pulsatile secretion of the gonadotropins LH and FSH from the anterior pituitary. Gonadotropin release stimulates the gonads. In girls, such maturation of reproductive neuroendocrine function eventually leads to the onset of ovulatory menstrual cycles. Appropriate gonadotropin regulation of ovarian function results in ovarian follicle secretion of ovarian androgens from theca cells and estradiol from granulosa cells before ovulation and secretion of progesterone from the corpus luteum and estradiol after ovulation. In boys, LH initiates the secretion of androgens (testosterone and androstenedione) from the testicular Leydig cells. Such onset of gonadal endocrine function is termed gonadarche. The initial molecular triggers of puberty onset remain unknown, although genetic and environmental factors are sus-pected.

Puberty is also associated with an independent physiologic event, adrenarche, or adrenal activation, that typically occurs between 6 and 8 years of age in both genders. Adrenarche leads to pubarche, the secondary sexual changes including pubic hair development, acne, and body odor. Adrenarche is marked by increased 17α-hydroxylase (17,20 lyase) activity of the P450c17 enzyme and increased cytochrome b activity resulting in increased dehydroepiandrosterone, dehydroepiandrosterone sulfate, and androstenedione production. These initial hormonal increases rise over time, resulting in a cumulative dosage of androgens. Adrenarche is a strictly primate phenomenon. Although little information is available, there is some evidence that adrenarche occurs before puberty in chimpanzees and during the neonatal period in rhesus macaques and baboons.3
Recently, much has been learned about the central mechanisms underlying the initiation of mammalian puberty. Rodents, nonhuman primates, and humans have been and remain the main species used to study the basic components and regulatory hierarchies that control the process of puberty. Traditionally assessed pubertal onset end points in rodents include the age of male preputial separation (PPS), an androgen-mediated event, and, in females, the age of female vaginal opening (VO), an estrogen-mediated event. Age at first estrus is an end point used to assess completion of the pubertal process in females because it occurs the day after the first preovulatory surge of gonadotropins. Some studies have assessed the age of breast cell differentiation events in the mouse and rat. Estrogen-mediated pubertal events that occur in nonhuman female primates include menarche, sex skin swelling and reddening, and nipple growth. Epiphyseal closure and cessation of long bone growth also have been assessed. In male monkeys, increases in testicular volume indicate onset of male puberty and reflect both gonadotropin- and testosterone-mediated events. Measurement of changes in testicular size seems to be a reliable, noninvasive procedure to detect the initiation of puberty in both human and nonhuman primates.

In addition to differences in the time frame, progression, and phenotypic landmarks of puberty, there is a fundamental difference in the neuroendocrine process that controls the initiation of puberty in rodents versus primates. In rodents and sheep, GnRH secretion is maintained at low levels during juvenile development by strong steroid inhibitory control, whereas a similar reduction in GnRH secretion is achieved in primates (including humans) by central mechanisms that operate independent of gonadal regulatory inputs. Thus, data indicate that the peripubertal regulation of the GnRH-releasing system is a more centrally controlled process in primates than in rodents that exhibit “gonadal” control of GnRH secretion. Despite this operational difference, the pubertal activation of GnRH release in rodents and primates is initiated and regulated by similar excitatory and inhibitory pathways, providing input to GnRH neurons (reviewed by Ojeda and Terasawa and Ojeda and Skinner). These inputs include both trans-synaptic and glia-to-neuron communication pathways. As neuronal networks that use excitatory amino acids and the newly discovered kispeptin peptide for neurotransmission or neuromodulation become activated, there is a concomitant reduction in the activity of those neurons using the inhibitory neurotransmitters \( \gamma \) amino butyric acid and opioid peptides for transsynaptic communication. In addition to this neuron-to-neuron flow of information, several trophic molecules have been identified as components of the cell–cell signaling process used by gial cells to regulate GnRH secretion. Prominent among these regulatory systems is a signaling complex that uses epidermal growth factor–like ligands and members of the epidermal growth factor receptor family for glia-to-neuron communication (reviewed by Ojeda et al). Although some gene products that are involved in glia and neuronal communication to regulate puberty onset have been identified, gene(s) upstream that provide the initial trigger of puberty timing remain largely unknown. The integrated use of genomics and proteomics using transgenic rodent models, nonhuman primates, and humans with alterations in the onset of puberty is beginning to uncover some of the common evolutionary conserved molecular components that are used by the neuroendocrine brain to control GnRH secretion and, in turn, the initiation of puberty in both rodents and higher primates. An example is the homeodomain gene TTF1, a transcription factor that was recently shown to be involved in the neuroendocrine control of both primate and rodent puberty. In addition, recent findings suggest that structural remodeling of GnRH neurons may play a key role in the onset of puberty.

Although the initiating trigger(s) for pubertal onset is unknown for rodents, nonhuman primates and humans, earlier molecular markers have been identified, such as the newly described kispeptin-GPR54 regulatory system. Humans who carry mutations of the GPR54 receptor and mice that lack this receptor each fail to reach puberty, indicative that activation of this signaling complex is essential for pubertal onset. Peptides that are produced by peripheral tissues, such as leptin and insulin-like growth factor 1, seem to be more important in maintaining pubertal progression rather than in its initiation despite that insulin-like growth factor 1 has been reported to accelerate pubertal initiation in rodents and monkeys (reviewed by Ojeda and Terasawa).

Understanding the mechanisms underlying the initiation, progression, and regulation of normal human puberty is essential for assessing the effect(s) of EDCs on human development. With limited empirical population-based data, it is important to consider other sources of data, such as those that arise from clinical populations. Although external validity may be limited from these data sources, useful information about potential cause is possible.

**CLUES FROM CLINICAL RESEARCH THAT SUPPORT ENVIRONMENTAL INFLUENCES**

A number of clinical conditions offer clues regarding the influence on environmental factors, such as nutrition and body size, on puberty. In fact, a recent clinical report attributed topical application of lavender and tea tree oils to gynecomastia in prepubescent boys on the basis of in vitro evidence supporting their weak estrogenic and anti-androgenic activities. A brief overview of environmental influences follows in relation to precocious puberty, delayed puberty, and polycystic ovary syndrome (PCOS).

**Precocious Puberty**

Precocious and delayed puberty in humans can have a genetic and/or environmental cause. Precocious puberty is defined herein as the development of secondary sexual characteristics before 8 years of age in girls and boys. Precocious puberty can be classified into “central,” or gonadotropin-dependent, precocious puberty; “peripheral,” or gonadotropin-independent, precocious puberty; or...
combined. Examples of genetic forms of peripheral precocious puberty are McCune Albright syndrome in girls and testotoxicosis (or familial male precocious puberty) in boys.

Premature thelarche and premature adrenarche may be regarded as partial forms of precocious puberty, in which the full spectrum of pubertal changes is absent and only specific changes occur. Premature thelarche is usually slowly progressing or even self-limiting but can occasionally transform into overt central precocious puberty. Although not precocious puberty, per se, premature breast development and gynecomastia after exposure to estrogen-containing products have been reported in girls and boys, respectively, with reversal on cessation of the exposure.

Pubertal Delay

Delayed puberty, defined as the absence of breast development by 13 years of age in girls or testicular volume <4 mL by 14 years of age in boys, can occur as a result of primary hypothalamic-pituitary dysfunction or primary gonadal failure. The most common cause of pubertal delay is constitutional, representing a transient delay in sexual development that is a variant of normal. Permanent hypogonadotropic hypogonadism may occur as an isolated hypothalamic-pituitary deficiency (eg, Kallman’s with or without cleft lip and/or palate) and may be associated with a variety of molecular genetic defects. All are congenital anomalies that are associated with multiple pituitary hormone deficiencies, not just those that affect puberty onset. Not all cases of delayed puberty have a known genetic cause, such as those that arise from head trauma or infection. Primary gonadal failure is associated with hypergonadotropism and is caused by numerous congenital or acquired disorders with a genetic (eg, gonadal dysgenesis, Klinefelter syndrome, molecular genetic defects of the steroidogenic pathway, galactosemia) or environmental cause (eg, gonadotoxic chemotherapy [alkylating chemotherapy impairs spermatogenesis but rarely affects Leydig cell function], radiotherapy, surgery). Autoimmune disorders can cause delayed puberty through 2 different mechanisms: hypogonadotropic hypogonadism as a result of hypophysitis or primary gonadal failure as a result of autoimmune gonadal destruction.

Polycystic Ovary Syndrome

PCOS is a common heterogeneous disorder that affects 5% to 10% of reproductive-aged women. Insulin resistance/hyperinsulinemia occurs in 50% to 60% of PCOS-affected women compared with a prevalence of 10% to 25% in the general population. Criteria for diagnosing PCOS vary and were defined by the 1990 National Institutes of Health–National Institute of Child Health and Human Development conference as including chronic anovulation and hyperandrogenism (including acne, hirsutism, and male pattern baldness) after exclusion of other disorders, such as congenital adrenal hyperplasia (CAH), hypercortisolism, and hyperprolactinemia. The more recent “Rotterdam criteria” for PCOS expanded the National Institutes of Health definition to include 2 of 3 of the following: (1) clinical or biochemical evidence of hyperandrogenism; (2) intermittent or absent menstrual cycles; and (3) polycystic ovary morphology as visualized by ultrasound.

Puberty timing is altered in some girls with PCOS, dependent, in part, on androgen secretion/exposure and nutritional status. Premature pubarche has been reported among some but not all girls with PCOS and is believed to arise from increased adrenal androgen secretion secondary to premature adrenal pubertal maturation in the absence of other known causes (eg, CAH, androgen-secreting tumors, Cushing syndrome). Androgen concentrations are elevated for chronologic age but appropriate for the stage of pubic hair development. Skeletal maturation may be normal or slightly advanced. The presence of insulin resistance/hyperinsulinemia, dyslipidemia, exaggerated GnRH-stimulated ovarian thecal cell androgen secretion in the early stages of puberty, and subsequent development of hyperandrogenic chronic anovulation during the later stages of puberty suggest that premature pubarche is related to the development of PCOS. In addition, the age at menarche is typically ~6 months earlier in girls with PCOS than in unaffected girls.

The relation between PCOS and adult-onset diseases is now recognized. For example, girls and women with PCOS are at increased risk for developing impaired glucose tolerance (IGT) and type 2 diabetes in comparison with unaffected women; however, the clinical manifestations of hyperandrogenemia (anovulation, hirsutism, acne, and androgenic alopecia) differentiate PCOS from type 2 diabetes and obesity. Obese adolescent girls with PCOS have greater insulin resistance than obese girls without PCOS. In PCOS, IGT, and type 2 diabetes, impaired metabolic activity leads to suppression of insulin-mediated glucose uptake and lipolysis, leading to compensatory hyperinsulinemia.

Potential mechanisms underlying PCOS include (1) early origins or in utero programming stemming from exposures during critical windows of development, (2) LH hypersecretion, (3) primary ovarian abnormality, (4) dysregulation of fat metabolism and adipogenesis, and/or (5) dysregulation of the inflammatory axis. Data arising from girls who present clinically with CAH and nonhuman female primates that are exposed in utero to androgens are suggestive of prenatal programming of gonadotropin secretory dynamics and PCOS. Women with CAH and PCOS have accelerated episodic release of LH and increased hypothalamic release of GnRH.

The persistence of the hyperandrogenic phenotype in cultured theca cells derived from women with PCOS suggests the possibility of a primary ovarian abnormality in PCOS possibly with an in utero origin. Investigation into the mechanism(s) responsible for insulin resistance in type 2 diabetes has suggested that abnormalities in fatty acid uptake and use lead to increased plasma free fatty acid concentrations followed by ectopic fat storage in skeletal muscle and liver; this aberrant fat storage (lipotoxicity) alters insulin signaling, insulin metabo-
lished, and pancreatic β-cell insulin secretion, leading to the development of insulin resistance with compensatory hyperinsulinemia. Oxidative stress may also contribute to the development of insulin resistance. Activation of the NF-κB/IRak-β/IKK-β pathway, a major pathway for inflammatory processes, by free fatty acid provides a potential link among coronary artery disease, type 2 diabetes, insulin resistance, and PCOS. Familial clustering of IGT, type 2 diabetes, and PCOS suggests a role for genetic determinants of this metabolic phenotype or in utero events such as decrements in fetal growth as measured by reduced birth size. The heterogeneity of PCOS may account for the lack of candidate genes identified to date.

**ENVIRONMENTAL INFLUENCES ON PUBERTY**

Consistent with our definition, environmental factors are defined to include both EDCs and body shape and adiposity. Consistent with the focus of the workshop, this review focuses on single chemicals by mode of action. A more comprehensive review describing the toxicology of puberty in rodents is found elsewhere.

Last, this review urges caution in assessing an individual pubertal marker given the highly interrelated and time-dependent nature of pubertal onset and progression as orchestrated by gonadal hormones under the control of gonadotropins. In the presence of exogenous endocrine-active agents, pubertal markers can be independently induced or blocked so that they are not necessarily in synchrony with centrally regulated pubertal maturation. For example, peripubertal exposure to the gonadotropin agonist Lupron and the estrogenic agent diethylstilbestrol both delayed menarche in monkeys, but other markers of puberty were delayed or accelerated depending on the agent. Therefore, the relative timing of all puberty-onset markers is the most informative, which argues for a complete pubertal assessment and related deviations in lieu of extreme categorization such as precocious puberty.

**Animal Studies**

A summary of selected examples of single environmental chemicals whose exposure at specific dosages may lead to puberty-timing alterations is presented in Table 1. This table is organized by mode of action (MOA). Estrogen receptor (ER) agonists (also called estrogen agonists or estrogens) and ER antagonists compete with the endogenous estrogen hormones estradiol and estrone for binding to the classical ER. Such agonists and antagonists bind to the ER and activate or repress, respectively, transcription of ER-dependent genes and thereby enhance or diminish estrogenic activity in vivo (eg, induce uterine weight increase/decrease, accelerate/delay puberty). After in utero exposure to a potent estrogen agonist, gross reproductive malformations may result in female rats, presumably by altering the development of reproductive organs that are responsive to estrogen action. Examples of estrogen agonists that are present as environmental contaminants include 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), a metabolite of the pesticide chemical methoxychlor, and some natural products such as isoflavonoids, flavonoids, stilbenes, lignans, equol, and phytoestrogens (eg, genistein, daidzein, coumestrol, 8-prenylarigenin, resveratrol). Gestational and lactational maternal oral exposure to dosages as low as 25 mg/kg per day methoxychlor resulted in an earlier age of VO and first estrus in the female rat. This was found to be pseudo-precocious rather than true precocious puberty because VO was accelerated but the onset of estrous cyclicity was not affected. Similarly, prenatal subcutaneous injections of BPA, genistein, resveratrol, zearalenone, or DES accelerated VO in mice. Although prenatal oral exposure to BPA did not significantly accelerate the age at VO or first estrus in either the rat or mouse, prenatal oral treatment of mice with BPA significantly reduced the number of days between VO and first vaginal estrus. When female rats were exposed after weaning throughout puberty to xenoestrogens like methoxychlor or 17 beta estradiol, early puberty onset was induced as measured by age at VO in the female rat. In addition, prenatal BPA exposure resulted in altered maturation of the mammary gland at puberty and increased sensitivity to endogenous estrogens.

Androgen receptor (AR) antagonists (also called androgen antagonists) compete with natural hormones testosterone and dihydrotestosterone for binding to the AR. Thus, a decrease in binding between the natural ligand and receptor occurs, which in turn inhibits AR-dependent gene expression in vivo. AR antagonist exposure in vivo can induce malformations in male reproductive tract and delay puberty in the male rat. AR antagonists include insecticides, herbicides, fungicides, toxic chemicals, and combustion byproducts. HPTE has also been shown to act as an AR antagonist. Peripubertal exposure to the AR antagonists methoxychlor or vinclozolin led to a delayed puberty in males as measured by the age of PPS in the rat.

Conversely, AR agonists (also called androgen agonists) compete with the endogenous hormones testosterone and dihydrotestosterone for AR, leading to an increase in AR-DNA binding in vitro and AR-dependent gene expression in vivo. Such environmental androgen contaminants induce malformations in the female reproductive tract and delay puberty in the rat. AR agonists include insecticides, herbicides, fungicides, toxic chemicals, and combustion byproducts. HPTE has also been shown to act as an AR agonist. Peripubertal exposure to the AR agonists methoxychlor or vinclozolin led to a delayed puberty in males as measured by the age of PPS in the rat.

Other receptors, including orphan receptors, are the targets of certain environmental agents. The environmental chemical 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has 1 of the best-characterized MOAs, binding to the aryl hydrocarbon (Ah) receptor that in turn initiates Ah-dependent gene expression changes. Even low dosages of TCDD in utero can lead to gene expression changes and malformations in the reproductive tract in rodents. Ah receptor ligands other than TCDD include other polychlorinated dibenzodioxins and some polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers, and pesticide contaminants as well as the pesticide methoprene, a juvenile hormone agonist. Exposure to TCDD in utero caused delayed puberty in male and female rats as measured by an older age at acquisition of PPS and VO.
respectively,\textsuperscript{64-67} and a delayed or incomplete differentiation of mammary epithelium.\textsuperscript{8}

Some environmental chemicals affect puberty via MOAs that are involved in the central nervous system/HPG axis, altering brain, hypothalamic, and/or pituitary function via direct interactions with the central nervous system neuroendocrine function. These include the pesticides thiram, molinate, metam sodium, chloridimeform, amitraz, triazoles, dichloracetic acid, atrazine, propazine, simazine, methanol, and linuron. Prepubertal exposure to atrazine causes delayed puberty in the male and the female, as evidenced by an older age at acquisition of PPS\textsuperscript{64,69} and VO,\textsuperscript{69} respectively. The modes of action for atrazine’s reproductive effects include puberty timing and a reduction in circulating LH and prolactin.\textsuperscript{70}

Perinatal exposure of mice to BPA results in a decrease of tricruetic preoptic area at 22 to 24 days of age (prepubertal), an important brain region that regulates estrous cyclicity and estrogen-positive feedback.\textsuperscript{71}

Chemicals that inhibit the synthesis of endogenous hormones (e.g., testosterone, 17\beta-estradiol, adrenal steroids) typically do so by competitively inhibiting the activity of \( \geq 1 \) P450 steroidogenic enzymes. The predominant effects are mediated through the steroidogenic enzymes cholesterol side chain cleavage enzyme, steroid 17,20 lyase, and aromatase (which converts androgens to estrogens). Some affect estrogen synthesis preferentially, while others affect adrenal and liver function. Examples of environmental and pharmaceutical aromatase inhibitors include conazole and imadazoles; fungicides (e.g., prochloraz, fenarimol, ketoconazole, fadrozole). Peripubertal exposure to the aromatase inhibitor fadrozole at dosages as

<table>
<thead>
<tr>
<th>MOA</th>
<th>Chemical</th>
<th>Species, Gender</th>
<th>Treatment Interval</th>
<th>Effects (at Dosages)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER agonist</td>
<td>E2</td>
<td>Rat, female</td>
<td>Peripubertal</td>
<td>Early VO (2 and 4 mg/kg per d)</td>
<td>Marty et al\textsuperscript{66} (1999)</td>
</tr>
<tr>
<td>ER agonist</td>
<td>DES</td>
<td>Rhesus monkey, female</td>
<td>Peripubertal</td>
<td>Early sex skin swelling and reddening; delayed nipple growth; completely suppressed menarche; increased serum estrogen activity (0.5 mg/kg per d)</td>
<td>Munoz de Toro\textsuperscript{124} (2005)</td>
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<tr>
<td>ER agonist</td>
<td>BPA</td>
<td>Mice, female</td>
<td>In utero</td>
<td>Increased lateral branching at 3 mo (25 and 250 mg/kg per d)</td>
<td>Markey et al\textsuperscript{12} (2001)</td>
</tr>
<tr>
<td>ER agonist</td>
<td>BPA</td>
<td>Mice, female</td>
<td>In utero</td>
<td>Pregnancy stage–like mammary gland differentiation (25 and 250 mg/kg) at PND 10, 1 mo, 6 mo</td>
<td>Howdeshell et al\textsuperscript{14} (1999)</td>
</tr>
<tr>
<td>ER agonist</td>
<td>BPA</td>
<td>Mice, female</td>
<td>In utero</td>
<td>Reduced interval between VO and first vaginal estrus (dose)</td>
<td>Gray et al\textsuperscript{62} (1989)</td>
</tr>
<tr>
<td>ER agonist and AR antagonist</td>
<td>Methoxychlor</td>
<td>Rat, female and male</td>
<td>Peripubertal</td>
<td>Female: early VO and first estrus (25, 50, 100, 200 mg/kg per d); VO was accelerated, but first estrus was not; F1 exposed in utero and lactation but not directly; VO acceleration noted Male: delayed PPS (100 and 200 mg/kg per d)</td>
<td>Marty et al\textsuperscript{66} (1999)</td>
</tr>
<tr>
<td>AR antagonist</td>
<td>Methoxychlor</td>
<td>Rhesus monkey, female</td>
<td>Peripubertal</td>
<td>Early sex skin swelling and reddening; delayed nipple growth and menarche; increased serum estrogen activity (25 or 50 mg/kg per d)</td>
<td>Golub et al\textsuperscript{66} (2003)</td>
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<tr>
<td>AR antagonist</td>
<td>Vinclozolin</td>
<td>Rat, male</td>
<td>Peripubertal</td>
<td>Delayed PPS (30 and 100 mg/kg per d)</td>
<td>Monosson et al\textsuperscript{66} (1999)</td>
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<tr>
<td>AR antagonist</td>
<td>p,p\textsuperscript{-}DDE</td>
<td>Rat, male</td>
<td>Peripubertal (PND 22–55)</td>
<td>Delayed PPS at 100 mg/kg per d</td>
<td>Kelce et al\textsuperscript{14} (1995)</td>
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<tr>
<td>AR antagonist</td>
<td>Ah agonist</td>
<td>p,p\textsuperscript{-}DDE</td>
<td>Rat, male</td>
<td>Peripubertal (PND 22–55)</td>
<td>Delayed VO and decreased/incomplete mammary epithelial differentiation (0.8–1.0 ( \mu )g/kg per d)</td>
</tr>
<tr>
<td>Aromatase inhibitor</td>
<td>Fadrozole</td>
<td>Rat, female</td>
<td>Peripubertal</td>
<td>Delayed VO (0.6, 1.2, and 6.0 mg/kg per d)</td>
<td>Markey et al\textsuperscript{12} (2001)</td>
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<tr>
<td>Steroidogenesis inhibitor</td>
<td>Ketonozacol</td>
<td>Rat, female</td>
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<td>Delayed VO (100 mg/kg per d)</td>
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<tr>
<td>Steroidogenesis inhibitor</td>
<td>Prochloraz</td>
<td>Rat, male</td>
<td>Peripubertal</td>
<td>Delayed PPS (125 mg/kg per d)</td>
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<td>CNS-HPG, reduces LH and prolactin</td>
<td>Atrazine</td>
<td>Rat, female</td>
<td>Peripubertal</td>
<td>Delayed PPS (12.5, 50, 100, 150, 200 mg/kg per d)</td>
<td>Markey et al\textsuperscript{12} (2001)</td>
</tr>
<tr>
<td>CNS-HPG, reduces LH and prolactin</td>
<td>Atrazine</td>
<td>Rat, female</td>
<td>Peripubertal</td>
<td>Delayed VO (50, 100 and 200 mg/kg per d)</td>
<td>Markey et al\textsuperscript{12} (1999)</td>
</tr>
<tr>
<td>GnRH agonist</td>
<td>Leuprolide acetate</td>
<td>Rhesus monkey, female</td>
<td>Peripubertal</td>
<td>Blocked menarche; delayed body growth (weight and height); decreased muscle mass; increased serum IGF-1 (0.75 mg/kg per month)</td>
<td>Golub et al\textsuperscript{66} (1999)</td>
</tr>
</tbody>
</table>
low as 0.6 mg/kg per day led to a delayed VO in female rats. Peripubertal exposure to 100 mg/kg per day of the steroidogenesis inhibitor ketoconazole also led to a delayed VO. The MOA of prochloraz is the inhibition of aromatase and 17,20 lyase apparently directly inhibiting steroidogenesis of estrogens and androgens. Prochloraz also delays puberty in male rats. The MOA of many phthalates is to inhibit fetal testis Leydig cell steroidogenesis and insulin synthesis, leading to male developmental and reproductive system malformations (eg, retained nipples, reduced or absent reproductive organs, undescended testes, hypospadias, delayed PPS) with in utero exposure and delays in puberty with peripubertal exposure. Administration of phthalates during puberty to marmosets induced ovarian and uterine alterations in females and reduced peripubertal testosterone levels in males; however, the effect on androgen levels was not significant because of the extreme variability in this measure in this species.

Human Studies

Many human studies have shown a positive relation between body fat, primarily measured by BMI or skin-fold thickness, and onset of puberty, as determined by the onset of the growth spurt, breast development, or pubic hair maturation. Body size measures also have been associated with early puberty and physical activity exposures and pubertal timing. Environmental exposure to persistent halogenated organic chemicals such as PCBs, dichlorodiphenyltrichloroethane/dichlorodiphenyl dichloroethylene (DDT/DDE), and brominated flame retardants have been associated with pubertal alterations in females and reduced peripubertal testosterone levels in males; however, the effect on androgen levels was not significant because of the extreme variability in this measure in this species.

Various studies have evaluated pubertal development among children who were exposed to DDT and its primary metabolite DDE. Two studies specifically assessed developmental exposure (in utero and/or postnatal via lactation) within a cohort. Gladen et al conducted a prospective cohort study of boys and girls who resided in North Carolina in relation to DDE concentrations that were previously measured in the mothers’ serum, cord serum, and the placenta (averaged for in utero exposure). Concentrations in breast milk also were determined (lactational exposure). Puberty-timing measures, Tanner stages (boys and girls), and menarche were assessed by annual questionnaires. For boys, no association was observed for either in utero or lactational DDE exposure. Among girls, there was no association with age at menarche; however, there was a suggestion of an association of higher in utero or lactational exposure and earlier breast and pubic hair development that was not statistically significant. Vasiliu et al also evaluated in utero exposure to DDT/DDE that was estimated using a decay model based on maternal measurements among daughters of a Michigan angler cohort. The authors observed a significantly earlier menarche among girls with an increased in utero exposure. Specifically, menarche was 1 year earlier for every 15-µg/L increase in in utero exposure. No association was seen with breastfeeding and age at menarche.

Several additional studies evaluated DDT/DDE exposure in childhood using various designs. Krstevska-Konstantinova et al measured DDE concentration among girls who were born in Belgium compared with those who were foreign-born. The foreign-born girls had significantly higher levels of DDE that were correlated with age at immigration and time since immigration than native-born girls. Other migratory effects have been seen in other populations. They also estimated the incidence of precocious puberty among native Belgium girls and foreign adopted girls from vital records and adoption registries and found the rate of precocious puberty to be 80 times higher than that for native-born Belgium girls. Although suggestive, this preliminary study was limited in size and scope, especially with regard to other differences unaccounted for between the 2 groups of children. Ouyang et al studied newly married female textile workers in China by measuring serum DDT/DDE concentration. Women were asked to recall age at menarche. A significant dose-response relation was found between serum DDT/DDE concentrations and earlier menarche. A 10 ng/g increase in exposure was associated with 0.2 years earlier onset of menarche. Because exposure occurred after pubertal development was complete, the causal order of exposure-puberty cannot be established because pubertal development may have affected the metabolism/distribution of DDT or other behaviors that influenced DDT exposure. Denham et al conducted a cross-sectional study among girls who were aged 10 to 17 years and resided in the Mohawk Nation...
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<th>Chemical Exposure (Biospecimen)</th>
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<td>US, Michigan girls aged 5–24, accidental in utero and/or lactational exposure (327)</td>
<td>CS/C, assessment of Tanner stages in female offspring; recalled age at menarche in postmenarchal offspring</td>
<td>Earlier menarche and pubic hair development among girls highly exposed in utero and breastfed; no association with breast development</td>
<td>Blanck et al95 (2000)</td>
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<td>DDE (maternal blood, cord blood, and placenta averaged; breast milk)</td>
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<td>C; assessed menarche, Tanner stages by annual questionnaire</td>
<td>Boys: no association; girls: suggestion of earlier breast and pubic hair development with high transplacental and with high lactational exposure</td>
<td>Gladen et al93 (2000)</td>
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<td>DDE (serum)</td>
<td>Girls with precocious puberty: foreign-born girls (26); Belgium girls (15)</td>
<td>CS; serum DDE compared between foreign-born and Belgium girls; all had precocious puberty</td>
<td>Foreign-born girls with precocious puberty had significantly higher DDE concentration than native Belgium girls with precocious puberty</td>
<td>Krstevska-Konstantinova et al99 (2001)</td>
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<td>DDT/DDE (serum in adulthood)</td>
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<td>Denham et al96 (2005)</td>
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<td>Dioxin-like activity (serum, CALUX)</td>
<td>17-y-old Belgium boys (78) and girls (120) from polluted and nonpolluted areas</td>
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<td>Boys: no association; girls: girls with high exposure were less likely to have reached the highest stage of breast development; no association with pubic hair development or age at menarche.</td>
<td>Den Hond et al106 (2002)</td>
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<td>Dioxin (serum)</td>
<td>Seveso, Italy, women exposed postnatally and prepuberty to dioxin industrial accident (282)</td>
<td>C; recalled age at menarche</td>
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<td>Endosulfan</td>
<td>India, boys aged 10–17 in exposed (117) and unexposed (90) areas</td>
<td>C/CS; Tanner stage by physical examination; serum hormones and endosulfan levels for 70 exposed, 45 unexposed.</td>
<td>Exposed boys were less mature and had lower testosterone and higher LH than unexposed boys</td>
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<td>HCB (serum)</td>
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<td>Lead (serum)</td>
<td>US 8- to 16-y-old girls NHANES III (2186)</td>
<td>CS, assessed age at menarche and Tanner stages; analyses stratified by ethnic groups</td>
<td>Later pubic hair and breast development associated with higher lead among blacks and Mexican Americans, suggested association among non-Hispanic whites; later menarche associated with higher lead among blacks, suggested association among Mexican Americans and non-Hispanic whites</td>
<td>Selevan et al112 (2003)</td>
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<td>Lead (serum)</td>
<td>US 8- to 16-y-old girls NHANES III (1706)</td>
<td>CS, assessed age at menarche and Tanner stages</td>
<td>Later menarche and pubic hair development with higher lead exposure; no association with breast development</td>
<td>Wu et al113 (2003)</td>
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<tr>
<td>Lead (serum)</td>
<td>US/Canada Mohawk Nation, girls 10–17 (138)</td>
<td>CS; menarche (yes/no)</td>
<td>Exposure to lead associated with lower likelihood of menarche</td>
<td>Denham et al102 (2005)</td>
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TABLE 2  Continued

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<td>PCBs (serum)</td>
<td>US/Canada Mohawk Nation, girls 10–17 (138)</td>
<td>CS; menarche (yes/no)</td>
<td>4 potentially estrogenic PCB congeners (52, 70, 101+90, and 187) significantly associated with a greater probability of having reached menarche</td>
<td>Denham et al (2005)</td>
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<td>PCBs (maternal blood, cord blood, and placenta averaged; breast milk)</td>
<td>US, North Carolina boys and girls (594)</td>
<td>C; assessed menarche, Tanner stages by annual questionnaire</td>
<td>Boys: no association; girls: suggestion of earlier breast and pubic hair development with high transplacental, suggestion of earlier pubic hair development with lactational exposure</td>
<td>Gladen et al (2000)</td>
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<tr>
<td>PCBs (maternal serum)</td>
<td>US, Michigan girls aged 5–24 (256)</td>
<td>CS; assessment of Tanner stages; recall of age at menarche in postmenarchal girls</td>
<td>No association</td>
<td>Blanck et al (2000)</td>
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<tr>
<td>PCBs (cord blood)</td>
<td>Faroe Islands boys (175)</td>
<td>C/CS; genital anomalies at birth, examination at age 14 for Tanner stage, testicular size, spermatotria, and serum hormones</td>
<td>No significant associations; suggestion of higher testosterone with higher PCB exposure</td>
<td>Mol et al (2002)</td>
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<tr>
<td>PCBs (serum, congeners 138, 153, and 180)</td>
<td>17-y-old Belgium boys (78) and girls (120) from polluted and nonpolluted areas</td>
<td>CS; assessment of Tanner stage by examination and testicular volume</td>
<td>Boys: high exposure to PCB 138 less likely to have reached highest stage of genital development, high PCB 153 less likely to have reached highest stage of pubic hair; girls: no associations</td>
<td>Den Hond et al (2002)</td>
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<tr>
<td>PCBs/PCDFs</td>
<td>Taiwan, girls aged 13–19 exposed to contaminated oil (Yucheng) in utero (27) and controls (21)</td>
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<td>No association with age at menarche; exposed girls reported shorter cycle length and more irregular cycles, exposed girls had higher levels of estradiol and FSH</td>
<td>Yang et al (2005)</td>
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<tr>
<td>PCBs/PCDFs</td>
<td>Taiwan, boys (mean age: 12.3) exposed to contaminated oil (Yucheng) in utero (61) and controls (60)</td>
<td>C/CS; Tanner stages by exam, testicular size, serum hormones</td>
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<tr>
<td>PCBs (serum congeners)</td>
<td>US/Canada Mohawk Nation, girls 10–17 (138)</td>
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<td>Exposure to estrogenic congeners (52, 70, 90, 101, 187, geometric mean) associated with greater likelihood of menarche; no association with other congener groups</td>
<td>Denham et al (2005)</td>
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<tr>
<td>Phthalates (serum)</td>
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<td>Case control</td>
<td>Serum phthalate (primarily DEHP) significantly higher in cases than controls</td>
<td>Colon et al (2000)</td>
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CS, cross-sectional study; C, cohort study; CS/C, cross-sectional assessment within a cohort; CALUX, chemically activated luciferase gene expression; PBB, polybrominated biphenyl; NHANES III, Third National Health and Nutrition Examination Survey; PCDFs, polychlorinated dibenzo-furans; PT, premature thelarche.

along the United States/Canadian border to assess serum concentrations and self-reported menarchal status (yes/no). No association was observed between DDT exposure and menarche despite the authors’ assumption that current serum concentrations were indicative of in utero and lactational exposures, given the long-standing con-
cern regarding consumption of contaminated fish among this population.

Five studies evaluated developmental PCB exposures in relation to pubertal development. Gladen et al reported no relation between in utero or lactational PCB exposure and age at menarche among girls. Higher lactational exposure was associated with earlier Tanner staging in boys and girls, although the results did not achieve statistical significance. Mol et al established a prospective cohort study of boys in the Faroe Islands, where PCB levels are known to be relatively high. PCB levels were measured in cord serum and tissue. Boys were examined at 14 years of age for Tanner staging, testicular size, spermaturia (sperm in urine), and serum hormones. No statistically significant associations were found between PCB concentrations and measures of pubertal development. A suggestion of a positive association between testosterone and PCB exposure was reported. Two cohort studies previously described also evaluated in utero exposure to PCBs among daughters of women who were exposed. Neither study found an association between PCB exposure and age at menarche.

Last, Yang et al and Hsu et al described no pubertal differences among boys or girls who were without exposure to PCB- and polychlorinated dibenzofuran–contaminated cooking oil.

Two cross-sectional studies of PCB exposure have been conducted. Den Hond et al measured PCB congeners in a sample of boys and girls who were aged 17 years and recruited from 1 of 2 exposed areas in Belgium: residence near a lead smelter or waste incinerator or a referent rural area. Puberty timing was measured using the Tanner staging as a part of a physical examination. Odds ratios were estimated for PCB concentration and odds for being in a puberty stage that indicated that puberty was not yet complete (ie, lower than stage 5). Exposed boys had completed pubertal development later, because odds ratios for higher PCB exposures (for both specific congeners and total PCBs) were associated with stage <5 for genital and pubic hair development. Because this study evaluated 17-year-olds, it is not clear whether puberty began later or the peripubertal duration was longer. No pubertal delays were seen in girls with PCB exposure. Among Akwesasne Mohawk Nation Girls, exposure to a group of 4 potentially estrogenic PCB congeners (52, 70, 101 + 90, and 187) was associated with a significantly greater likelihood of having reached menarche.

Two studies assessed childhood exposure to dioxins and puberty. Den Hond et al measured dioxin-like activity with the chemically activated luciferase gene expression assay and reported an association with Tanner stage <5 breast development; however, pubic hair stage in boys and girls and genital stage in boys was not significantly associated with dioxin-like activity. Warner et al examined pubertal development among girls who were exposed postnatally or during childhood to dioxin in Seveso, Italy. TCDD was not associated with age of menarche (self-reported at interview). None of the women was exposed prenatally.

Colon et al studied phthalate esters exposure and premature thelarche. Forty-one girls with thelarche were compared with 35 control girls with regard to serum pesticides and phthalate esters. Significantly higher phthalate levels were found among the girls with thelarche than comparison girls. The authors concluded that the findings were suggestive of a possible association between exposures and premature breast development in girls. Interpretation of these findings may be limited by residual confounding and concerns about the analytical method of measuring the phthalate parent compound instead of the metabolites. Saiyed et al found that boys who were exposed to endosulfan in India were less mature and had lower testosterone and higher LH than unexposed boys.

Three cross-sectional published articles assessed the relation between lead exposure and pubertal measures using either the Third National Health and Nutrition Examination Survey or the Mohawk Nation Study. In the Third National Health and Nutrition Examination Survey analyses, blood lead levels were analyzed in relation to age at onset for Tanner stage 2 for breast and pubic hair and menarche among US girls aged 8 to 18 years. Selevan et al estimated odds ratios for lead levels in relation to progression of puberty (stages 2–5) among girls. Combined, these studies suggested an inverse relation between blood lead levels and the onset and progression of puberty in girls, especially for subgroups within the population. An association also was found between higher blood lead level and delayed menarche, but this association was statistically significant only for black girls. The cross-sectional analysis among Mohawk nation girls also found later puberty among girls with higher lead exposures than girls with lower exposure.

**Cross-species Concordance of Pubertal Timing Effects**

Most chemicals have not been consistently studied in both animals and humans, with a few notable exceptions. Even when such research is available for a particular chemical, interpretation is exceedingly challenging given issues pertaining to dosage and timing of exposure and limited measurement of other relevant covariates. When such data are available (eg, TCDD, lead, DDE, PCBs, estrogens), similar findings tend to be observed across species. For example, pharmaceutical estrogens (eg, diethylstilbestrol, ethinyl estradiol) are the best examples of accelerated female breast development in humans, but the effect tends to be reversible and isolated from other pubertal effects. This is similar to the effects of estrogen exposure in the female rat and the monkey, which cause pseudoprecocious or dysregulated puberty markers. In male humans, pharmaceutical agents that are used to halt early puberty also cause similar effects in male rats. In the rat, in utero TCDD exposure resulted in delayed puberty as measured by a late age of VO and a delayed or incomplete differentiation of mammary epithelium. Similarly, Den Hond et al reported an association between high dioxin blood levels and later completion of breast development. This cross-species concordance may indicate that the Ah receptor agonism is active across species. For developmental lead exposure, animal and human studies both show a delay in...
pubertal development,\textsuperscript{112-115} suggesting a similar mechanism for rodents and humans. P,p'-DDE exposure in “foreign-born” girls was associated with earlier puberty relative to native-born Belgians\textsuperscript{99} and, possibly, increasing DDE in utero exposure with earlier puberty in girls\textsuperscript{97,98}; however, no animal studies of DDE exposure and female puberty timing were identified, limiting additional interpretation of the human data. In male rats, peripubertal DDE exposure delays PPS,\textsuperscript{116-118} although no relation to lower exposures in boys after in utero DDE exposure.\textsuperscript{97}

The weight of animal and human data, although suggestive, remains inconclusive for establishing a causal relation between EDCs and human pubertal disturbances. As reviewed, much of the available literature focuses on a single chemical or class, ignoring the mixture of compounds to which humans are exposed. Much of the data are derived from cross-sectional studies, which are unable to establish the causal ordering of environment in relation to puberty. To this end, timing and dose at critical windows of human development have not been purposefully assessed. Other relevant covariates such as lifestyle, genetic determinants, and other xenobiotics in the chemical mixture have not been well studied, if at all, in the context of EDCs.

**RESEARCH RECOMMENDATIONS**

The panel was asked to make research recommendations that are responsive to critical data gaps regarding purported environmental factors that may adversely affect puberty, to prioritize research initiatives including meth-

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<td>Etiologic Research</td>
<td>Prospective epidemiologic research inclusive of existing cohort currently being followed (e.g., Seveso, Yucheng, Yusho, PBB, DES grandchildren) or newly designed epidemiologic research capturing environmentally relevant exposures (single chemical and mixtures) Cross-sectional studies for monitoring population changes particularly across geographic areas and inclusive of high-risk subgroups of the population</td>
<td>Humans</td>
</tr>
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<td>1. Are environmental levels of EDCs affecting onset and progression of puberty and aberrant pubertal development such as delayed or precocious puberty in children?</td>
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<td>2. Do body shape and adiposity affect onset and progression of puberty and aberrant pubertal development such as delayed or precocious puberty in children?</td>
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<td>Humans</td>
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<tr>
<td>Critical windows of exposure</td>
<td>Prospective research comprising couples attempting pregnancy to capture parentally mediated exposures from periconception window to prospective pregnancy cohorts Research focusing on the early origins of human health and disease</td>
<td>Humans and nonhuman primates</td>
</tr>
<tr>
<td>3. What are the critical windows, ranging from periconception through adolescence, for human pubertal development in relation to environmental exposures?</td>
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<tr>
<td>Mechanistic</td>
<td>Genomics and proteomic studies using nonhuman primates and humans with alterations in the onset of puberty</td>
<td>Nonhuman primates and humans</td>
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<tr>
<td>4. What is the primary signal(s) for the onset of GnRH-dependent (central) puberty?</td>
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<tr>
<td>5. What is the primary signal(s) for the onset of GnRH-independent (peripheral) puberty?</td>
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<td>Rodents</td>
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<td>6. What is the molecular basis for sexual dimorphism in puberty onset and progression?</td>
<td></td>
<td>Rodents, nonhuman primates</td>
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<td>7. How is the tempo of puberty progression including the temporal relationship among the different markers regulated?</td>
<td></td>
<td>Rodents, nonhuman primates, humans</td>
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| TABLE 3: Research Recommendations for Focusing on Environmental Influences on Puberty |
|----------------------------------------|---------------------------------|---------------------------------|
| Priority Research Questions | Recommended Studies | Recommended Species |
| Etiologic Research | | |
| 1. Are environmental levels of EDCs affecting onset and progression of puberty and aberrant pubertal development such as delayed or precocious puberty in children? | Prospective epidemiologic research inclusive of existing cohort currently being followed (e.g., Seveso, Yucheng, Yusho, PBB, DES grandchildren) or newly designed epidemiologic research capturing environmentally relevant exposures (single chemical and mixtures) Cross-sectional studies for monitoring population changes particularly across geographic areas and inclusive of high-risk subgroups of the population | Humans |
| 2. Do body shape and adiposity affect onset and progression of puberty and aberrant pubertal development such as delayed or precocious puberty in children? | Prospective epidemiologic research inclusive of existing cohort currently being followed (e.g., Seveso, Yucheng, Yusho, PBB, DES grandchildren) or newly designed epidemiologic research capturing environmentally relevant exposures (single chemical and mixtures) Cross-sectional studies for monitoring population changes particularly across geographic areas and inclusive of high-risk subgroups of the population | Humans |
| Critical windows of exposure | Prospective research comprising couples attempting pregnancy to capture parentally mediated exposures from periconception window to prospective pregnancy cohorts Research focusing on the early origins of human health and disease | Humans and nonhuman primates |
| 3. What are the critical windows, ranging from periconception through adolescence, for human pubertal development in relation to environmental exposures? | | |
| Mechanistic | | |
| 4. What is the primary signal(s) for the onset of GnRH-dependent (central) puberty? | Genomics and proteomic studies using nonhuman primates and humans with alterations in the onset of puberty | Nonhuman primates and humans |
| 5. What is the primary signal(s) for the onset of GnRH-independent (peripheral) puberty? | Genomic and proteomic studies using transgenic rodent model. | Rodents |
| 6. What is the molecular basis for sexual dimorphism in puberty onset and progression? | Identifying the similarities and differences in molecules involved from puberty initiation to final outcome (described in 4 and 5) in males and females | Rodents, nonhuman primates |
| 7. How is the tempo of puberty progression including the temporal relationship among the different markers regulated? | Environmental exposure and puberty-timing studies that measure >1 puberty-timing end point/outcome. | Rodents, nonhuman primates, humans |

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ods development, and to identify avenues for research collaboration.

Environmental Factors Identified as Important for Human Puberty

After reviewing the weight of evidence described herein, the panel agreed that EDCs and body size or adiposity may be associated with pubertal disturbances in humans. The panel further recognizes that the 2 broad exposures may be interrelated in that exposure to EDCs can affect fat metabolism (eg, organotin compounds and diethyl hexyl phthalate act as peroxisome proliferator–activated receptor agonists in adipose tissue). Although humans can do little to minimize exposure to ubiquitous EDCs other than change dietary practices, particularly with regard to fish consumption and preparation, body size is potentially amenable to behavioral intervention. Carefully designed epidemiologic research, particularly that with longitudinal capture of exposures and relevant covariates at critical windows and the sensitive and specific measurement of markers of pubertal onset, progression, or deviations from expected normative standards, is needed to answer remaining questions about the role of environmental influences on developmental milestones. Such studies are rightfully complex and require a priori consideration and capture of other environmental determinants of puberty such as those that are believed to be attributed to body size and composition and possible genotypes and phenotypes that may emerge. Further complicating future research will be the challenges afforded by environmental mixtures, including those that arise from “natural” sources such as phytoestrogens.

Research Priorities and Future Directions

The panel identified 7 priority research questions reflecting 3 broad research domains: etiologic, exposure assessment, and mechanistic (Table 3). The panel strongly supported additional etiologic work aimed at answering lingering questions about the impact of EDCs and body size and adiposity on the onset and progression of puberty and aberrant clinical manifestations (ie, delayed or precocious puberty). The natural history of puberty requires delineation and ordering of a multitude of factors such as lipids, body shape, and diet in relation to pubertal onset and progression. With regard to exposure assessment, continued work is needed to capture the timing and dosage of maternal, paternal, or parental exposures across sensitive and critical windows of human development to identify agents that may disrupt normal embryonic or fetal development resulting in fetal (re)programming with lifetime effects.115,120 Last, the panel continued to support basic research to identify the primary signal(s) for the onset of GnRH-dependent and GnRH-independent puberty in humans, nonhuman primates, and other animals.

The increasing methodologic complexities that are associated with capturing exposures, including environmental mixtures, in the context of lifestyle and behavior across critical windows of human development argue for prospective epidemiologic studies whose utility and feasibility have been demonstrated.121,122 Ideally, such studies would incorporate longitudinal biospecimen collection at timed intervals for the quantification of chemical mixtures and lifestyle exposures that may affect growth and development inclusive of pubertal milestones. Efforts to develop a suite of sensitive pubertal markers, rather than a sole marker, that are consistent with the manner in which humans are exposed are needed. Future research needs are discussed elsewhere in this issue but underscore the need to develop behavioral, psychosocial, and neurologic end points along with the social and psychological consequences of precocious or delayed puberty.11

Last, regardless of the research question, newly designed animal and human research must use the best available methods. With regard to animal research, careful attention to choice of the most appropriate animal model, relevant critical or sensitive windows of exposure, route and duration of exposure, timing and relevant dosages, assumptions regarding the dose–response curve, and an analytic statistical plan that is responsive to the study design, particularly assessment of mixtures and litter effects, is needed. Epidemiologic research also needs to address these same issues while recognizing the highly correlated nature of reproductive outcomes and the need to model appropriately previous history of adverse reproductive outcomes, a strong predictor, in the context of more subtle environmental effects.123

CONCLUSIONS

A concerted body of multidisciplinary research is needed to answer lingering questions about the impact of EDCs, body size, and adiposity on human puberty. This research can be grounded within a broader research base inclusive of human reproduction given the highly interrelated and timed series of critical events that are characteristic of human growth and development, of which puberty is 1 of many key milestones. Basic research targeting the primary signals for onset is critical so that epidemiologic investigators can design research that is sensitive to the capture and measurement of key biomarkers of pubertal onset. Additional basic research is needed regarding comparative species physiology of puberty, the primary signal of puberty onset for central versus peripheral signaling, and the basis for sexual dimorphism.

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Grumbach, Marcia Herman-Giddens, John Himes, Anders Juul, Paul Kaplowitz, Carole Kimmel, Peter Lee, Robert Lustig, Tony Plant, Ed Reiter, Steve Schrader, Sherry Selevan, Richard Sharpe, Thorkild Sorensen, and Patricia Whitten. We especially thank Anders Juul for critical reading of the manuscript.

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