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Initiation and progression of puberty in girls, and environmental exposures in a contemporary longitudinal cohort

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Initiation and progression of puberty in girls, and environmental exposures in a contemporary longitudinal cohort

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B.A., Northwestern University, 2003
M.P.H., Emory University, 2004

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An abstract of a dissertation submitted to the Faculty of the Graduate School of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Epidemiology

2009

Abstract

Initiation and progression of puberty in girls, and environmental exposures in a contemporary longitudinal cohort

By Krista Yorita Christensen

Puberty is occurring earlier than in the past, a secular trend which may be due in part to exposure to endocrine disrupting chemicals such as persistent organic pollutants. In this dissertation, the initiation and progression of pubertal development were described for female offspring participating in the Avon Longitudinal Study of Parents and Children (ALSPAC). Self-reported breast and pubic hair and age at menarche were collected via mailed questionnaire from 8-14 years. Ages at entry into each stage of breast and pubic hair development, age at menarche, and duration of puberty were estimated using survival analysis. The correlation between age at beginning breast and pubic hair development was estimated using a maximum likelihood approach. Factors associated with initiation pathway, and with breast and pubic hair stage were assessed using polytomous logistic regression, and ordinal probit analyses, respectively. The association between age at menarche and exposure to persistent organic pollutants was assessed in two ways. First, a nested case-control study was conducted among ALSPAC participants to determine association between earlier (<11.5 years) or later age at menarche, and maternal serum concentration of polyfluoroalkyl compounds (PFCs). Second, data from the 2003-2004 National Health and Nutrition Examination Survey (NHANES) were used to construct survival analysis models for the association between age at menarche, and serum concentrations of polybrominated diphenyl ethers (PBDEs), polybrominated biphenyl (PBB) 153, polychlorinated biphenyls (PCBs), and PFCs.

On average, girls in the ALSPAC began breast and pubic hair development at the ages of 10.2 and 10.95 years. The correlation between these ages was estimated to be 0.5. Most girls had synchronous initiation of breast and pubic hair development (46.3%), or started breast development first (42.1%). Age at menarche (mean=12.87 years) and duration of puberty (mean=2.7 years) both varied by initiation pathway. Both maternal and child characteristics were associated with initiation pathway, and with breast and pubic hair stage. PCBs and PBDEs were associated with altered age at menarche, but no relationship was seen for PBB 153 or PFCs. Further research is needed to determine the impact of these and other pollutants on other growth parameters, and periods of vulnerability for exposure.

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Chapter 1. Study Motivation

I. Study Motivation

Despite the importance of the timing and progression of pubertal development, the process has not been well described in a contemporary cohort. Puberty is occurring earlier than in the past, and accelerated puberty can have adverse consequences, both physiological and emotional. Understanding patterns of development is necessary to elucidate underlying biological mechanisms and potential pathways for variation in timing and progression of developmental milestones. Methodological difficulties in this field include (1) appropriate utilization of repeated measures on individuals, (2) interval censored data, (3) ordinal outcomes and (4) lack of information on the interrelationship of pubertal milestones.

There is substantial evidence both from animal and from human studies, that exposure to environmental chemicals in early life may disrupt normal endocrine system and gamete development, with subsequent consequences for sexual maturation, growth and function. However, evidence from studies of human female pubertal development is limited and sometimes conflicting. Exposure to chemicals such as persistent organic pollutants (POPs) is nearly ubiquitous; it is critical that we understand how these exposures may affect human health, including potential to alter the initiation and progression of puberty.

II. Study Aims

My first dissertation aim is to describe patterns of pubertal initiation and progression in a contemporary population of girls in Avon, England. The second is to determine the association between exposure to persistent organic pollutants and age at menarche.

III. Study Contributions

The contributions of the first study aim are two-fold. First, data from a representative, longitudinal cohort will be used to describe puberty in contemporary girls. Second, methods used will address some of the difficulties encountered in utilizing growth data, and in particular data from repeated assessments of developmental status. Repeated measures ordinal probit analysis has not been used in previous studies of growth and development, but is an appropriate and flexible approach for studying. Finally, a maximum likelihood technique adapted for interval censored data will be used to estimate correlation of event times for various developmental markers.

The effects of environmental chemicals on the timing and progression of puberty in humans is not known, although exposure to these chemicals is nearly ubiquitous. This dissertation will determine the association between age at menarche and polyfluoroalkyl chemicals using a nested case-control study design, and the association between age at menarche and polyfluoroalkyl chemicals, polychlorinated biphenyls, brominated flame retardants and dioxins using a cross-sectional survey. Findings from these studies may elucidate mechanisms of action for these chemicals, and reproductive health effects of exposure.

Chapter 2. Literature review

I. Pubertal development of girls

(1) Events and processes

(a) Events and processes in utero

Puberty is the process through which the reproductive system matures. We can think of this time, which is marked by numerous physical and behavioral changes, as the transition period between childhood and adolescence. The sexual growth and maturation of humans actually begins in utero, as the endocrine system and reproductive organs are developed. The hypothalamus, a large gland which sits just above the brain stem, develops during the first trimester of pregnancy.[1] The hypothalamus is described as the 'link' between the nervous system and the endocrine system; it responds to sensory and other inputs (including olfactory stimuli, light, steroids, and blood-borne stimuli) by regulating the excretion of hormones. Hypothalamic hormones include corticotropin releasing hormone (CRH), dopamine, growth hormone releasing hormone (GHRH), melatonin, somatostatin and thyrotropin releasing hormone. Through these hormones, the hypothalamus regulates body temperature, appetite and thirst, fatigue, and emotional behaviour. The hypothalamus also produces gonadotropin releasing hormone (GnRH). During early gestation, approximately one thousand neurons form in the olfactory placode, and migrate through the forebrain to the hypothalamus. These neurons form the GnRH pulse generator, which regulates the production of GnRH. Gonadotropin releasing hormone regulates the stimulation and excretion of gonadotropins (leutinizing hormone [LH] and follicle

stimulating hormone [FSH]), and thus partially regulates pubertal development and reproduction.

The pituitary gland begins to develop around 7 weeks gestation.[1] It also sits at the base of the brain, and is linked to the hypothalamus. The pituitary gland is comprised of three sections, which each produce certain hormones. The anterior lobe is of main interest for pubertal development, as it produces growth homone, prolactin, adrenocorticotropic hormone (ACTH), thyroid stimulating hormone, and the gonadotropins FSH and LH. The intermediate lobe produces melanocyte stimulating hormone, and the posterior lobe produces antidiuretic hormone and oxytocin. The pituitary is referred to as the 'master' gland, as its products regulate the function of other endocrine glands, such as the adrenal glands. The adrenal glands are located on top of the kidneys, and produce corticosteroids (in the adrenal cortex) and catecholamines (in the adrenal medulla). Corticosteroids include androgens such as testosterone, androstenedione, and dehydroepiandrosterone (DHEA).

Around 6 weeks gestation, the urogential ridge forms the mullerian ducts.[1] These ducts, in the absence of anti-mullerian hormone (AMH), will form the fallopian tubes, uterus, and vagina. The follicles develop at approximately 20 weeks of gestation, and the uterus completes development by week 22. Females are born with their entire supply of ova, numbering approximately 1-2 million ova at birth, but by the time puberty begins this number has decreased to roughly 400,000. Conditions in the womb during gestation (including nutritional status, exposure to environmental chemicals, and illness) can impact the developing reproductive and endocrine systems of the fetus, at varying points

throughout pregnancy. Such exposures may affect the timing and course of pubertal development and reproduction.

(b) Events and processes in childhood

The hypothalamic-pituitary-adrenal (HPA) axis regulates many of the physiological changes of puberty (Figure 2.1). Adrenarche usually begins before puberty, and continues throughout pubertal development. During these years, the adrenal cortex increases in size and mass and completes differentiation into the zona glomerulosa, zone fasciculate, and zona reticularis (the primary site of androgen production).[2, 3] Adrenal androgens are produced and released through a complex signaling process, beginning with the central nervous system (CNS). The CNS signals the hypothalamus to produce corticotropin releasing hormone (CRH), which causes the pituitary gland to produce adrenocorticotropic hormone (ACTH). This in turn leads to the release of the adrenal androgens androstenedione and DHEA (as well as cortisol). These androgens stimulate physical changes, most notably pubic hair development (pubarche) and axillary hair development.

Further pubertal changes are initiated by the hypothalamic-gonadal (HPG) axis (Figure 2.1). During infancy, GnRH is released from the hypothalamus, but is only produced in minute quantities during childhood.[1] Reactivation of the GnRH pulse generator, the network of hypothalamic neurons which stimulate the release of GnRH, occurs during puberty. At this time, GnRH is released in increasing quantities, in a pulsatile fashion. GnRH stimulates cells in the pituitary gland to produce LH and FSH. Leutinizing hormone initiates follicular growth, and triggers ovulation. Follicle stimulating hormone stimulates growth of granulosa cells, which surround the developing oocyte. In

addition, both LH and FSH stimulate the ovaries to produce estradiol. Estradiol in turn stimulates the growth of the uterus, endometrium and breasts.

Progression through puberty for girls is often measured in clinical and research settings using three markers: breast development, pubic hair development and achievement of menarche. Marshall and Tanner developed a 5 stage method to evaluate breast and pubic hair development for girls[4] which is used in both clinical and research settings (Figure 2.2). In the Tanner scheme, stage 1 represents the prepubertal stage, with no breast tissue or pubic hair present. In stage 2 of breast development, breasts begin budding and areolar size increases; breasts and areolae continue to increases in size in stage 3, and the secondary mound of the areolae is observed in stage 4. Stage 5 represents the adult breast configuration. For pubic hair, stage 2 is marked by a small amount of coarse, pigmented hair along the labia majora, while in stage 3 hair growth extends to the mons pubis. Stage 4 is marked by a nearly adult appearance, although pubic hair has not yet spread to the medial thighs and the area covered is slightly smaller than in stage 5 (the adult configuration).

(2) Timing

There is no one set of ages at which the events of pubertal development occur; rather, individuals vary widely in the initiation and relative timing of developmental milestones. However, there is a typical sequence of events, and average ages at which each is observed. Adrenarche typically begins around 7 or 8 years of age.[1] In females, the first sign of pubertal development is usually breast development (thelarche), which occurs around the age of 10 and is closely followed by the development of pubic hair (pubarche).[5, 6] About a year later, a somatic growth spurt is observed, with linear growth continuing until about the

age of 14-18[7-9] when final adult height is attained. Menarche, or the first menstrual bleeding, typically occurs 2 years after thelarche, around age 12.5.[10, 11] Ovulation does not necessarily commence immediately with menarche, but may begin after the first few menstrual periods.[1]

There are numerous factors which affect the timing of pubertal initiation and development. The reactivation of the HPG axis is not a discrete event, but a gradual transition, and the mechanism for this suppression and reactivation is not known. Although the greatest increases in GnRH, LH and FSH activity occur during puberty, small increases in production can also occur during childhood.[1] Therefore, variations in timing and magnitude of hormone activity and regulation of the HPG axis are likely to explain much of the variation in pubertal timing. Such disruption of the HPG axis may be caused directly or indirectly by environmental signals including light, energy availability, and endocrine disruptor exposure.

Nutritional status—both pre- and post-natal—partially determine timing of pubertal development.[12] In rat models, intrauterine growth retardation (IUGR) led to delayed vaginal opening, a marker of sexual maturation. In contrast, postnatal food restriction did not affect timing of puberty onset.[13] Human studies have shown mixed evidence for the effect of IUGR on pubertal development. In some studies, IUGR is associated with earlier pubarche[14] and age at menarche,[15] while a study in France found that IUGR was associated with delayed puberty.[16] In girls experiencing post-natal malnutrition (including those with anorexia nervosa) puberty is often delayed or arrested.[17] Chronic malnutrition has been shown to delay menarche by approximately 2 years.[18] On the other end of the spectrum, overweight and obese girls tend to experience an earlier age at puberty onset and age at

menarche.[19] This indicates that initiation of pubertal development may be sensitive to attainment of a particular level of somatic growth and/or fat mass, rather than being a function of chronological age.[20, 21] The hormone leptin is thought to mediate this relationship to some extent. In both human and animal studies, leptin deficiency prevents initiation of pubertal development. However, evidence suggests that leptin may be permissive for puberty, rather than the initiating mechanism.[22]

Genetic factors are thought to play a significant role in determining initiation and progression of pubertal development. The single best predictor of a girl's age at menarche is her mother's age at menarche,[23, 24] and monozygotic twins show greater correlation in age at menarche, age at growth spurt, and Tanner staging than dizygotic twins. Recent genome-wide association studies have identified several alleles that are associated with timing of menarche, including some that have been previously identified as being associated with adult height, and body mass index.[25-29]

Timing of puberty also varies by race/ethnicity. In the US, Black girls experience earlier initiation of puberty than white girls, and Latina girls experience slightly delayed pubic hair development compared to white girls.[30] At every stage of development, Black girls tend to be at more advanced stages of breast and pubic hair development compared to white girls, and the age at menarche is significantly earlier, as well. However, this difference by race/ethnicity is a relatively new phenomenon; McDowell et al report that among women born before 1950, the age at menarche was similar among Mexican American and non-Hispanic White women, and older among non-Hispanic Black women.[31] However, non-Hispanic Black women had a more rapid decline in the age at menarche compared to the other two groups, so that for women born in later years, they had an earlier

age at menarche (12.2 in 1980-1984) compared to non-Hispanic White women (12.5 in 1980-1984).

There are several clinical syndromes which are known to disrupt timing and precedence of markers of pubertal development, which give some insight into potential pathways for endocrine disruption. These conditions may arise from altered timing of constitutional growth or specific alterations in timing of sexual maturation, and may refer to either generalized maturation, or to acquisition of specific pubertal changes such as thelarche or adrenarche.

(3) Precocious puberty

(a) Definition of precocious puberty

Precocious puberty is usually defined as the onset of puberty before the age of 8 years in girls. By this definition, a US study of girls seen in physician office practices estimated that approximately 8% of white girls and 25% of Black girls experience precocious puberty in the United States.[5] Based on these findings, the Lawson Wilkins Pediatric Endocrine Society recommended that the age limit for precocious puberty be reduced to 7 years for white girls, and 6 years for Black girls.[32] However, there has been some discussion over the wisdom of adopting new standards based on a single study, particularly since study participants were not a random sample of the population, and the new standards are not universally recognized.

Precocious puberty may encompass early somatic growth, early menarche, and early development of secondary sexual characteristics. Accelerated puberty may be gonadotropin dependent ("central"), gonadotropin independent ("peripheral"), or a combination of both (Table 2.1). In gonadotropin dependent precocious puberty, GnRH is

produced at an earlier age than usual, while in gonadotropin-independent precocious puberty, estrogen is produced and released earlier than usual, without the intervening action of GnRH. In both central and peripheral precocious puberty, the underlying condition may be permanent or transient.

(b) Causes of precocious puberty

(i) Causes of central precocious puberty

Central precocious puberty (CPP) in girls is often idiopathic, but in a minority of cases may be due to specific clinical conditions. Hypothalamic hamartomas, tumor-like lesions in the brain, are capable of causing CPP, with pubertal development evident as early as infancy.[33, 34] Other CNS conditions such as cranial radiation therapy, infection, supracellar cysts, arachnoid cysts, and optic gliomas, may also lead to CPP.[1] It is thought that in these cases, GnRH secretion may be increased due to disruption of the 'brake' on the GnRH pulse generator.

Congenital adrenal hyperplasia (CAH) is the most common instance of CPP caused by sustained exposure to sex steroids. In individuals with CAH, the adrenal glands are unable to produce sufficient amounts of corticosteroids. Low levels of cortisol and aldosterone cause the pituitary to secrete ACTH, stimulating the adrenal cortex to release more adrenal androgens. If CAH is caused by a deficiency of 21-hydroxylase (~90% of CAH cases), progesterone and 170H-progesterone accumulate, leading to early pubertal development.

(ii) Causes of peripheral precocious puberty

Peripheral precocious puberty (PPP), in which sex steroid levels are increased while gonadotropin levels are not, is less common than CPP. Peripheral precocious puberty may be due to disorders of the gonad or adrenal gland, or to exposure to external estrogens (including estrogen containing creams or lotions). Estrogen-secreting tumors, often located in the adrenal or pituitary glands, may cause PPP, as can ovarian cysts and tumors. McCune-Albright syndrome is caused by a genetic mutation of the GNAS1 gene—this mutation causes gonadotropin receptors to become active, leading to increased estrogen secretion. Although the mechanism is unclear, primary hypothyroidism may also lead to early breast development and isolated vaginal bleeding.[35]

(c) Treatment of precocious puberty

Precocious puberty treatment may be indicated for a variety of reasons, including preservation of adult height and the child's psychological well-being. The most common treatment of CPP is administration of GnRH agonists, such as leuprolide and histrelin. The treatment of PPP is more complicated, but anti-androgenic compounds such as cyproterone acetate, or aromatase inhibitors may be used to decrease circulating levels of sex steroids by blocking the conversion of androgens to estrogens.[36, 37]

(4) Delayed puberty

(a) Definition of delayed puberty

Delayed puberty, for girls, is usually defined as the lack of pubertal development by the age of 13 years. This may be due to delayed overall growth (constitutional delay), hypogonadotrophic hypogonadism, or hypergonadotrophic hypogonadism (primary gonadal failure; Table 2.2). In hypogonadotrophic hypogonadism, there is a defect in

GnRH (and subsequent LH and FSH) secretion. Contrarily, in hypergonadotrophic hypogonadism, the ovaries are unable to produce sufficient estrogen, leading the hypothalamus and pituitary to overproduce GnRH, and subsequent LH and FSH.

(b) Causes of delayed puberty

(i) Constitutional delay of puberty

In individuals with constitutional delay, delayed puberty is considered to be a variant of normal development rather than a pathological condition. A history of slow linear growth is key to this diagnosis, as is a family history of delayed puberty.

(ii) Hypogonadotrophic hypogonadism

About half of the cases of idiopathic hypogonadtrophic hypogonadism are due to Kallman's syndrome, an X-linked condition in which GnRH neurons do not migrate to the hypothalamus during development, leading to deficient production of GnRH later in life. Other genetic conditions which can lead to delayed puberty are defects in the GnRH releasing gene, FSH-β gene, or LH-β gene. Other causes of delayed puberty in this class include malnutrition, non-anosmic hypogonadotrophic hypogonadism, intracranial tumor, mid-line congenital CNS malformation, and cranial radiotherapy

(iii) Hypergonadotrophic hypogonadism

Turner's syndrome, or a 45X karyotype, results in lack of development of the ovaries and consequent lack of pubertal development. Other genetic conditions which may lead to delayed puberty include 46,XY karyotype, aromatase deficiency, mutations in the androgen receptor gene, and FSH releasing gene mutation. While females with aromatase deficiency

exhibit absence of breast development and progressive virilization, FSH releasing gene mutations often lead to pubarche in the absence of menarche, but may not affect breast development. Androgen receptor gene mutations can lead to partial or complete androgen insensitivity. Individuals with partial androgen insensitivity usually present as under-virilzed males, while those with complete androgen insensitivity may have female external genitalia. Delayed puberty may also occur secondary to surgery, chemotherapy, radiation, or infectious disease.

(c) Treatment of delayed puberty

Estrogen replacement is the primary treatment for delayed puberty in girls. Estrogen therapy is usually begun around 12 years of age, to prompt age-appropriate menarche. Progesterone is also used in treatment, usually after a year of unopposed estrogen therapy, or after menstrual bleeding begins. This is most often accomplished using hormonal forms of contraception, including oral contraceptives, transdermal patches, or a combination of the two.[1]

(4) Secular trends in pubertal timing

The average age at menarche is currently 12.7[4] among American girls, and has decreased[4, 5, 10, 11, 31, 38] over the last century. Breast and pubic hair development also appear to be occurring at earlier ages. In Marshall and Tanner's 1948 cohort, breast development was reported to have begun, on average, at 11.15 years of age, and pubic hair development at 11.69 years.[4] In contrast, Hermann-Giddens found in a more contemporary US cohort that the ages of initiation of breast and pubic hair development had decreased by approximately 1.2 years, and age at menarche by 0.6 years.[5] Although these two studies were different in

terms of population and design, the results are quite striking. A recent panel review found sufficient evidence to conclude that in the US, age at initiation of breast development and menarche have decreased between 1940 and 1994; however, there is insufficient evidence to determine whether age at initiation of pubic hair development has decreased, or whether progression through stages of breast and pubic hair development has accelerated.[39] Similar secular trends may be occurring in Europe, as evidenced by findings from a recent Danish study.[40] Between 1991-1993 and 2006-2008, age at entry into breast stage 2 decreased significantly (10.88 and 9.86 years, respectively), even after adjusting for BMI; age at entry into breast stages 3, 4+ and pubic hair stages 3 and 4+ were also significantly advanced. Although there was a trend towards age at entry into pubic hair stage 2 and age at menarche as well, these differences were not significant after adjusting for BMI.

(5) Consequences of disrupted puberty

Disruptions in timing and progression of puberty may have negative consequences. Children experiencing precocious puberty may be perceived as more mature by adults and other children, altering normal social relationships. In some cases, unwanted adult attention may also result from precocious development.[41] Consequently, precocious puberty has been associated with multiple adverse outcomes, including earlier age at first sexual activity, increased risk-taking behavior (including smoking, drinking, and use of illicit drugs), increased depression, and decreased physical activity.[42-46] Both altered parent-child relationships, and composition of social network are thought to mediate these associations.[47] In terms of impact on adult health, precocious puberty may lead to premature fusion of the epiphyses, reducing adult height.[35] Girls who experience earlier age at menarche are at increased risk for breast cancer, presumably due to increased

lifetime exposure to estrogen.[48, 49] Premature adrenarche is a marker of risk for polycystic ovarian syndrome (PCOS), and for metabolic syndrome.[50] Complications of PCOS include menstrual irregularity, impairment of fertility, increased risk for type II diabetes, obesity, dyslipidemia, and cardiovascular disease.[14, 50]

Alteration in age at pubertal development also has implications for policy and clinical recommendations. Based on findings from the PROS study reported by Herman-Giddens et al,[5] the Lawson Wilkins' Pediatric Endocrine Society advised changes to the guidelines for the clinical evaluation of girls presenting with early breast development.[32] The suggested guidelines lowered the age for defining 'precocious' breast development to 7 years for Caucasian children, and 6 years for Black children (previously the age limit was 8 years regardless of race). However, these recommendations were controversial both because of the race-specific age limits, and because they were based on a single study which may have been affected by recruitment bias and other design issues. In addition, these recommendations increase the amount of healthcare resources (and accompanying burden on children and their families), since a greater number of girls are classified as having precocious puberty and referred for further clinical evaluation. Of course, this consideration is weighed against the need to correctly identify and treat girls whose early development is indeed pathological rather than a normal variation in timing. Therefore, it is important to understand the secular trends for pubertal development and elucidate potential mechanisms for altered timing of puberty.

(6) Longitudinal studies of pubertal development

(a) Longitudinal studies in the United States

(i) Bogalusa study

The Bogalusa study took place in Bogalusa, Louisiana.[51] Between the periods 1973-1974 and 1992-1994, seven cross-sectional assessments were undertaken of schoolchildren in the Washington Parish; some children were assessed in more than one of these, yielding a mixed longitudinal/cross-sectional design (Table 2.3). In this cohort, 9158 girls had at least one examination, and 2058 girls had at least two assessments, one before and one after the age of 10. Information on menarche was collected through self-report; breast and pubic hair development were only assessed in the 1973-1974 cohort, using visual inspection.[52] In this earlier group, age at entry into breast stage 2 was estimated to be 10.37 (SD=0.105) among white girls, and 10.22 (SD=0.125) among Black girls. Age at entry into pubic hair stage 2 was estimated to be 10.86 (SD=0.095) and 10.13 (SD=0.125) among white and Black girls, respectively. For the entire cohort, median age at menarche was reported to be 12.7 (12.6-12.8) for white girls, and 12.5 (12.4-12.6) for Black girls; this age decreased over the study period for both races. Among white girls, median age at menarche was 12.7 in 1973-1974, increased slightly to 12.9 from 1976-1982, before dropping to 12.6 in 1984-1988 and to 12.5 in 1992-1994. A more variable pattern was noted for Black girls, with median age at menarche ranging from 12.1 (1984-1985 and 1992-1994) to 12.9 (1976-1977). When focusing on the longitudinal cohort, median age was 12.6 for white girls and 12.3 for Black girls.[51] Among a subset of girls who were observed at adulthood in addition, menarche before 12 years of age was associated with greater weight, BMI, and skinfold thickness compared to those reporting menarche after 13.5 years. However, the

bulk of this effect appeared to be due to the association between childhood overweight (which is associated with earlier menarche), and adult overweight.[53]

(ii) Fels Longitudinal study

The Fels Longitudinal study was begun in 1929, in order to determine the effects of the Great Depression on childhood growth and later risk for certain disease conditions (Table 2.3). The study cohort consists of individuals residing in Ohio, born between 1929 and 1990. Participating children were seen every 6 months between the ages of 2-18; at each clinic visit, girls were asked whether they had begun menstruation in the interval since the last clinic visit, and the date of first menstruation.[54, 55] In this cohort, girls born in each decade from the 1930's to the 1970's reported similar mean ages at menarche (ranging from 12.57-12.99). In the next decade, however, a significantly lower age at menarche was reported (12.34). Although average BMI also increased over the study period, this secular trend did not explain the decreased age at menarche in the most recent time period.[54] When girls were categorized by age at menarche, those with early age at menarche (before 12 years of age) showed increased levels of insulin and glucose, increased systolic blood pressure, increased percent body fat, and decreased fat-free mass, when compared to girls experiencing average or later age at menarche.[55] These factors confer increased risk for cardiovascular disease and other chronic conditions later in life.

For a sample of Fels participants, timing of sexual maturation was assessed more fully through clinic examinations (also taking place at 6 month intervals) in which information including the girl's Tanner stage of breast and of pubic hair development and menarche status was collected. In the first such subsample, reported in 1948, 49 girls were examined.[56] Girls ranged in age from 8 to 18, with an average age of 14. Among these

girls, the average age at entry into breast development was 10.8 (SD=1.1), and the average age at entry into pubic hair development was 11.0 (SD=1.1). Over half (53.1%) of girls had breast development preceding pubic hair development, while a third (32.7%) had pubic hair development preceding breast development; the remainder (14.3%) had simultaneous development. Very few girls had reached stage 5 for either marker, but among these the average age at entry into breast stage 5 was 13.7, and 13.9 for pubic hair. The average age at menarche was reported to be 12.9 (SD=1.4), and the authors noted that a similar amount of time elapsed between initiation of puberty and menarche, for both early and late maturers (~2.5 years). In a later subsample, exams were conducted between 1985 and 1993, and participants ranged in age from 9.5 to 17.[57] Among 67 girls (all white), the average age at entry into breast development was 11.2 (SD=0.7), slightly later than that reported in the earlier study. Average age at beginning of pubic hair development was unchanged from the previous study, 11.0 (SD=0.5). For this subset, the average age at which stage 5 of pubic hair was observed was 13.1 (SD=0.8); average age at reaching breast stage 5 was not reported due to a small sample size.

(iii) Lee study

Peter Lee conducted a longitudinal study of pubertal development among a small group of males and females in the United States (Table 2.3). From 1969-1974, participants were assessed every 6 months for signs of pubertal development.[58] The 18 female participants entered at various stages of development (ages 8.6-17.8), so age at entry into puberty and age at menarche were not assessed for all girls. In this cohort, girls tended to begin breast development before pubic hair development (average ages of 11.2 [SD=1.6] and 11.9 [SD=1.5], respectively). The average age at menarche was 13.3 (SD= 1.3), and axillary

hair growth was not observed until the age of 13.1 (SD=0.8). These values are within the range reported from other studies, although the small sample size limits this comparison.

(iv) NHLBI Growth and Health study

The National Heart, Lung, and Blood Institute Growth and Health study enrolled children in 3 clinical centers in California and Cincinnati, and followed them to study the development of obesity and other cardiovascular disease risk factors (Table 2.3).[59, 60] Children were enrolled in 1987 at the age of 9 or 10, and seen annually for 10 visits. Breast and pubic hair development were assessed by female examiners, and menarche status by self-report. Among white participants, mean age at menarche was significantly greater compared to Black participants (12.7 and 12.0 respectively, $p<0.001$). When categorizing girls as 'early,' 'mid-onset,' and 'late' maturers, girls in the 'early' category were shorter and heavier compared to their peers. However, there was no association between timing of puberty and adult height.[60] A sub-analysis of girls who were 9 years of age at recruitment showed similar results.[61] Median age at menarche and at entry into pubertal stages was estimated using survival analysis; age at onset of puberty was significantly lower for Blacks compared to whites (13.55 and 14.26, respectively), and duration of puberty was shorter for Blacks (4.16) compared to whites (4.03), although this difference was not significant. Median age at menarche was 12.7 among white participants in this group, significantly lower than that for Black participants (12.0). The most commonly observed initiation pathway was synchronous (43.3%), followed by thelarche (37.3%) and pubarche (19.5%). Correlation between developmental milestones (menarche, age at peak height velocity, age at adult height, onset of puberty, completion of puberty) was also assessed; correlation was lower for this study compared to previous studies from the 1920's and

1960's. The authors conclude that menarche and puberty onset may represent non-parallel processes, and that age at menarche and age at puberty onset may not be interchangeable.[61]

(b) Longitudinal studies in Europe

(i) First Zurich longitudinal study

The First Zurich longitudinal study was conducted from 1954-1980 (Table 2.4).[62] In this cohort, 142 girls between the ages of 8 and 18 were assessed for breast and pubic hair development, and for menarche. Onset of puberty ranged from 8 years to 15 years. Just over half (53%) of girls had pubic hair development as the initial marker of puberty, while 18% had initial breast development. About one third (29%) of girls had synchronous initiation of breast and pubic hair development. Few girls omitted any stage of pubic hair or breast development (i.e. stage was not observed by the researchers); breast stage 4 was the most common stage to be omitted. A small number of girls regressed in developmental stage, mostly in later stages of breast development. Age at completion of puberty ranged from 12 to 18. The mean age at menarche was 13.4 (SD=1.0), and occurred on average 2.2 years after beginning breast development, and 2.8 years after beginning pubic hair development. The duration of breast development was slightly shorter than that for pubic hair development (mean 3.2 [SD=1.4] and 3.6 [SD=1.1], respectively); however, there was no clear relationship noted between timing and duration of puberty.[62]

(ii) Young-HUNT study

The Young-HUNT study is a prospective study of adolescents in Norway, examined once between 1995-1997 when participants were in middle school (ages 12-17), and once again between 2000-2001 when participants were in high school (ages 16-20; Table 2.4). Age at

menarche was assessed by self-report; breast and pubic hair development were not assessed. In this cohort, the mean age at menarche was 13.2 (SD=1.2). Girls who showed central adiposity in early adolescence and had earlier age at menarche, were more likely to be overweight in late adolescence.[63]

(iii) Swedish Longitudinal Growth Study

Starting in 1955, 212 Swedish children (90 girls) were examined starting at the age of 8 years, and continuing through late adolescence as part of a longitudinal growth study in Sweden (Table 2.4).[64] Clinic exams were performed annually, with Tanner stage of breast and pubic hair development assessed by visual inspection. Menarche was assessed via status quo self-report every 3 months. Among these girls, the mean age at entry into breast stage 2 was estimated to be 10.99 (SD=0.04), while mean age at entry into pubic hair stage 2 was estimated to be 11.48 (SD=0.04) years. In this cohort, 45% of girls experienced breast development as the first marker of puberty, while 8% experienced pubic hair as the first marker; the remainder of girls (47%) had synchronous development. The average age at menarche was 13.03 (SD=0.03) years.

(iv) Harpenden Growth Study

The first longitudinal study of children in Great Britain to assess pubertal development was begun in 1948 by the Ministry of Health. Dr. James Tanner was invited to head up the study, which was sited in Harpenden, close to London. The original purpose of the study was to describe the effects of malnutrition on growth, the study later broadened its scope to describe growth and development in general. In 1956, Tanner along with W.A. Marshall moved the study to the Institute of Child Health, where the study continued until 1971

(Table 2.4). Participants were drawn from a children's home, and consequently represented mainly lower socio-economic classes. Although none of the children had physical abnormalities, it was possible that not all children had received routine medical care before entering the children's home. Most study participants were followed between the ages of 5 and 16 years, with examinations conducted every six months of normal growth, and every three months during pubertal growth spurts. Photographs were used to document pubertal changes, along with self-report of age at first menses (in 3-month intervals). From these observations, a 5-stage classification system (the Tanner stages) was developed for categorizing breast and pubic hair development.

In total, 192 girls (all white) were included in analyses of pubertal development. For pubic hair development, all girls passed from stage 1 to stage 5 in a sequential fashion, with no stages 'skipped' and no reversal of stage. Breast development was more variable, as a small number of girls went from stage 5 to stage 4 in subsequent examinations, and stage 4 was not observed in a few girls, indicating rapid transition from stage 3 to stage 5. The authors caution that due to the photographic methods used, it was difficult to discern presence of pubic hair in earlier stages; thus, the calculated ages at entry into stage 2 of pubic hair development are likely to be overestimated. Time spent in each stage of breast and pubic hair development assumed an approximately log-normal distribution; in general, relatively slower breast development in earlier stages did not predict greater time spent in stage 4. Although the largest proportion of girls were concordant for breast and pubic hair stage (i.e. were in the same stage for both indicators at examination), a substantial number were discordant. However, no girls were stage 1 for one indicator and stage 5 for another at any given examination. The mean age at menarche was 13.47 in this cohort, and the

majority of girls were in stage 4 for both breast and pubic hair development at first menses. Breast development began, on average, at 11.2 years of age, with pubic hair development beginning slightly later (11.7 years, or 6 months later). Average age at entry into stages 3 and 4 occurred at 12.2 and 13.1 for breast development, and at 12.4 and 13.0 for pubic hair development, respectively.

(v) National Child Development Study

The National Child Development Study (NCDS), the second major longitudinal study measuring pubertal development in Great Britain, started as the Perinatal Mortality Survey. Information was gathered on all the individuals born in one week in England, Scotland and Wales in 1958. Since that year, six attempts have been made to gather information on study participants; two of these attempts (in 1969 [11 years of age] and in 1974 [16 years of age]), collected data on pubertal development. In the 1969 sweep, participants were examined by a medical officer, who determined Tanner stage of breast and of pubic hair development. In addition, the child's parent or guardian was asked whether the girl had started menstruating, and if so, at what age (before 5 years, between 5 and 8 years, 9 years, 10-10.5 years, 10.5-11 years, 11 years or older). In the 1974 sweep, there were significant changes in the questionnaire. Medical officers rated girls' breast development on a 3 stage basis (absent, intermediate, adult), and pubic hair development on a 4 stage basis (absent, sparse, intermediate, adult); axillary hair was assessed using the same stages. Age at menarche was entered in integer years, instead of in categories.[65]

For the 1969 sweep (age 11), information is available on pubic hair and breast development. The majority of girls assessed were in Tanner stage 1 (36.3%) or stage 2 (35.8%) of breast development, with fewer girls in stage 3 (20.5%), and very few in stages

4 and 5 (6.5% and 0.8%, respectively). Pubic hair development showed a similar distribution, with most girls assessed as stage 1 (41.3%) or stage 2 (36.3%). Fourteen percent of girls were in stage 3, 7% in stage 4, and fewer than one percent were in stage 5. More information was available in the 1974 sweep (age 16). Age at first menstruation was reported as either 12 or 13 years of age for most girls (22.8% and 31.6%, respectively). A handful of girls reported menarche at age 9, and 2% reported menarche at age 10. More girls reported later ages (age 14, 18.6%; age 15, 4.6%, age 16, 0.1%) and 2% had not yet begun menstruating. Virtually all girls were in either 'intermediate' or 'adult' stage of breast development (35.9% and 61.1%, respectively), with fewer than one percent having no breast development. A similar pattern was seen for both pubic hair and axillary hair development; the majority of girls were in the 'intermediate' stage (32% and 29.5%, respectively) or the 'adult' stage (56.6% and 47.7%, respectively), with few girls showing no pubic or axillary hair (0.3% and 2.2%, respectively). A larger proportion of girls were assessed as having 'sparse' axillary hair (17.1%) compared to pubic hair (5.8%).[65] Due to the cross-sectional nature and time lag between the NCDS sweeps, age at entry into Tanner stages can not be assessed. Also, since the first sweep occurred at 11 years of age, early developers were not characterized by the assessment of breast and pubic hair development.

(vi) Buckler Study

The most recent longitudinal study to assess pubertal development in Great Britain is that conducted by John Buckler. Buckler began his study in 1972, recruiting children aged 9-10 years attending state middle schools in Leeds, England (as well as one boy's boarding school in Berkshire; Table 2.4).[66] The study sites were selected to represent a variety of socio-economic environments, but analysis was limited to white children only.

Considerable loss to follow-up occurred during the study, in part due to matriculation into various high schools and to children leaving school at the age of 16. Children who were at an advanced stage of puberty at the time of enrollment were also excluded from analysis, thus preventing assessment of precocious developers. After these losses and exclusions, the final sample size was 102 girls.[66, 67] Participants were examined approximately three times a year in the schools' medical rooms; assessments were performed by Buckler for earlier ages of girls, and by his colleague J. Wild for later ages.[66] Pubertal development of girls was assessed using the Tanner stages for breast and pubic hair development, and by asking the girl to recall age at first menses. Tanner stages were used conventionally for some results, but in others are presented as a 10-stage system, allowing for intermediate stage of development between the 5 originally defined stages.

Buckler's results were largely consistent with the earlier study of Marshall and Tanner—the most commonly observed first sign of puberty was breast budding (93%), while for 4% of girls pubic hair development occurred first, and for 5% the two markers occurred simultaneously. The median age at entry into stage 2 was 11.05 for breast development, and 11.66 for pubic hair development, a difference of about 6 months. Median ages at entry into stages 3 and 4 were 12.10 and 13.01 for breast development, and 12.37 and 13.08 for pubic hair development, respectively. Ages at entry into stage 5 were not defined, although it was reported that for both boys and girls, time from initiation of puberty to full maturation was approximately 5 years. Menarche most often occurred when both breast and pubic hair development were in Tanner stage 4, and the median age at menarche was 13.27 (about 3 months earlier than observed in Marshall and Tanner's study).[67] Buckler also separated the cohort into 'early developers' (earliest 20% to reach

peak height velocity [PHV]), 'average age developers' (middle 60% to reach PHV), and 'late developers' (last 20% to reach PHV). Among girls, small sample size prevented detailed analyses, but early developers seemed to progress through the early stages of puberty more rapidly compared to average and late developers, but had a longer period of growth after menarche and achievement of PHV. Early developers experienced menarche about 1.3 years earlier than average developers (median ages of 12.02 and 13.30, respectively), and about 3 years earlier than late developers (14.87 years). Age at initiation of breast and pubic hair development, and age at entry into subsequent stages, followed a similar pattern, although the differences between the three groups were less pronounced.[67]

(7) Cross-sectional studies of pubertal development: United Kingdom

There have been several cross-sectional studies of female pubertal development in Great Britain. One of the first was conducted among matriculating university students at the University College of Swansea (1959-1970) and the University of Warwick (1971-1986).[68] During the required medical exam, girls were asked to recall their age at first menses. There appeared to be a small decrease in age at menarche over the study period, although the change in location and study population make direct yearly comparisons challenging. Restricting to Warwick students, the mean age at menarche was 12.83 (SD=1.28),[69] slightly lower than seen in Marshall and Tanner's study. Two further studies by Roberts among school girls in Cumbria[70] and Northumberland[71] showed similar results (mean ages of 13.31 (SD=0.06) and 13.31 (SD=0.03, respectively). In the 1970's, Billewicz et al assessed a group of girls in Newcastle upon Tyne, ranging in age from 9-17. The authors found that the mean age at thelarche was 10.78 (SD=1.63),[72] and the mean age at menarche was 13.37 (SD=1.14);[73] pubic hair development was not assessed. The most recently conducted

cross-sectional study was performed in 1998-1999, among school children attending secondary school in 10 British towns.[74] The median age at menarche was determined using probit analysis of self-reported menarcheal status; the median age was 12.9 (95% CI: 12.8-13.1), and did not vary significantly by region (Southern England, Northwest England, South Wales). Based on this and the previous cross-sectional studies, there appears to be a slight decrease in mean and median age at menarche, from 1959-1999.

(8) Methodological approaches for studies of pubertal development

A variety of approaches have been used in the analysis of data from longitudinal studies of pubertal development. Some of the challenges in analyzing data from longitudinal studies include accommodation of correlated effects arising from repeated measures on individuals, incorporation of time dependent covariates, incomplete data, and modeling of multiple outcomes.

Survival analysis is one of the most popular and flexible approaches, as it can be employed in parametric, semi-parametric, and non-parametric forms. Time dependent covariates can be included, by including interaction terms for the covariate with time. Further, incomplete data is accommodated in the form of censoring. Non-uniform times of entry into a study and loss to follow up are accounted for with right and left censoring, allowing maximal utility of longitudinal data. This is sufficient for many situations, when the time of event (i.e. developmental milestone, for example menarche) is known with some certainty. However, with other markers such as transition into Tanner stages of breast and pubic hair development, the exact age at which a girl enters a given stage is not known exactly. More commonly, the stage of development is observed or known only for certain points in time (such as clinic visits or assessments); for example, a girl may be in

stage 1 at the first clinic visit and stage 2 at the second. This is a case of interval censoring; although exact age at transition is not observed, it is known what stage a girl is in at different points in time, and mean (via parametric assumptions) and median ages at entry into each stage may be estimated for the population using interval censored survival analysis. One disadvantage of the survival analysis approach is that typically only one outcome is modeled at a time, while simultaneous modeling of related outcomes may be desirable. Extensions of survival analysis models are available for multiple outcomes, but generally take the form of stratified models, which remove the correlation between related outcomes rather than allowing estimation of the covariance structure between them.

A second approach, and one which has not been utilized in studies of growth and development, is ordinal probit analysis with repeated measures. Probit analysis is used when the observed outcome is a discrete variable characterizing an underlying continuous distribution. In standard probit analysis, there is a single event (for example, menarche) which is observed. In the case of multiple events, for example progression through the 5 Tanner stages of breast and pubic hair development, standard probit is not sufficient. It is reasonable to assume that breast and pubic hair development are both continuous processes; once a 'threshold' level of development is achieved, it is recognized as entry into the next Tanner stage. In this situation, where there are multiple ordered outcomes, an ordinal probit analysis is appropriate. However, in the case of longitudinal data, one must also consider the auto-correlation between observations made on the same individual. Hedeker and Giddons propose a random-effects ordinal probit model, which accommodates clustering of data and ordered outcomes.[75] Time dependent covariates may be included via interaction terms of the covariate with the time variable. This approach is more

comprehensive and flexible in some regards than survival analysis, in that all of the ordered events are considered at once instead of separately, utilizing all data from the study population in model estimation. It also allows calculation of probability estimates of each ordered event for a given vector of covariates. A potential drawback is the assumption that covariate effect estimates remain constant for each stage transition.

Parametric survival analysis will be used to evaluate the age distribution at entry into each Tanner stage, and at menarche. The introduction and application of ordinal probit analysis with repeated measures to longitudinal studies of pubertal development will provide a useful alternative to more common analytical approaches.

II. Environmental exposures

(1) Overview

Exposure to many persistent environmental chemicals is ubiquitous, but level of exposure varies across populations, and the effects of long-term and potentially low-level exposure are unknown. Exposure to environmental pollutants begins in utero and continues throughout life, affecting growth and development. In particular, persistent organic pollutants (POPs) are thought to impact a variety of reproductive and developmental outcomes. However, the evidence for the effect of such exposures on pubertal development in humans is limited, and sometimes conflicting. Human studies have generally used special populations to assess the effects of chemical exposures on development, including cohorts exposed to certain chemicals through industrial accidents. As one example, in 1978-1979, individuals in Yucheng, Taiwan were exposed to polychlorinated biphenyls (PCBs) via contaminated cooking oil.[76] This population has

been studied to determine health effects of high levels of exposure to PCBs. Variation in exposure also occurs by geographic location, occupation and socio-economic status. One example is the Faroe Islands, where residents have a high intake of seafood. This leads to dietary exposure to chemical compounds in contaminated seafood, including methylmercury and PCBs.[77] Exposure status also varies by age and time of assessment relative to the exposure incident. Timing of exposure, whether in utero or postnatal, may alter the effects of chemical exposure. Over time, policy measures to regulate the production and use of certain chemical compounds create a cohort effect when assessing exposure status.

"Organic" in this context simply means that these substances have a carbon based skeleton, and "persistent" indicates their resilience and slow degradation in the environment. POPs as a class of chemicals tend to have low water solubility and high lipid solubility; these properties allow POPs to accumulate in lipid tissue. Due to this lipophilic property, POPs may be stored for many years, and may be mobilized during pregnancy, breastfeeding, and weight loss. Many POPs are halogenated, often with chlorine groups, which increase environmental persistence. As molecular mass increases, POPs become more persistent, more toxic, and tend to have less reversible effects.

Due to their persistence, POPs have a tendency to accumulate over time in body tissue, and to "biomagnify." That is, as higher organisms consume lower organisms on up the food chain, the concentration of POPs increases, with consequent health effects also becoming more pronounced. In the US and in many other nations around the world, legislative action has been taken to reduce the production and use of some persistent pesticides and fungicides. One of the most famous examples is that of dichlorodiphenyltrichloroethane

(DDT), a synthetic pesticide. After the discovery of DDT's insecticidal abilities in 1939, DDT was heavily used both in the US and abroad. During World War II, DDT was used to control mosquito populations in Europe and the Pacific, and was also used in many anti-malaria campaigns. However, observations that DDT use seemed to coincide with reduced bird populations led to concern over the effects of DDT. Rachel Carson's book Silent Spring was published in 1962, amid growing public resistance to DDT.[78] In addition to its detrimental effects on wildlife, DDT has also been found to have a range of adverse effects in humans, including increased incidence of asthma and diabetes, increased risk of several cancers, and disruption of normal growth and development. Norway and Sweden were the first countries to ban DDT in 1970, and the US followed in 1972. Throughout the next decade, most developed nations also banned DDT, although the United Kingdom did not ban usage until 1984. DDT is still used in limited settings, particularly in areas where malaria and typhus are endemic. A "dirty dozen" list of POPs was compiled by the United Nations Environment Programme Governing Council in 1995, with the intent of investigating the health effects of 12 POPs, including DDT and PCBs.

(2) Brominated flame retardants

Brominated flame retardants (BFRs) are used in both industrial applications and consumer products. They comprise two main groups of chemicals—polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs), which have nearly identical structure, differing only by one oxygen atom linking two carbon rings. Both are also structurally similar to polychlorinated biphenyls, which are described below. PBBs were first produced in 1970, but were banned in 1979 due to increasing evidence of adverse health effects.[79] PBDEs, on the other hand, are still in widespread use. PBDEs are not

covalently bonded to the consumer products they are applied to, and over time dissociate and are released into household dust and other environmental media.[80] Humans are thus exposed dermally and through inhalation. Due to the wide variety in congener structure among BFRs, their activity may be estrogenic, anti-estrogenic or anti-androgenic.

(a) Evidence from animal studies

Endocrine disruption by BFRs has been demonstrated in multiple animal species (Table 2.5). Some effects of BFRs are believed to be mediated through binding of the aryl hydrocarbon receptor,[81, 82] similar to dioxin and other related compounds. Other potential mechanisms include direct estrogenic activity,[83] and disruption of thyroid hormone production.[84, 85] Rhesus monkeys exposed to a commercial mixture of PBBs exhibited altered progesterone production, leading to increased menstrual cycle length.[86] In Sprague-Dawley rats, direct estrogenic effect of PBDE 47 is suggested by the increase expression in CaBP-9k, as well as increased uterine wet weight in animals exposed pre-pubertally. A delay in vaginal opening was observed in Wistar rats exposed to PBDE 71, along with decreased serum T4, which suggest a thyroid-dependent mechanism.[85] A similar delay was seen for Long-Evans rats exposed to PBDE 99.[87]

(b) Evidence from human studies

The first evidence that POPs can impact timing of puberty came from the polybrominated biphenyl (PBB) contamination incident in Michigan in the 1970's (Table 2.6). Girls who were exposed to PBBs in utero and through breastfeeding began menarche approximately a year earlier than non-exposed girls, and also had earlier ages at pubic hair development.[88] Among women who had been exposed solely through dietary route, a decreased cycle

length was noted among those who had recently lost weight, and had high serum PBB levels.[89] There have been few studies assessing health effects of PBDE exposure in humans; however, a study of adolescents in the Netherlands did not find any association between PBDE exposure and age at menarche, or secondary sexual characteristics.[90]

(3) Pesticides, fungicides and dioxins

Pesticides and fungicides are substances used to prevent, control, or decrease damage done by pests and fungi. POPs in this class include the following pesticides: hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH), gamma- hexachlorocyclohexane (γ-HCH), p,p'-DDT, p,p'-DDE, o,p'-DDT, oxychlor, trans-nonachlor, mirex; and fungicides: prochloraz, fenarimol, ketoconazole and fadrozole.

Dioxins are chemicals formed as byproducts of the burning of organic material in the presence of chlorine. They are halogenated organic compounds, with a basic structure comprising a deoxygenated ring flanked by two benzene rings. The most toxic, and probably best recognized dioxin is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which was a contaminant of the herbicide Agent Orange, used during the Vietnam war. In the US, the current main sources of dioxins are diet, coal-fired plants, diesel fuel, and the burning of treated wood and waste.[91] Industrial accidents are another source of dioxin exposure, with one of the most important being the Seveso, Italy incident. In 1976, an industrial plant accidentally released approximately 1 kilogram of TCDD into the air; this resulted in airborne concentrations of ~100 ppm, and the highest known human exposures to TCDD.[92] TCDD is considered to be carcinogenic in both animals and humans,[93] and it, along with other dioxins, also has effects on other health outcomes including disorders of the nervous system,[94] thyroid,[95] endocrine (diabetes) and immune systems,[96, 97] and reproductive

function.[98, 99] Dioxins are also important because other chemicals, including some polychlorinated biphenyl (PCB) congeners, dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans, are considered 'dioxin like compounds' in terms of their chemical structure and activity.

(a) Evidence from animal studies

Persistent organic pesticides and fungicides, and dioxins may act as endocrine disruptors in a variety of ways: by mimicking a naturally occurring hormone, by blocking the effects of hormones, through alteration of gene expression, or by acting directly on the endocrine system. Another potential mode of action is steroidogenesis inhibition, which decreases the amount of endogenous hormone produced by competitively inhibiting the activity of steroidogenic enzymes. Fungicides including prochloraz, fenarimol, ketoconazole and fadrozole, are examples of steroidogenesis inhibitors; fadrazole and ketocanazole exposure have been shown to delay vaginal opening in female rats.[100] Alterations in gene expression may also affect pubertal growth and development; TCDD for example, binds aryl hydrocarbon receptors which then alters aryl hydrocarbon dependent gene expression.[101-103] In rodents, these changes have been shown to lead to malformations in the reproductive tract and disruption of reproductive parameters (Table 2.7).[104-108] High TCDD exposure has been associated with delayed vaginal opening in rats,[48, 109, 110] as well as in hamsters.[111] However, effects of TCDD on pubertal development may be influenced by dosage. A recent study exposing Long Evans rats pre-natally to high doses of TCDD found accelerated development; the authors speculate higher doses may act directly on the central nervous system,[112] accounting for the difference in their findings compared to those of Fenton et al,[48] Franczak et al[109] and Gray et al.[108]

(b) Evidence from human studies

DDT, one of the first environmental chemicals shown to affect the health of humans and animals, has been linked with changes in pubertal development in girls (Table 2.8). A US study found that high transplacental and lactational exposure to the DDT metabolite dichlorodiphenyldichloroethylene (DDE) via in utero exposure and breast feeding, was associated with earlier breast and pubic hair development. However, this finding was not statistically significant,[113] and a later study looking at serum levels of DDE found no association with timing of breast development.[114] Vasiliu et al found that in a cohort of girls born to mothers participating in the Michigan anglers' cohort, higher estimated maternal serum DDE at conception was associated with earlier age at menarche.[115] Similarly, a study of female textile workers in China found that high serum levels of DDT were associated with earlier age at menarche.[116] Den Hond et al found that increased serum dioxin in Belgian adolescents was associated with a delay in breast development.[117] Another Belgian study found that among girls with precocious puberty, foreign-born girls had higher serum DDE levels compared to native Belgian girls.[118] A study conducted in Seveso, Italy found a suggestion of earlier age at menarche among girls exposed to dioxin before the age of 5, but this finding was not significant.[119] Finally, similarly to the Belgian study by Den Hond et al, a study in a small group of Dutch girls found an association between pre-natal and lactational exposure to PCDDs, and delayed breast development.[90]

(4) Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are a class of commercially synthesized chemicals, and consist of 209 different congeners. Although PCBs have a wide range of industrial applications, the most prevalent use is in electronic equipment manufacture, where they act

as dielectric fluids (transformers, capacitors), coolants, lubricators and stabilizers. Production of PCBs was banned in the 1970's due to concerns about their toxic effects, but because of their persistence, levels of many PCB congeners are still elevated in the US population.

(a) Evidence from animal studies

Certain PCBs resemble dioxin in their structure and activity, and depending upon congener structure, may either mimic or block estrogen activity, with subsequent adverse reproductive effects.[120] Certain PCBs may also bind Ah receptors, with similar effects to those described for TCDD. While most studies have shown PCB exposure to delay vaginal opening in female rats,[121-124] some have shown an earlier age at vaginal opening among highly exposed rats (Table 2.9).[125, 126] In some instances, age at first estrus was also delayed in highly exposed rats.[123-125] First estrus in mink and first progesterone rise in goats are markers of attainment of sexual maturity; in mink, high transplacental and translactational PCB exposure has been associated with delayed age at first estrus.[127] Among goats, high transplacental exposure to PCB 153 was associated with delayed age at first progesterone rise.[128]

(b) Evidence from human studies

The evidence for effect of PCBs on female reproductive function is mixed (Table 2.10). A US study found that among North Carolina girls, high transplacental exposure was associated with earlier breast and pubic hair development, while high lacatational exposure was only associated with earlier pubic hair development, although these were not statistically significant associations.[113] Conversely, a study among girls in New York City

found that elevated PCB levels were associated with delayed breast development, but only among girls with BMI below the median for the group.[114] One study has reported earlier age at menarche among Mohawk Nation girls exposed to PCBs,[129] and in Taiwan girls poisoned by PCB contaminated cooking oil are reported to have abnormal menstrual bleeding twice as often as unexposed girls.[130] Axmon et al and Medola et al reported shortened duration of menstruation to be associated with PCB exposure,[131, 132] while Cooper et al reported increased cycle length.[133] Among female participants of the New York State Angler cohort study, exposure to both estrogenic and anti-estrogenic PCBs was associated with reduced fecundability ratio and longer time to pregnancy, although the confidence intervals included unity.[134] However, total PCB exposure was associated with increased fecundability and shorter time to pregnancy.

(5) Polyfluoroalkyl chemicals

Polyfluoroalkyl chemicals (PFCs) are a class of commercially synthesized chemicals, used as surfactants, surface coatings, and in other applications to decrease staining and sticking. The manufacture of PFCs began in the 1940's, and although there are many different polyfluoroalkyl chemicals, the two major congeners to which humans are exposed are perfluorooctanoic acid (PFOA; also called C8) and perfluorooctane sulfonate (PFOS). PFOA is found in non-stick coatings such as Teflon® (DuPont, Wilmington DE). PFOS is largely known for having been a main component of Scotchgard® (3M, St. Paul MN). Production of PFOS is being downregulated due to concerns about toxic effects.[135] Similarly, the 8 major producers of PFOA in the US have agreed to drastically reduce levels of PFOA in products under the EPA's 2010/2015 PFOA Stewardship Program.

However, due to the high persistence of PFCs and continued production of both PFOA and PFOS, there is ongoing exposure to both compounds.

(a) Evidence from animal studies

Animal studies have provided evidence for direct endocrine disruption after exposure to PFCs (Table 2.11). A study on the neuroendocrine effects of PFOS exposure in female rats found that exposed animals exhibited anorexia and subsequent decreased body weight, increased corticosterone and norepinephrine levels, decreased leptin levels, and disruptions in estrous cycles.[136] Perfluorododecanonic acid (PFDoA), a long chain PFC, has also been linked to decreased estradiol and altered expression of genes involved in estrogen production.[137] Direct endocrine disruption has also been observed in fish. Du et al demonstrated that zebrafish exposed to waterborne PFOS had growth delays (both somatic growth and gonad growth), increased rates of embryo malformation and mortality, and increased expression of the estrogen-responsive vitellogenin (VTG) gene.[138] The developmental toxicity of PFOA in animal studies appears to be somewhat similar to that of PFOS. Exposed male mice exhibit accelerated sexual maturation as indicated by preputial separation, despite decreased body weight; a slight delay in first estrus and vaginal opening was noted in female mice, but the difference was not significant. However, the effects may be strain-dependent; Yang et al demonstrated that in Balb/c mice, exposure to PFOA causes decreased mammary gland differentiation and uterine weight, as well as delayed vaginal opening.[139] In contrast, among C57BL/6 mice, opposing effects were seen at different doses; lower doses of PFOA caused increased mammary gland and uterine development, while higher doses had inhibitory effects and were also associated with delayed vaginal opening.[139] Further studies in mice have similarly demonstrated an

effect of PFOA exposure on mammary glands; F0 and F1 mice exposed to PFOA during pregnancy both showed stunted mammary epithelial branching and growth,[140] and incidence of mammary fibroadenomas was increased in rats exposed during adulthood.[141] In addition PFOA exposure in rats leads to decreased testosterone and increased estradiol.[142-144] Similarly to effects seen in fish exposed to PFOS, Wei et al found that exposure to PFOA caused increased expression of VTG and estrogen receptor beta (ER-β), both products of estrogen-receptive hepatic genes.[145] Further, exposure to PFOA in males caused oocytes to appear in the testes, and ovary degradation was observed among females.

(b) Evidence from human studies

Much of what is known about human health effects of exposure to PFCs is from studies of occupationally exposed cohorts (Table 2.12). Among workers at the 3M plant in Alabama, an increase in bladder cancer was reported, although rates of other cancers did not differ from that of the general US population.[146] Other studies reported alterations in both lipid and liver biomarkers.[147-149] Increased estradiol levels and 17[alpha]-hydroxyprogesterone (a testosterone precursor) levels were elevated in the most highly exposed male 3M employees.[150]

Since most occupational exposure to PFCs occurs among males, there is little known about effects specific to females (including reproductive function and development). It is not known whether PFCs are metabolized differentially by sex, as appears to be true for mice/rats. However, the C8 Science Panel and associated research program, which collects information on persons residing near the DuPont Washington Works plant in West Virginia, is currently conducting studies to determine health effects of exposure to PFOA released from the plant into the air and water.[151] Preliminary results indicate that serum

PFOA levels in this cohort are significantly higher than those for the general US population. Cholesterol levels in this cohort were elevated,[152] but there was no significant association with diabetes and PFOA exposure.[153] Some immunoglobulins were decreased as PFOA exposure increased (IgA, IgE), while others were increased (C reactive protein, antinuclear antibodies).[154] A study of birth outcomes showed no evidence for effect of either PFOA or PFOS on miscarriage or preterm delivery.[155] PFOS was associated with low birthweight, however, and both PFOA and PFOS were associated with preeclampsia and birth defects. Other studies are in progress to assess hormonal disorders in the cohort.

Recent studies from Denmark have demonstrated an association between maternal exposure to both PFOA and PFOS, and a decreased infant birthweight, ponderal index and head circumference,[156, 157] as well as increased time to pregnancy for exposed mothers.[158] However, there was no evidence of effect on APGAR score, or on maternally reported developmental milestones at 6 and 18 months of age for infants born to exposed mothers.[159] These findings were similar to a study of newborns in Maryland, which found that both PFOS and PFOA were associated with a decreased birthweight, ponderal index and head circumference.[160]

(6) Exposure to persistent organic pollutants in the United States

Evidence from nationally representative surveys and studies of special populations shows that there is significant exposure to POPs in the United States. Examples of occupationally exposed cohorts include factory workers exposed to PFCs,[151] agricultural workers exposed to various pesticides and fungicides,[161] and electronic recycling workers exposed to brominated flame retardants.[162] Exposure to many chemicals also occurs through non-occupational routes; consumers are exposed to PCBs and BFRs through handling of

consumer goods such as mattresses, carpets, furniture, and electronic devices.[163, 164] A retrospective comparison of serum samples taken from US residents in the Northwest and Southeast showed that PBDE levels are increasing over time, while PCB and PBB levels are decreasing. However, even though PBB and PCB production has been restricted, these chemicals are still present in most US residents due to a long half-life. In a nationally representative survey of US residents in 2003-2004,[165] PBDEs were detected in up to 97% of subjects, and PBB 153 was detected in 83% of subjects; PCBs were detected in up to 100% of subjects.[166] In this same population, there was considerable exposure to pesticides and fungicides as well, including hexachlorobenzene (99.9%), trans-nonachlor (92.6%), DDE (99.7%).[166] There is also significant PFC exposure among the US population, despite increased regulation and decreased production of PFOA and PFOS. When compared to the 1999-2000 US population, serum levels of PFOA had decreased by 25%, while PFOS had decreased by 32%. However, levels of both chemicals were detectable in over 98% of samples analyzed in the later cohort.[167] Other studies performed in the United States have shown that children in some communities may be more highly exposed to PFCs than adults.[168, 169]

One reason for higher PFC levels among children may be additional dietary exposure through breast milk.[170-176] A study performed among nursing mothers in Massachusetts found that PFOA and PFOS were detectable in 98% and 100% of milk samples, respectively; other compounds detected in these breast milk samples included PBDEs, DDTs, chlordane, hexachlorocyclohexane isomers, and hexachlorobenzene.[175] Levels of PFOA were higher among first-time nursing mothers compared to mothers who had nursed previously, and (unlike lipophilic POPs) concentration of PFCs in breast milk increased

over the first 6 months of nursing. This increase could be due to increased dietary consumption and changing composition of breast milk during this time period, and emphasizes the importance of lactation as a route of exposure to PFCs.[174] In comparison to breast milk samples from Asian and European countries, concentrations of PBDEs were between 10 and 100-fold greater, while pesticide levels were somewhat lower.[175]

(7) Exposure to persistent organic pollutants in the United Kingdom

As in the US, British residents may be exposed to POPs through both occupational and non-occupational routes. Although there is little systematic or representative assessment of exposure to environmental chemicals in the United Kingdom, there is evidence to suggest that residents do have measurable and significant exposure to many chemicals (Table 2.13). Thomas et al measured levels of various organohalogens in 154 volunteer blood donors from across the UK. PCBs 153, 180 and 138, along with hexachlorobenzene (HCB), p,p'-DDE, p,p'-DDT, and beta-hexachlorocyclohexane (HCH) were detected in most samples.[177] A recent analysis of breast milk samples from mothers residing in two regions of the United Kingdom also found evidence of exposure to POPs. Polybrominated diphenyl ethers (PBDEs), PCBs, and other organochlorine contaminants were detected, and PBDE levels were on average higher than those found in other European countries.[178] Further, there was regional variation; mothers residing in London had higher average levels of each contaminant compared to mothers residing in northwest England (Lancaster). The Food Standards Agency for the United Kingdom has also performed an analysis of dietary exposure to PFOA and PFOS.[179] PFOA was detected in potatoes (along with PFOS and ten other fluorinated compounds), while PFOS was also detected in canned vegetables, eggs, sugars, and preserved foods. Based on these results, the Agency estimated that on average,

UK adults intake 0.13 (+/- 0.03) and 0.07 (+/- 0.01) μg/kg/day of PFOS and PFOA, respectively. However, average intake varied by age group, with higher levels estimated for toddlers and school-aged children.

There is also evidence of exposure to POPs from other European countries. Several studies of mother-infant pairs have found measurable levels of contaminants (including PCBs) in breast milk and in maternal serum, in the Netherlands,[180] Germany,[181] and in Denmark.[77] Body burden of persistent chemicals such as PCBs does not appear to change during pregnancy[182, 183] so levels observed in samples taken at a single time point during gestation should be representative of levels through the duration of the pregnancy.

The Avon Longitudinal Study of Parents and Children (ALSPAC) is one of several study sites comprising the European Longitudinal Study of Parents and Children (ELSPAC). The United States Centers for Disease Control and Prevention (CDC) collaborated with ALSPAC researchers to conduct a pilot study of environmental exposures in the ALSPAC cohort. The goals of this pilot study were to determine the quality, volume and utility of available biological samples, and to estimate levels of environmental chemicals in a representative sample of participating mothers and children. Pooled serum and urine samples from 20 eligible mothers and urine samples from 20 eligible offspring (age 9, 11 boys and 9 girls) were analyzed. To avoid depleting the available biological samples, serum and urine from individuals not in the core ALSPAC cohort were selected. However, these individuals were representative of the ALSPAC study population. Pooled specimens were analyzed by the Division of Laboratory Sciences at the National Center for Environmental Health, Centers for Disease Control and Prevention. Samples were analyzed using analytical methods described in detail

elsewhere.[178, 184-188] Detectable levels of perfluorochemicals, persistent pesticides, and PCBs were present in maternal serum samples; further, levels of HCB, beta-HCH, oxychlor, p,p'-DDE, and PCBs 153, 180 and 138 were higher in the pooled ALSPAC samples than values for the US adult female population in 1999-2000,[189] and higher than previous UK studies (Table 2.13).[177, 178]

Table 2.1. Overview of precocious puberty.

Class	Gonadotropin levels	Sex steroid levels	Common causes	Treatment
Gonadotropin dependent	Elevated	Elevated	Idiopathic, congenital adrenal hyperplasia	GNRH agonist
Gonadotropin independent	Not elevated	Elevated	Disorder of gonad or adrenal gland, McCune-Albright syndrome	Aromatase inhibitor, anti-androgen

Table 2.2. Overview of delayed puberty.

Class	Gonadotropin levels	Sex steroid levels	Common causes	Treatment
Constitutional delay	Lowered	Lowered	Family history, history of slow growth	Usually none
Hypogonadotropichic hypogonadism	Lowered	Lowered	Kallman's syndrome, defects in GnRH releasing, FSH-β or LH-β genes	Estrogen/progesterone therapy
Hypergonadotrophic hypogonadism	Elevated	Lowered	Turner's syndrome, aromatase deficiency	Estrogen/progesterone therapy

Table 2.3. Longitudinal studies in the United States

Study	**Study period**	**Study population (n)**	**Method of assessment**	**Age at B2***	**Age at PH2***	**Age at menarche***
Bogalusa[52]	1973-1974	White and Black, LA (1680)	Visual inspection	White: 10.37 (0.105) Black: 10.22 (0.125)	White: 10.86 (0.095) Black: 10.13 (0.125)	White: 12.69 (0.085) Black: 12.83 (0.11)
Bogalusa[51]	1992-1994	White and Black, LA (653)	--	--	--	White: 12.5 (12.4-12.8) Black: 12.1 (11.9-12.3)
Fels[56]	1948	White, OH (49)	Photos	10.8 (1.1)	11.0 (1.1)	12.9 (1.4)
Lee[58]	1969-1974	Mainly white, MD (18)	Palpation, visual inspection	11.2 (1.6)	11.9 (1.5)	13.3 (1.3)
NHLBI[59, 60]	1987-1997	White and Black, CA (2379)†	Visual inspection	10.7 (0.7) in thelarche group	10.7 (0.9) in pubarche group	White: 12.7 Black: 12.0

*Age given is median when available, mean if median was not presented.
†Sample size for breast and pubic hair is 781

Table 2.4. Longitudinal studies in Europe

Study	**Study period**	**Study population (n)**	**Method of assessment**	**Age B2***	**Age PH2***	**Age menarche***
First Zurich[62]	1954-1980	White, Switzerland (142)	Visual inspection	10.9 (1.2)	10.4 (1.2)	13.4 (1.0)
Young-HUNT[63]	1995-2001	White, Norway (864)	--	--	--	13.2 (1.2)
Swedish longitudinal[64]	1955-1972	White, Swedish (90)	Photos	10.99 (0.04)	11.48 (0.04)	13.03 (0.03)
Harpenden[4]	1948-1971	White, UK (192)	Photos	11.15 (1.1)	11.69 (1.21)	13.47 (1.02)
Buckler[67]	1972-1979	White, UK (102)	Visual inspection	11.05	11.66	13.27

*Age given is median when available, mean if median was not presented.

Table 2.5. Animal studies of brominated flame retardants, and female pubertal development.

Chemical exposure	**Animal**	**Route of exposure**	**Doses**	**Findings for female development**
PBB mixture[86]	Rhesus monkeys	Post-pubertal	Daily 0.3, 1.5, or 25 ppm over 15 months	Altered progesterone levels, increased menstrual cycle length in 0.3 ppm group
PBDE 47[83]	Sprague-Dawley rats	Pre-pubertal	Single injection of 50, 100, or 200 mg/kg	Increased uterine weight, dose-dependent increase in CaBP-9k expression
PBDE 71[85]	Wistar rats	Pre-pubertal	Daily 3, 30, or 60 mg/kg postnatal d22-41	Delayed vaginal opening in highest dose group
PBDE 99[87]	Long-Evans rats	Transplacental, translactational	F0: Daily 1, 10 mg/kg gest d10-18	Delayed vaginal opening in higher dose group

Table 2.6. Human studies of brominated flame retardants, and female pubertal development.

Chemical exposure (biospecimen)	**Population (sample size)**	**Study Design (Endpoint assessed)**	**Findings for female development**
PBB (estimated in utero and lactational)[88]	US, MI girls aged 5-24 (n=328 for menarche, n=201 for Tanner stage)	Cross-sectional (Self-reported age at menarche and Tanner stage)	Accelerated age at menarche and pubic hair development among most highly exposed girls
PBB (estimated from previous serum measurements)[89]	US, MI women aged 24-56 (n=337)	Cross-sectional (self-reported menstrual cycle length)	Decreased cycle length, increased bleed length, among women with high PBB exposure and recent weight loss
PBDE (serum)[90]	Netherlands (n=18)	Cross-sectional (self-reported age at menarche, clinical assessment of Tanner stage)	No associations

Table 2.7. Animal studies of fungicides and dioxin, and female pubertal development.

Chemical exposure	**Animal**	**Route of exposure**	**Doses**	**Findings for female development**
Ketoconazole[100]	Sprague-Dawley rats	Pre-pubertal	Daily 24, 50, or 100 mg/kg postnatal d21-40	Delayed age at vaginal opening for highest dose only
Fadrazole[100]	Sprague-Dawley rats	Pre-pubertal	Daily 0.6, 1.2, or 6.0 mg/kg postnatal d21-40	Delayed age at vaginal opening
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[112]	Long Evans rats	Transplacental, translactational	F0: single; 200, 800 ng/kg gest d15	Earlier age at vaginal opening, first estrus, and accelerated ovarian compensatory hypertrophy (dose-dependent)
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[108]	Long Evans rats	Transplacental, translactational	F0: single; 0.05, 0.20, or 0.80 µg/kg gest d15	Delayed age at vaginal opening
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[111]	Syrian hamsters	Transplacental, translactational	F0: single, 2 µg/kg gest d11.5	Delayed age at vaginal opening
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[48]	Long Evans rats	Transplacental, translactational	F0: single, 1µg/kg gest d15	Delayed age at vaginal opening
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[109]	Sprague-Dawley rats	Pre-pubertal	Single, 10 µg/kg on postnatal d29	Delayed age at vaginal opening
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[190]	Holtzman rats	Transplacental, translactational	F0: single, 1 µg/kg gest d11, 15, 18	Delayed age at vaginal opening, but not significant
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[110]	Long Evans rats	Transplacental, translactational	F0: single, 1 µg/kg gest d8, 15	Delayed age at vaginal opening (not significant); vaginal opening occluded by vaginal threads

Table 2.8. Human studies of DDE, dioxin and dioxin like compounds, and female pubertal development.

Chemical exposure (biospecimen)	**Population (sample size)**	**Study Design (Endpoint assessed)**	**Findings for female development**
DDE (serum)[114]	US, NY girls (n=192)	Cross-sectional (Clinical assessment of Tanner stage)	No associations
DDE (maternal blood, cord blood and placenta averaged; breast milk)[191]	US, NC boys and girls (594)	Cohort (Menarche, Tanner stages via annual questionnaire)	Suggestion of earlier breast and pubic hair development with high transplacental and with high lactational exposure
DDE (serum)[118]	Girls with precocious puberty (foreign born [26] and Belgian [15])	Cross-sectional (Serum DDE)	Foreign-born girls with precocious puberty had significantly higher DDE concentration than native Belgian girls with precocious puberty
DDE (estimated maternal serum DDE at conception)[115]	US, MI daughters of women who consumed Great Lakes fish (151)	Cohort (Recalled age at menarche)	Higher DDE associated with earlier menarche
DDT/DDE (serum in adulthood)[116]	Chinese female textile workers, newly married (466)	Cross-sectional (Recalled age at menarche)	Higher DDT/DDE associated with earlier menarche
DDE (serum)[129]	US/Canada Mohawk Nation, girls aged 10-17 (138)	Cross-sectional (Menarche [yes/no])	No associations
Dioxin-like activity (serum; CALUX)[117]	17 yr old Belgian boys (78) and girls (120) from polluted and non-polluted areas.	Cross-sectional (Clinical assessment of Tanner stage, recalled age at menarche)	High exposure girls less likely to have reached the highest stage of breast development; no association with pubic hair development or age at menarche

Dioxin (serum)[119]	Seveso, Italy women exposed to dioxin post-natally/pre-puberty (282)	Cohort (Recalled age at menarche)	No associations, but suggestion of earlier menarche among girls exposed before the age of 5
PCDD (prenatal and lactational [breast milk], current [serum])[90]	Amsterdam, Netherlands girls aged 14-19 years (18)	Cohort (Tanner stage, recalled age at menarche)	High exposure to PCDD associated with delayed breast development; no association with pubic hair development or age at menarche

Table 2.9. Animal studies of PCBs and female pubertal development.

Chemical exposure	**Animal**	**Route of exposure**	**Doses**	**Findings for female development**
Aroclor 1254[125]	Wistar rats	Transplacental, translactational	F0: daily; 30 mg/kg for 1 month	Earlier age at vaginal opening; delayed age at first estrus
Aroclor 1254[192]	Long Evans rats	Transplacental	F0: daily; 10 mg/kg, gest d10-d18	No associations
PCB 77, 126[121]	Wistar rats	Transplacental	F0: single, 100 µg/kg (PCB77) or 10 µg/kg (PCB126) on gest d15	Delayed age at vaginal opening for PCB126
Aroclor 1221, 1242, 1254, 1260[193]	Sprague-Dawley rats	Neonatal	Single dose of 1 or 10 mg/kg on 1 or 2 day old neonates	Earlier age at vaginal opening in A1221 high dose group
PCB 153, 126[128]	Goats	Transplacental	F0: from gest d60 until parturition, 98 µg/kg/d (PCB153), 49 ng/kg/d (PCB126)	Delayed age at 1st progesterone rise for PCB 153
Clophen A50[122]	Dunkin-Hartley guinea pigs	Transplacental, translactational	F0: daily; 1.8-3.2 mg/kg, gest d18-d60	Delayed age at vaginal opening; delayed age at first ovulation not significant
PCB 126[123]	Sprague-Dawley rats	Transplacental	F0: daily; .025, 2.5, 250 ng/kg, 7.5 µg/kg, gest d13-19	Delayed age at vaginal opening in 250ng, 7.5µg; delayed age at first estrus in 2.5ng, 250ng, 7.5µg
PCBs in Great Lakes carp[127]	Mink	Transplacental, translactational	F0: 0.25, 0.5, or 1.0 ppm/d for approx 6 months	Delayed age at first estrus
Aroclor 1254[124]	Holtzman rats	Translactational	F0: 8, 32, 64 µg/g on d1,3,5,7,9 postpartum	Delayed age at vaginal opening in 32, 64 µg groups; delayed age at first

				estrus in 32, 64 µg groups
PCB 126, 138, 153, 180[126]	Sprague-Dawley rats	Transplacental, translactational	F0: 10 mg/kg gest d15-19	Earlier age at vaginal opening

Table 2.10. Human studies of PCBs and female pubertal development.

Chemical exposure (biospecimen)	**Population (sample size)**	**Study Design (Endpoint assessed)**	**Findings for female development**
PCBs (serum; congeners 118, 153, 138, 180)[114]	US, NY girls (n=192)	Cross-sectional (Clinical assessment of Tanner stage)	Delayed breast development among girls with high PCB exposure and low BMI
PCBs (serum; congeners 138, 153,180)[117]	17 yr old Belgium boys (78) and girls (120) from polluted and non-polluted areas.	Cross-sectional (Clinical assessment of Tanner stage, recalled age at menarche)	No associations
PCBs/PCDFs[76]	Taiwan, girls aged 13-19 exposed to contaminated oil (Yucheng) in utero (27) and controls (21)	Cohort; recalled age at menarche, menstrual cycle characteristics, serum hormones	No association with age at menarche; exposed girls reported shorter cycle length and more irregular cycles, and had higher levels of estradiol and FSH
PCBs (estimated maternal serum PCBs at conception)[115]	US, MI daughters of women who consumed Great Lakes fish (151)	Cohort (Recalled age at menarche)	No associations
PCBs (serum congeners)[129]	US/Canada Mohawk Nation, girls 10-17 (138)	Cross-sectional (Menarche [yes/no])	Exposure to estrogenic congeners associated with greater likelihood of menarche; no association with other congener groups
PCBs (maternal blood, cord blood and placenta averaged; breast milk)[113]	US, NC boys and girls (594)	Cohort (Menarche, Tanner stages via annual questionnaire)	Suggestion of earlier breast and pubic hair development with high transplacental, suggestion of earlier pubic hair development with lactational exposure
PCBs (maternal serum)[88]	US, MI girls aged 5-24 (256)	Cross-sectional (Self-assessment of Tanner stages; recalled age at menarche)	No associations

Dioxin-like PCBs (prenatal and lactational [breast milk], current [serum])[90]	Amsterdam, Netherlands girls aged 14-19 years (18)	Cohort (Tanner stage, recalled age at menarche)	No associations
PCBs (serum congeners)[134]	US, NY women aged 18-44 (n=83)	Cohort (time to pregnancy)	Suggestion of reduced fecundability odds ratios for estrogenic and anti-estrogenic PCBs and increased ratios for all other PCB congeners; significantly increased ratio for total serum PCB

Table 2.11. Animal studies of PFCs and female pubertal development.

Chemical exposure	**Animal**	**Route of exposure**	**Doses**	**Findings**
PFOS[136]	Sprague-Dawley rats	Injection	Daily; 1 or 10 mg/kg for 14 days	Disrupted estrous cyclicity
PFOA[194]	CD-1 mice	Oral gavage during pregnancy, transplacental	F0: daily; 5 mg/kg for 6-18 days (different timing)	Decreased mammary gland differentiation and branching
PFOA[144]	CD rats	In vitro (Leydig cells)	21 hours	Increased estradiol production
PFOA[142]	CD rats	Oral (dietary)	Daily; 2 years	Increased estradiol production; no change in testosterone, FSH, LH or prolactin
PFOA[139]	Balb/c mice	Oral gavage	Daily; 1, 5 or 10 mg/kg for 20 days	Decreased mammary gland differentiation, decreased uterine weight; delayed or absent vaginal opening
PFOA[139]	C57BL/6 mice	Oral gavage	Daily; 1, 5 or 10 mg/kg for 20 days	Decreased mammary gland differentiation and uterine weight at highest dose; increased differentiation and weight at lower doses; delayed or absent vaginal opening
PFOA[145]	Rare minnows	Waterborne	Daily; 3, 10, or 30 mg/L for 14 or 28 days	Degradation of ovaries
PFDoA[137]	Sprague-Dawley rats	Oral gavage	Daily; 0.5, 1.5 or 3 mg/kd for 28 days	Decreased estradiol (3 mg/kg), reduced estrogen receptor expression (0.5, 5 mg/kg)

Table 2.12. Human studies of PFCs and reproductive health effects.

Chemical exposure (biospecimen)	**Population (sample size)**	**Study Design (Endpoint assessed)**	**Results**
PFOA (serum)[150]	US, occupationally exposed male workers (n=123)	Cross-sectional (2 time points; cortisol, DHEAS, estradiol, FSH, 17[alpha]-hydroxyprogesterone, testosterone, LH, prolactin, TSH, sex hormone-binding globulin)	Increased estradiol among most highly exposed group; increased 17-hp in 1995 cohort
PFOS (job matrix)[195]	US, AL occupationally exposed workers (n=1400)	Retrospective cohort (cancer, liver disease, pregnancy outcomes)	No significant associations
PFOA and PFOS (serum)[152-155]	US, OH and WV residents potentially exposed through water and air (n varies by outcome)	Cohort (miscarriages, preeclampsia, PTD, LBW, birth defects; diabetes; cholesterol, LDL, HDL, triglycerides; IgA, IgG, IgE, IgM, ANA, CRP)	Increased odds of preeclampsia and birth defect; PFOS associated with LBW; cholesterol, LDL and triglyceride levels elevated; PFOA associated with elevated IgA and IgE and lowered CRP and ANA
PFOA, PFOS (1^{st} trimester serum, cord blood)[156]	Random sample of Danish National Birth Cohort participants (n=1400)	Retrospective cohort (Birthweight, gestation, stability of levels of PFOS and PFOA during pregnancy)	Increased levels among overweight/obese women and decreased levels with increasing parity; PFOA associated with low birthweight, PFOS and PFOA associated with preterm birth (3^{rd} and 2^{nd} quartiles, respectively); serum levels are stable over pregnancy, but cord blood levels significantly lower than maternal levels
PFOA and PFOS (serum)[158]	Denmark, pregnant women (n=1240)	Cohort (time to pregnancy)	Longer TTP, trend for lower fecundity

Table 2.13. Comparison of ALSPAC pilot study results to previous studies of exposure in the UK.

Analyte in ng/g lipid	**ALSPAC Pilot Study**	**Thomas[177]**	**Kalantzi[178]**	**CDC[189]**
Persistent organochlorine pesticides				
	Arithmetic mean of pooled samples	**Median (range)**	**Median (range)**	**Median**
Hexachlorobenzene (HCB)	50.31	11 (<4.8-72)	18 (<LOD-180)	<LOD (<31.74)
Beta-hexachlorocyclohexane (β-HCH)	74.24	12 (<0.68-80)	17 (1.2-1500)	<LOD (<6.76)
Gamma-hexachlorocyclohexane (γ-HCH)	4.18	<1.7 (<1.7-110)	0.6 (<LOD-7.7)	<LOD (<10.5)
p,p'-DDT	13.16	2.9 (<0.64-73)	6.2 (1.1-760)	<LOD (<17.4)
p,p'-DDE	302.49	100 (<11-2600)	150 (22-1600)	224
o,p'-DDT	0.95	<0.51 (<0.51-8.4)	0.6 (<LOD-55)	<LOD (<17.4)
Polychlorinated biphenyls				
PCB 138	43.16	27 (<7.1-110)	37 (4.2-100)	21.9
PCB 153	435.26	41 (<9.3-200)	49 (4.3-130)	32.9
PCB 180	44.20	33 (<4.7-200)	25 (1.8-120)	24.8
Polyfluoroalkyl chemicals				
Analyte in ng/mL	**ALSPAC Pilot Study**		**CDC[167]**	
	Arithmetic mean of pooled samples		**Median (95% CI)**	
PFOSA	0.3		<LOD (<0.2)	

Et-PFOSA-AcOH	0.6	<LOD (<0.2)
Me-PFOSA-AcOH	0.4	<LOD (<0.2)
PFOS	16.0	21.1 (19.8-22.4)
PFOA	2.9	4.0 (3.8-4.4)
PFHxS	1.9	1.9 (1.6-2.1)

Figure 2.1. Regulation of puberty initiation and progression by the hypothalamic-pituitary-gonadal (HPG) axis and the hypothalamic-pituitary-adrenal (HPA) axis.

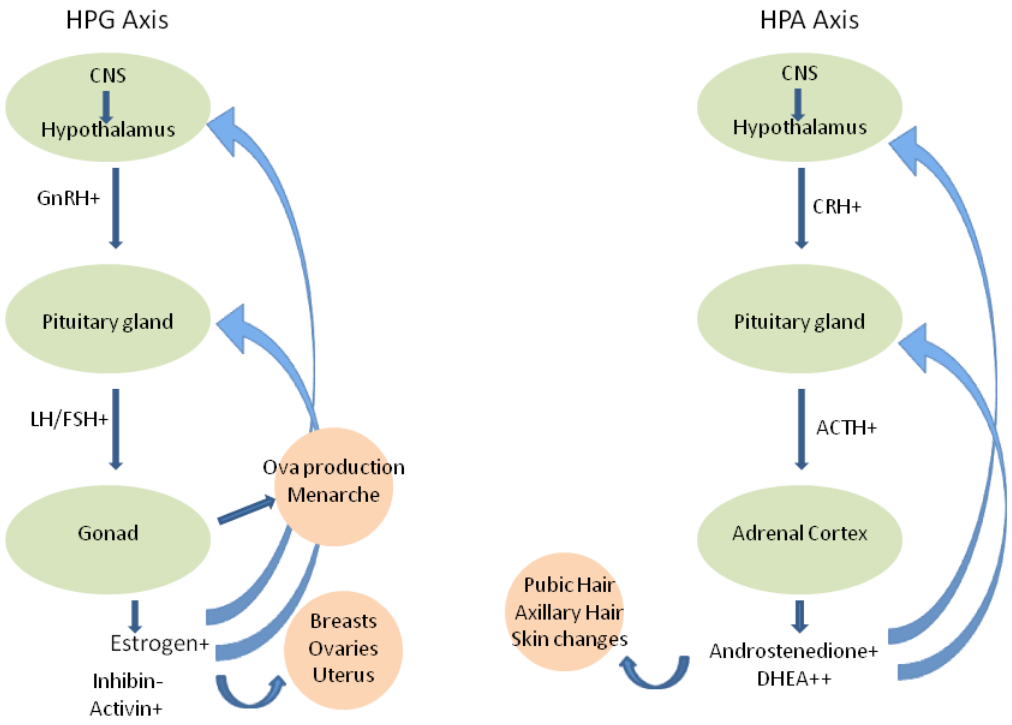
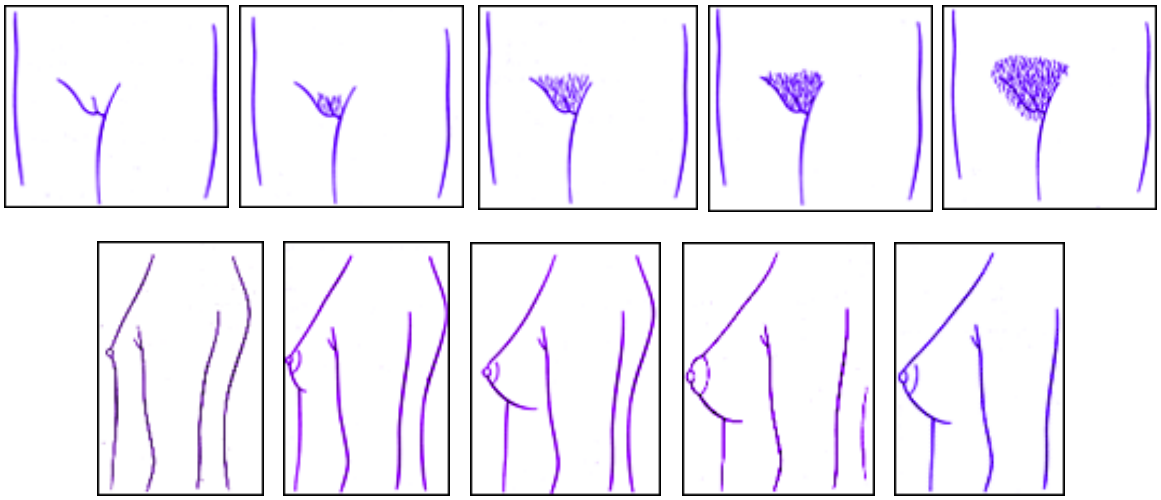


Figure 2.2. Tanner stages for female pubic hair and breast development. *Images used with permission from www.afraidtoask.com*



Chapter 3. Study Design

I. Study Design

(1) ALSPAC study population

The University of Bristol's Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective cohort study of 14,541 pregnant women. ALSPAC was designed to determine the effects of genetics, and the physical and social environment, upon health, behavior and development of children.

Women were eligible for inclusion if they were residing in Avon during pregnancy, and had an expected delivery date between April 1, 1991 and December 31, 1992.[196] General practitioners, obstetricians, pediatricians, and midwives were consulted during the design of enrollment materials and strategies, and methods for enrollment were developed including posters (displayed in chemist shops, libraries, mother and toddler groups, preschools, physician waiting rooms, antenatal clinics, etc.), approach during routine ultrasounds, mailed information from hospitals, approach during midwife consultations, media coverage (press, radio, and television), and approach after delivery. Once interested mothers sent back an initial information card or contacted the study office, a brochure explaining the study protocol and objectives was sent to the mothers. If the mother did not refuse participation at this point, the first questionnaire was mailed to her, with the choice of questionnaire dependent upon the mother's gestation.[196] In addition, study personnel were able to access medical and educational records for the

mother and child. Further information was collected using additional mailed questionnaires, and periodic clinic visits.

The study area is geographically well-defined, and includes women residing in the section of the county of Avon which is under the South West Regional Health Authority. This comprises a total population of approximately 1 million persons, and a range of environments (rural, urban [the city of Bristol], and suburban). The cohort was designed to be representative of the county of Avon, as well as Great Britain in 1991, as Avon was similar in many respects to the overall population of Great Britain at the time of the study inception. Compared to mothers in Great Britain, mothers in Avon were slightly more likely to own their own home (68.7% vs. 63.4%) and to have a car in the household (83.7% vs. 75.6%), and slightly less likely to be non-white (4.1% vs. 7.6%) and to have more than one person per bedroom (26.0% vs. 30.8%).[197] They were equally likely to be married (71.7% vs. 71.8%).[197] Compared to mother in Avon, mothers participating in ALSPAC were more likely to own their own home (79.1%), to have more than one person per bedroom (33.5%), to have a car in the household (90.8%), and to be married (79.4%). They were less likely to be non-white (2.2%).[197] Based on these comparisons, the study population is somewhat more heavily weighted toward more affluent mothers (based on housing, car, and marital status) and toward white mothers, both compared to Avon, and to Great Britain.

Of the initial cohort, 14,472 pregnancies had known outcomes, and 14,273 were singleton births, leading to at total of 14,676 fetuses in the cohort. Nearly all (95.8%) of the pregnancies led to live births, for a sample of 14,062 infants. Analyses were restricted to singleton births, who were included in the core sample of ALSPAC

(n=14,273). The number of participants varied by year, as the number of eligible participants returning the relevant questionnaires changed (details below; Table 3.1).

(2) ALSPAC data collection

(a) Pubertal development ascertainment

The Centers for Disease Control and Prevention (CDC) has collaborated with ALSPAC researchers to explore the association between environmental exposures and pubertal development; a 'Growing and Changing' questionnaire was developed and distributed to children annually starting at 8 years of age. The questionnaire collects information on age at menarche and Tanner stages of development. For all ages, parents or guardians could choose to complete the questionnaire themselves, complete it with their child or allow the child to complete it herself. Menarche is determined using the following questions: 'Has your daughter started her menstrual periods yet?' (parent-completed version) and 'Have you started your periods yet?' (child-completed version). If the answer was 'yes,' the questionnaire then asked 'How old (was your daughter/were you) when (she/you) had (her/your) first period?' and 'When exactly was (her/your) first period?' The former question is answered in integer number of years of age, and the latter is answered with the month and year of first period. Physical development is assessed using Tanner stages;[4] parents/guardians and girls completing the questionnaire were given a series of pictures showing different stages of breast and pubic hair development, along with written descriptions of each stage (Figure 3.1a, 3.1b).

These diagrams were developed at the University of North Carolina's Population Center, and have been previously validated.[198] Each characteristic (breast and pubic hair development) has 5 possible stages, ranging from pre-pubertal (stage 1) to fully

developed (stage 5). The parent/guardian or girl then selected the stage which most closely aligns with the girl's own stage of development.

There are some limitations to the Growing and Changing questionnaire. Since the stages of breast and pubic hair development are self-reported, there is increased potential for misclassification. Although Morris and Udry[198] reported good correlation between physician and self-assessment of breast and pubic hair development using the drawings included in the ALSPAC questionnaires, physician assessment is still considered a 'gold standard' in determining Tanner stage. In general, girls tend to overestimate stage in early puberty, and to underestimate stage in later puberty.[199] Another issue with self-reported stage is the effect of weight and adiposity on breast assessment; girls with greater levels of adipose tissue may be mistakenly categorized as having more advanced breast development.[32] The difference between adipose and breast tissue is evident only with palpation, not with visual inspection alone. Menarche status and age at menarche are less likely to be misclassified in self-report, due to the 'hard' nature of this outcome, and the short period of time elapsed between event and recall.[200]

Response rates were greatest in the earlier years of questionnaire mailing (Table 3.2). Some children answered questionnaires out of the intended age range (for example, a 9-year old may have returned the 8-year questionnaire); these were excluded, so that only responses in the correct age range were analyzed. From years 8-14, 4462 girls returned at least one Growing and Changing questionnaire.

Among those eligible girls who did return Growing and Changing questionnaires, some did not report valid information on breast development, pubic hair development, and/or menarche status. Relatively few (<5%) questionnaires had missing information;

the greatest amount of missing information on breast development was seen in the 8-year questionnaire, while the greatest amount of missing information on pubic hair development was seen in the 14 year questionnaires; menarche information was missing most often on the 11-year questionnaire.

After excluding girls with missing or out of range (i.e. >5) responses for breast and pubic hair Tanner stages, girls who regressed in stage were identified. Girls who reported a stage of development ≥ 2 in year x, then a lower stage in year y>x were flagged as having 'gone backward' in developmental stage. Among all girls, there were 320 girls who 'went backward' in breast development, and 223 girls who 'went backward' in pubic hair development. Girls who reported having achieved menarche in year x, then reported not yet having had their first period in year y>x were flagged as having 'gone backward' in menarche status. Among all girls, there were 17 girls who 'went backward' in menarche status.

Age at menarche was self-reported on the Growing and Changing questionnaire. There were 15 girls who reported an age at menarche that was greater than the age at completion of questionnaire; for 13 of these girls, the difference was only 1 month. There were 1160 girls who reported more than one age at menarche (in different questionnaires). Of these, 667 girls reported discrepant ages at menarche. The discrepancies ranged from -59 months to 49 months; 65% of these girls (n=434) reported discrepancies of less than 1 year, while 35% (n=233) reported discrepancies of a year or more. In the case that more than one age at menarche was reported, the first reported age at menarche will be used in all analyses, on the assumption that it is closer to the time of event and therefore more accurately reported.[23]

(b) Environmental exposure ascertainment

Biologic samples were collected from mothers (urine and blood) during pregnancy. The selection of samples for analyses is described in the 'Objectives and Methods' chapter. Maternal serum samples will be analyzed for persistent pesticides and fungicides, and PCBs. Serum sample analyses will be performed at the Division of Laboratory Sciences of the National Center for Environmental Health (NCEH), Centers for Disease Control and Prevention (CDC). Since serum levels of POPs should be relatively stable throughout pregnancy, the earliest available serum sample will be taken for each mother. Requested samples will be shipped to the NCEH in Atlanta, GA, and analyzed using gas chromatography.[201] For this dissertation, the following polyfluoroalkyl chemicals will be analyzed: perfluorooctane sulfonamide (PFOSA), 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH), 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), perfluorononanoate (PFNA), and perfluorodecanoate (PFDeA).

(c) Additional variables of interest

Additional variables of interest have been collected through maternal questionnaires and clinic visits. These include mother's pre-pregnancy BMI (<18.5 [underweight], 18.5-24.9 [normal], 25-29.9 [overweight], and ≥30 [obese]), mother's age at delivery (<20, 20-29, ≥30), mother's age at menarche (8-11, 12-14, 15+), mother's ethnic background (white, Black Caribbean, Black African, Black other, Indian, Pakistani, Bangladeshi, Chinese, other), mother's educational level (CSE/none, vocational, O-level, A-level,

degree), mother's social class (lower, middle, upper), gestational diabetes (1st trimester, 2nd/3rd trimester, 1st and 2nd/3rd trimester, none), breastfeeding (any, none), child's birthweight (<2500 g, ≥2500 g), child's ethnic background (white, non-white), birth order (first born, second born, third born or later) and child's BMI at ages 8-11, 13, and 14 (<5th percentile for month of age [underweight], 5th-85th percentile [normal], 85th-95th percentile [overweight], and ≥95th percentile [obese]). Percentiles of BMI for age were defined using female-specific growth standards from a representative sample from the United Kingdom.[202] In addition, child's BMI at age 8 was obtained from the Focus at 8 clinic visit; because child's height and weight were measured by a health professional at the clinic, the BMI data from this visit was used when available, and self-reported BMI data from the Growing and Changing questionnaire were used for children whose clinic data were not available.

Some of these variables had missing information; for non-time dependent variables, missing information included: mother's education (n=158, 4.0%), mother's age at menarche (n=538, 13.7%), mother's social class (n=738, 18.7%), mother's pre-pregnancy BMI (n=382, 9.7%), girl's birth order (n=100, 2.5%), girl's birthweight (n=54, 1.4%), girl's race/ethnicity (n=218, 5.5%) and girl's initiation pathway (n=417, 10.6%). Girl's BMI at 8 years of age (combined clinic and self-reported data) was missing for 969 (27.5%) of girls. Information was missing for some questionnaire-dependent observations, including girl's BMI (n=4399, 31.4%) and exercise level (n=279, 2.0%) at a given age.

Human subject protection for these analyses was assessed and approved by the ALSPAC Law and Ethics Committee, the Local Research Ethics Committees, and the CDC Institutional Review Board.

(3) NHANES study population

For this dissertation, information from the 2003-2004 cycle of the NHANES are used to describe POP exposure among women of reproductive age, and effect of these exposures on age at menarche. The National Health and Nutrition Examination Survey (NHANES) is a series of complex sample surveys of the civilian, non-institutionalized US population.[203] Information from NHANES participants may be used to generate nationally representative, robust estimates. Certain groups are over-sampled, including adolescents and those ≥60 years of age, African Americans and Mexican Americans. Information on age, sex, race/ethnicity, and other demographic characteristics are collected in household interviews; information on health status indicators and biological samples are collected during a medical examination.

Over 10,000 people participated in the 2003-2004 NHANES (Figure 3.2). Of these, just over half (50.9%) were female, and 2088 were within the desired age range of 12-40 years. The lower limit of 12 years was chosen because lab analysis was only performed on participants aged 12 and over; the upper limit of 40 years was chosen because most of the POPs assessed were not in widespread production until the 1970's. Therefore, older women would not have been exposed until after menarche. A small number of women were interviewed but did not undergo the full examination (n=69), leaving a final sample size of 2019 women. The weighted sample size is 58,840,778 (standard deviation=4,251,374) women.

(4) NHANES data collection

(a) Pubertal development ascertainment

Self-reported age at menarche in integer years was collected in the Reproductive Health Questionnaire, administered during the household interview to participants aged ≥12 years. Women were asked "How old were you when you had your first menstrual period?" Information on breast and pubic hair development is not available in this questionnaire.

(b) Environmental exposure ascertainment

Biological samples are collected from NHANES participants, including serum.[204] For participants aged ≥12 years, serum samples were analyzed for different groups of POPs, including coplanar compounds (dioxins, furans, and coplanar PCBs), non-dioxin like PCBs, PBDEs, 2,2',4,4',5,5'-hexabromobiphenyl (PBB 153), and PFCs. Each group of chemicals was analyzed in a 1/3 subsample of the NHANES cohort, with non-overlapping subsample assignment.

(c) Other variables of interest

To investigate possible influence of secular trends in exposure to POPs and in age at menarche, age at screening, household poverty income ratio (PIR; calculated as the ratio of family income to poverty threshold) and race/ethnicity were included as potential confounders and effect modifiers. Age at screening was dichotomized as 12-25 and 26-40 years of age; PIR was categorized as low (<1), medium (1 to <2), or high (≥2). Race/ethnicity was coded as non-Hispanic white, non-Hispanic Black, Mexican American, other/mixed, and other Hispanic. The other/mixed category included those

with unknown race. PIR was missing for 97 records (4.8%) while race/ethnicity was coded as other/mixed for 82 records (4.1%).

Table 3.1. ALSPAC participant eligibility

Year	Subjects	Singletons	Singletons in core*	Returned questionnaire†	Correct age range	Females
8	15024	14628	14273	5906	5836	3073
9	15024	14628	14273	6596	6411	3331
10	14962	14566	14273	6199	5946	3120
11	14941	14547	14273	5889	5727	3018
13	15140	14742	14273	5677	5636	2955
14	5158	5036	4814	4814	4480	2490

*There was no 'in_core' variable on the 10 year and 11 year datasets; the 10 and 11 year data were merged with the 8 and 9 year old data to determine core status.
†There was no 'in_pub' variable on the 13 year or the 14 year dataset to identify those who actually returned a questionnaire. For the 13 year dataset, the variable pub585 was used instead (indicates questionnaire was returned, not blank). For the 14 year dataset, only those individuals who had a value of '1' for pub685 (i.e. the questionnaire was returned, not blank), had been included in the dataset sent from ALSPAC.

Table 3.2. ALSPAC participant response rates by year

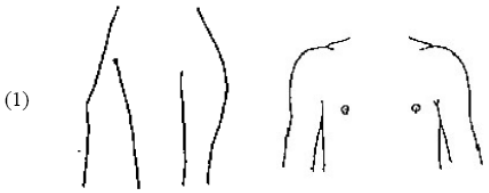
Year	Females	Missing breast stage	Unsure of breast stage	Missing pubic hair stage	Unsure of pubic hair stage	Missing menarche status
	N	N (%)	N (%)	N (%)	N (%)	N (%)
8	3073	114 (3.7)	0 (0)	34 (1.1)	0 (0)	22 (0.7)
9	3331	44 (1.3)	0 (0)	32 (1.0)	0 (0)	26 (0.8)
10	3120	31 (1.0)	6 (0.2)	22 (0.7)	22 (0.7)	19 (0.6)
11	3018	36 (1.2)	24 (0.8)	39 (1.3)	84 (2.8)	28 (0.9)
13	2955	71 (2.4)	79 (2.7)	86 (2.9)	228 (7.7)	13 (0.4)
14	2490	65 (2.6)	0 (0)	91 (3.7)	0 (0)	12 (0.5)
TOTAL	17987	361 (2.0)	109 (0.6)	304 (1.7)	334 (1.9)	120 (0.7)

Figure 3.1a. Tanner stages of breast development.

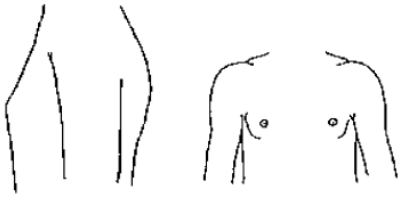
SECTION B (girls)

*The drawings below show stages of the way the **breasts** develop. A girl can go through each of the five stages shown, although some girls skip some stages. Please look at each of the drawings. It is also important to read the descriptions.*

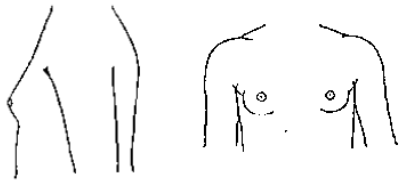
*Put a tick in the box to the right of the drawing that is **closest** to your daughter's current breast stage.*



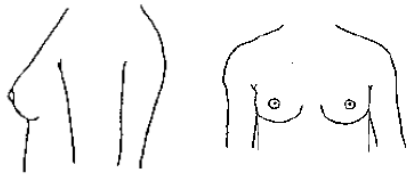
(1) *The nipple is raised a little in this stage.*
The rest of the breast is still flat



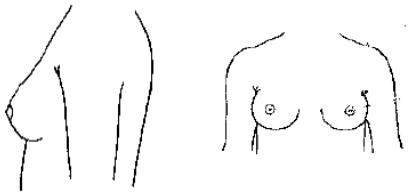
(2) *This is the breast bud stage.*
In this stage the nipple is raised more than in stage 1. The breast is a small mound.
The dark area around the nipple (areola) is larger than in stage 1.



(3) *The areola and the breast are both larger than in stage 2. The areola does not stick out away from the breast.*



(4) *The areola and the nipple make up a mound that sticks up above the shape of the breast. (Note: This stage may not happen at all for some girls. Some girls develop from stage 3 to stage 5 with no stage 4.)*



(5) *This is the mature adult stage. The breasts are fully developed. Only the nipple sticks out in this stage. The areola has moved back in the general shape of the breast.*

(6) *Not sure*

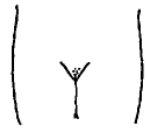
Figure 3.1b. Tanner stages of pubic hair development.

SECTION C (girls)

*The drawings below show different amounts of **female pubic hair**. A girl can go through each of the five stages shown. Please look at each of the drawings. It is also important to read the descriptions.*

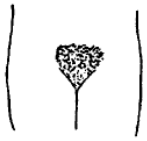
Put a tick in the box to the right of the drawing that is the closest to the amount of pubic hair your daughter has.

(1)  *There is no pubic hair*

(2)  *There is a little long, lightly coloured hair. This hair may be straight or a little curly.*

(3)  *The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a bigger area.*

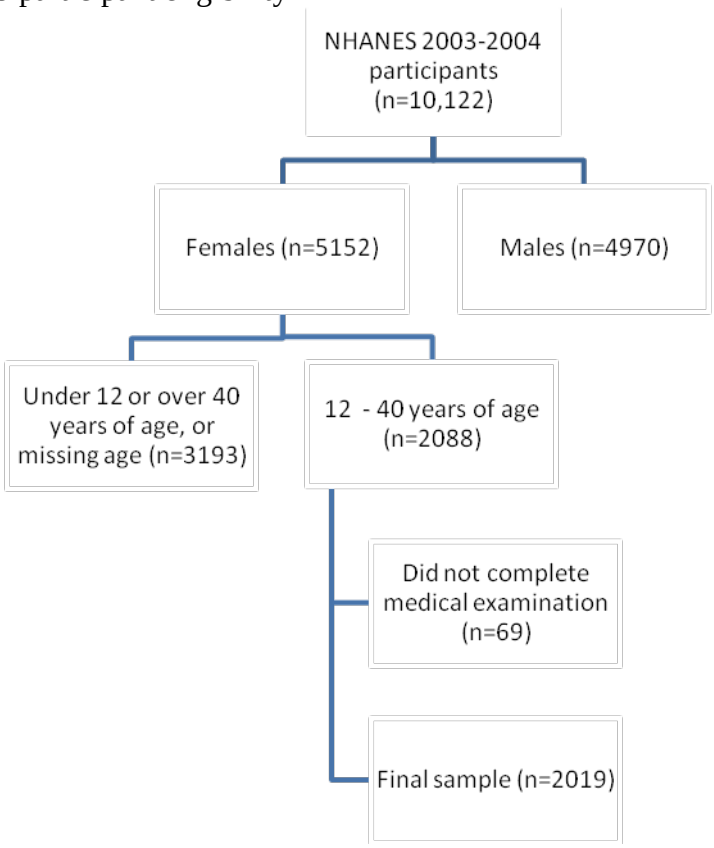
(4)  *The hair is now as dark, curly, and coarse as that of an adult woman. However, the area that the hair covers is not as large as that of an adult woman. The hair has not spread out to the legs.*

(5)  *The hair now is like that of an adult woman. It also covers the same area as that of an adult woman. The hair usually forms a triangular pattern as it spreads out to the legs.*

(6) *Not sure*

NOTE: Your daughter's pubic hair stage may or may not be the same as her stage of breast development.

Figure 3.2. NHANES participant eligibility



Chapter 4. Research Objectives and Methods

This dissertation has two research objectives: first, to describe patterns of pubertal initiation and progression in a contemporary population of girls in Avon, England and second, to determine the association between exposure to persistent organic pollutants and age at menarche.

I. Objective 1

(1) Initiation of pubertal development

The first aim of this dissertation is to explore patterns of pubertal development in the ALSPAC cohort, using the markers of menarche, pubic hair and breast development. Information from the 'Growing and Changing' questionnaire was used to describe initiation and progression of pubertal development of girls participating in ALSPAC; in addition, an alternative technique for analysis of pubertal developmental data is introduced.

(a) Initiation pathways

Initiation pathway, whether synchronous, thelarche, or pubarche, has been shown to vary among populations and is associated with differences in progression of pubertal development. The first research question for objective (1) is to determine the proportion of girls reporting synchronous, thelarche, and pubarche pathways in the ALSPAC cohort. This question was answered by examining the combined years of data, and identifying the first instance in which a girl reported being in stage 2 or higher for breast and for pubic hair development. If the initial report of being in a stage ≥ 2 was reported for breast development only or for pubic hair development only, the girl was categorized as being

in the thelarche or the pubarche pathway, respectively. If the initial report of being in stage ≥2 was for both breast and pubic hair development, the girl was categorized as being in the synchronous pathway. Age at menarche was compared among groups using a parametric survival model with initiation pathway included as a predictor. Factors assessed for potential association with initiation pathway include the following maternal and child characteristics: mother's pre-pregnancy BMI (<18.5 [underweight], 18.5-24.9 [normal], 25-29.9 [overweight], and ≥30 [obese]), mother's age at delivery (<20, 20-29, ≥30), mother's age at menarche (8-11, 12-14, 15+), mother's ethnic background (white, Black Caribbean, Black African, Black other, Indian, Pakistani, Bangladeshi, Chinese, other), mother's educational level (CSE/none, vocational, O-level, A-level, degree), mother's social class (lower, middle, upper), gestational diabetes (1^{st} trimester, $2^{nd}/3^{rd}$ trimester, 1^{st} and $2^{nd}/3^{rd}$ trimester, none), breastfeeding (any, none), child's birthweight (<2500 g, ≥2500 g), child's ethnic background (white, non-white), birth order (first born, second born, third born or later), and child's BMI at age 8 ($<5^{th}$ percentile [underweight], 5^{th}-85^{th} percentile [normal], 85^{th}-95^{th} percentile [overweight], and $\geq 95^{th}$ percentile [obese]). Percentiles of BMI for month of age were defined using British reference percentiles from female-specific growth standards.[202]

Polytomous logistic regression models were used to determine which of these factors were significantly associated with initiation type. Polytomous logistic regression allows the modeling of multiple, nominal outcomes (i.e. initiation pathway), and leads to estimates of odds ratios and associated confidence intervals. The outcome modeled is the log odds of the g^{th} outcome, where g ranges from 1 to m; the exponentiated beta

coefficients are interpreted as odds ratios. In this case there are 3 outcomes, representing the 3 initiation pathways.

$$\ln\left(\frac{P(D=g \mid x)}{P(D=0 \mid x)}\right) = \alpha_g + \beta_{gj} t_j$$
$$g = 1....m,\ j = 1...n$$

Each characteristic was entered into the logistic regression model singly; those that were associated with initiation pathway at the $p<0.30$ level in univariate analysis ere assessed for effect modification using appropriate interaction terms. These variables and any significant interaction terms were then entered into a multivariate logistic regression model; an alpha of 0.05 was used to identify characteristics to be retained in the full model, using a backward selection algorithm. Finally, the analysis was repeated excluding BMI as a covariate, due to the large number of girls missing information on BMI at 8 years of age (27.5%).

Since the ALSPAC data were collected on an annual basis, the exact age at which a girl entered a stage of development is not known. However, it is known what stage a girl reports at different points in time (i.e. the questionnaire administration times); this may be thought of as a special case of ordinary survival time data, in which there is interval censoring. That is, the event (entry into a stage of development) is known to occur between two questionnaires (not necessarily subsequent), although the timing of the event within the interval is not observed. Girls' information could also be right or left censored, if the ending and starting points of the interval were not known, respectively. The likelihood model accommodating right, left and interval censoring is as follows:

$$L = \Pi_{i=1}^{n}\{F_i(t_{Li})^{\delta_{Li}}[1-F_i(t_{Ri})]^{\delta_{Ri}}[F_i(t_{Ri})-F_i(t_{Li})]^{\delta I}\}$$

where t_{Li} indicates left-censoring time, t_{Ri} right-censoring time, and δ as in indicator denoting right (R), left (L), and interval censoring at time t_i.[205] This survival model was used to estimate median age at entry into each stage (2-5) of breast and of pubic hair development, separately, assuming a normal distribution. Median age at entry into stage 2 was compared between breast and pubic hair for all girls, and median age at entry into stages 2 of the initial marker of development was estimated for each initiation pathway. Age at entry is not compared between pathways, as it is not independent from pathway assignment. That is, girls who experience asynchronous initiation of puberty by definition have, on average, earlier age at initiation compared to their counterparts experiencing synchronous initiation.

(b) Distribution of Tanner stages and age at menarche

The second research question is to describe the distribution of Tanner stages, and of age at menarche in the ALSPAC cohort. Using the Growing and Changing questionnaire data, the proportion of girls in each stage of breast and of pubic hair development, and the proportion of girls reporting menarche, were determined for each year. Combining data across years, the distribution of ages for girls reporting each stage of breast and of pubic hair development, and the distribution of age at menarche were estimated. Age at menarche was compared for the three initiation pathway groups using survival analysis models including initiation pathway as an independent predictor.

(c) Characterization of the correlation between age at entry into breast and pubic hair development

It is reasonable to think that the age at which a girl enters breast development (i.e. enters breast stage 2), and the age at entry into pubic hair development, are not independent. When looking at these two outcomes separately (as in the parametric survival analyses outlined above), age at the time of each event may be treated as a survival time, and is thus a random variable following some distribution. In the above analyses, the univariate normal distribution is used. In order to characterize the relationship between ages at the two events, a natural extension is the bivariate normal distribution. Parametric survival analyses allow the estimation of mean and median ages at each event, as well as standard deviations for age at event. However, the correlation between ages at occurrence of the two distinct events is not easily obtained. Most extensions of survival analysis models to multiple outcomes rely on stratification or competing risk scenarios, in which the correlation is either accounted for but not estimated, or only one event is observed per individual (so that correlation is not estimable). A further difficulty is introduced by interval censoring, where the event is known to occur within a certain interval, but the exact timing of the event is not known. In order to estimate the covariance structure Σ of ages at the time of occurrence of two distinct and observed events, a maximum likelihood approach is used, with modification to accommodate the interval censored nature of the available data.

In the general case, a pair of event times, defined as x for the first event and y for the second event, follow some distribution f(x, y). The cumulative distribution function

(CDF) of the paired variables, F(x, y) = P(X≤x, Y≤y). In the case that x and y follow a bivariate normal distribution, f(x, y) takes the following form:

$$f(x,y)=\frac{1}{2\pi\sigma_x\sigma_y\sqrt{(1-\rho^2}}\exp\left[\frac{-1}{2(1-\rho^2)}[\left(\frac{x-\mu_x}{\sigma_x}\right)^2+\left(\frac{y-\mu_y}{\sigma_y}\right)^2-2\rho\left(\frac{x-\mu_x}{\sigma_x}\right)\left(\frac{y-\mu_y}{\sigma_y}\right)]\right]$$

while F(x, y) takes the following form:

$$F(x,y)=\int\int\ f(x,y)dxdy$$

As there is no closed expression for F(x, y), the double integrand is approximated, usually using iterative, numerical techniques.

However, x and y are not observed; rather, for each individual, x and y are known to fall within a certain interval; in the case of left and right censoring, the endpoint of the interval is negative or positive infinity, respectively. These intervals will be indicated as (L1, R1) for the interval containing x, and (L2, R2) for the interval containing y. Given these intervals, a region can be obtained which contains the pair of event times (x, y) as follows: [F(R1, R2) – F(R1, L2) – F(L1, R2) + F(L1, L2)].[206-208] Evaluating the bivariate normal CDF for the area contained in this region of support approximates the likelihood contribution for each individual. For this step, the PROBBNRM function in SAS is used to estimate the CDF at each point defining the region of support. To obtain a likelihood function for the cohort, the individual likelihood contributions are multiplied together, and the resulting function maximized with respect to Σ. In order to increase efficiency without loss of specificity, two alternatives are: (1) maximize the natural log of the likelihood, (2) minimize the negative of the natural log of the likelihood. Taking the

natural log of the likelihood transforms products into summation, and (due to the monotonicity of the log function) yields the same optimum value of the variables of interest. This method was used to estimate the correlation coefficient and corresponding covariance, between ages at entry into breast and pubic hair stage 2.

(2) Progression of pubertal development

(a) Concordance of stages

Concordance and discordance of stages were assessed both cross-sectionally and longitudinally. For each year, the proportion of girls who are in the same stage for breast and pubic hair development (concordant), 1 stage apart (mildly discordant), 2-3 stages apart (moderately discordant) and 4 stages apart (extremely discordant), was determined.

(b) Progression through stages of development

To characterize the factors affecting breast and pubic hair stage, a repeated-measures ordinal probit model was used to model the probability of being in each of the Tanner stages for the two markers, given a certain configuration of covariates (including age). Probit analysis is used when the observed outcome variable—in this case, Tanner stage—is a discrete variable characterizing an underlying continuous distribution. It is reasonable to assume that breast and pubic hair development are both continuous processes; once a 'threshold' level of development is achieved, it is recognized as entry into the next Tanner stage. Since there are 5 Tanner stages (corresponding to 4 thresholds [entry into stages 2-5]), with an ordered progression to the stages, an ordinal probit analysis is appropriate.[209] In the ALSPAC data, individuals have multiple observations; this within-individual correlation must be accounted for when modeling

breast and pubic hair stage. Hedeker and Giddons propose a random-effects ordinal probit model, which accommodates such clustering of data.[75] Given this random-effects ordinal probit model for the underlying continuous variable, the probability that a response Y occurs in category k or lower takes the following form:

$$P(Y_j \leq k \mid \beta_0, \beta_1) = \Phi[(y_k - z_j)/\beta_0]$$

Where Φ represents the standard normal cumulative distribution function, and $z_{ij} = x_{ij}\beta_1 + w_{ij}\beta_0$. In the probit formulation, $\gamma_1 = 0$ and $\sigma = 1$. The y term represents the mean value of the intercept (i.e. the 'cutoff' value for the underlying variable) for transition into stage k, while the β coefficients represent the change in the Z-score (unit is standard deviations), for each one-unit increase in the covariate x. Thus, a positive coefficient value indicates an increased probability of being in higher stages, while negative coefficient values indicate increased probability of being in lower stages.

The GLIMMIX procedure in SAS was used to fit random-effects ordinal probit models, where Tanner stage of breast or pubic hair development was the ordinal outcome, and correlation within girl is accounted for using random effects. First, models were fit which incorporate data from all girls as follows:

$$y_{ij} = \beta_0 + \beta_1 t_j + u_i + e_{ij}$$
$$i = 1....m,\ j = 1...n$$

where there are *m* girls, who each have n_i assessments (this number will vary by individual). y_{ij} represents the underlying, continuous variable describing breast or pubic hair development, for girl i at age j; however, we only observe the discrete values indexed by Tanner stage for breast and for pubic hair development. Therefore, in the

actual model, y_{ij} is entered as a discrete integer with possible values of 1 through 5. β_0 is the intercept, β_1 is the fixed effect for age at assessment, and t_j represents the age at assessment for girl i. The model also contains a normally distributed random effect u_i for each individual, to account for within subject correlation across assessments. The error term (e_{ij}) is normally distributed with mean 0 and variance σ_e^2. The thresholds of the underlying continuous variables describing breast and pubic hair development are estimated, representing the values at which the individual is identified as having entered into Tanner stages 2 through 5. These thresholds are treated as fixed effects (i.e. they do not change depending on the individual being assessed).

From the random-effects ordinal probit model, probability of being in each breast and pubic hair stage, for a given age, is calculated as follows:

$$P(Y_j = k \mid \beta_0, \beta_1) = \Phi[(y_k - z_j)/\beta_0] - \Phi[(y_{k-1} - z_j)/\beta_0$$

These probabilities are compared to those resulting from parametric survival analysis (objective 1(a)), in order to assess agreement between the two methods.

Next, this model is extended to incorporate the effect of covariates as follows:

$$y_{ij} = \beta_0 + \beta_1 t_j + \beta_2 g_{i2} + \ldots + \beta_k g_{ik} + u_i + e_{ij}$$
$$i = 1 \ldots . m,\ j = 1 \ldots n$$

In addition to terms included in the previous model, there are additional terms ($\beta_k g_i$) to represent the effects of maternal and child characteristics (as listed above) which may affect probability of being in each Tanner stage. β_k represents the effect of the kth covariate, while g_{ik} represents the covariate value for girl i. Covariates were assessed singly (along with age) for association with breast or pubic hair stage; factors associated

at the alpha=0.30 level were assessed for effect modification by age by the inclusion of appropriate interaction terms. Next, a multivariate model was fit including all covariates significant in preliminary analyses (including interaction terms). An alpha of 0.05 was used to select covariates remaining in the multivariate model.

As described above, parametric survival analysis was used to estimate median age at entry into stages 2-4 of breast and of pubic hair development. Estimation of age at entry into each stage also allows for the calculation of average time spent in a stage (by taking the difference between age at entry into subsequent stages), and duration of puberty (by taking the difference between age at entry into stage 2 and menarche). This is appropriate since the normal distribution is assumed when modeling ages at entry into each stage, and age at menarche and therefore, the difference of the means is the mean difference. Median ages at entry into stages 2-4 were compared between breast and pubic hair for all girls, and median age at entry into stages 3-4 were compared by initiation pathway.

II. Objective 2

The second research objective, to determine the association between exposure to persistent organic pollutants and age at menarche, will take the form of two studies. First, a nested case-control study of *in utero* exposure to polyfluoroalkyl chemicals and age at menarche was conducted among girls participating in the ALSPAC. Second, NHANES data were used to describe the association between exposure to PFCs, PCBs, dioxins and brominated flame retardants, and age at menarche.

(1) Nested case control study of *in utero* exposure to PFCs and age at menarche

(a) Study objective

The first component of the second objective of this dissertation is to compare *in utero* exposure to polyfluoroalkyl chemicals (PFCs) for girls experiencing earlier menarche, relative to their peers. This analysis was performed as a nested case-control study within the prospective cohort of ALSPAC.

Biologic samples were collected from mothers (urine and blood) during pregnancy. Since serum levels of PFCs are relatively stable throughout pregnancy,[156] the earliest available serum sample is used in the event that multiple samples are available. Exposure in utero is biologically relevant because this represents the period of organ and brain development; further, the fetus is more susceptible to exposures due to lack of a complete blood-brain barrier, absence of metabolizing enzymes, and genes which are ordinarily under steroid control may be prematurely activated.

The specific PFCs analyzed were: perfluorooctane sulfonamide (PFOSA), 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH), 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), perfluorononanoate (PFNA), and perfluorodecanoate (PFDeA). The following categories were used to group PFC analytes, based on chemical structure: sulfonamide (PFOSA), sulfonamide esters (Et-PFOSA-AcOH, Me-PFOSA-AcOH), sulfonates (PFOS, PFHxS), and carboxylates (PFDeA, PFNA, PFOA).

(b) Study design

Case and control series were selected from girls who had completed at least two puberty staging questionnaires (including self-assessed Tanner stage of pubic hair and breast development, and reported menarcheal status/age at menarche) between the ages of 8 and 13 (5 possible questionnaires returned). Girls meeting eligibility criteria were ordered according to reported age at menarche, at the time of the 13 year old data became available. A cut-off of 11.5 years was established as defining 'earlier' menarche. Of girls reporting menarche before the age of 11.5 (n=338), 71% (n=240) of these had at least one maternal serum sample available. Among these, 90.8% (n=218) of the serum samples were viable for analysis. These 218 girls represent the cases.

Among girls who reported menarche at or after the age of 11.5, a random sample of 394 was chosen. Of these potential controls, 71.6% (n=282) had at least one maternal serum sample available, of which 81.6% (n=230) were viable for analysis. These 230 girls represent the controls. Controls were randomly selected from the cohort of non-cases so that the case-control study population may be weighted to represent the entire study population.

Potential effect modifiers, or those covariates which alter the association between exposure and outcome, were identified during the analysis stage from variables previously reported to be associated with age at menarche and/or exposure to PFCs. Potential confounders, or those covariates which are associated with both exposure and outcome and may distort estimated measures of association, were identified after effect modification was assessed.

From previous analyses of the ALSPAC data and other studies of development, age at menarche has been reported to be associated with maternal age at menarche,[210, 211] maternal pre-pregnancy BMI, maternal smoking in the first trimester,[212] socio-economic status, [213-215] birth order,[216] race/ethnicity,[30, 51, 61, 217] and girl's BMI.[18, 19] In order to be considered as a potential confounder, these variables must also be associated with maternal exposure to PFCs, and must not be in the causal pathway between exposure and outcome. Variables meeting these criteria include maternal age at menarche, socio-economic status, birth order, and race/ethnicity. Maternal age at menarche may be related to maternal PFC exposure, if exposure in utero is a proxy for chronic exposure to chemicals, and is not in causal pathway. Socio-economic status is potentially related to exposure, through association with area of residence, and occupational exposures related to education/occupation. It is also not likely to be in the causal pathway. Birth order is related to PFC exposure in utero; previous studies have found that maternal PFC concentration decreases over the course of pregnancy, possibly due to changing PFC pharmacokinetics and tissue disposition,[218] and plancental transfer to the fetus.[219] Birth order is not likely to be in the causal pathway. Race/ethnicity is potentially related to exposure status, as it is often a proxy for socio-economic status and consequently of occupation, education and residence, and is not likely to be in the causal pathway. Other of these variables which are associated with the outcome (age at menarche), are not likely to be related to exposure. These include maternal smoking during pregnancy, and maternal pre-pregnancy BMI. Birthweight and girl's BMI are related to both exposure and outcome, but may be in the causal pathway, and therefore do not meet the criteria for confounding. Therefore, the following variables from the ALSPAC cohort were

evaluated for potential effect modification and confounding: mother's pre-pregnancy BMI (<18.5 [underweight], 18.5-24.9 [normal], 25-29.9 [overweight], and ≥30 [obese]), mother's age at delivery (<20, 20-29, ≥30), mother's age at menarche (8-11, 12-14, 15+), mother's educational level (CSE/none, vocational, O-level, A-level, degree), mother's social class (lower, middle, upper), child's ethnic background (white, non-white), and birth order (first born, second born, third born or later).

The power for this analysis depends on the prevalence of exposure (high levels of POP exposure in utero) in the control group, as well as effect size (Table 4.1). For an assumed prevalence of exposure of 0.30 among cases and an odds ratio of 2.0, power to detect a statistically significant association is 0.94. If prevalence of exposure increases to 0.40, or to .5 we will have at least 80% power to detect odds ratios of 1.7 and 1.8 respectively. Given the results of previous research on the effect of POPs on female pubertal development,[88, 116, 117] effect measures of this magnitude or greater were expected.

(c) Analysis

Maternal serum samples were collected from storage facilities at the University of Bristol, and sent to the NCEH at CDC in Atlanta, GA. Samples were then analyzed using gas chromatography.[201] As with many environmental exposures, distribution of the PFC analytes was skewed, and a natural log transformation was used to approximate normality in continuous analyses. In addition, each analyte was treated as a binary exposure, with values categorized as being either at or above the median of values among cases, or below the median.

Analysis of the case-control study yields odds ratios as the main effect measures. To accommodate a multivariate analysis, the LOGISTIC procedure in SAS was used to fit both unadjusted and adjusted models. The following regression model was used to compare PFC exposure between cases and controls. L_i is the log odds of having early menarche for girl *i*:

$$L_i = \beta_0 + \beta_1 X_{1i} + \sum_{a=1}^{k_1} \delta_a Y_{ai}$$
$$a = 1,\ldots,k_1$$
$$i = 1,\ldots,m$$

where there are *m* girls. β_0 is the intercept, β_1 is the effect of exposure to high levels of POPs in utero, and X_{1i} represents the exposure status for girl i. δ_a are effects of the k_1 covariates for each of *m* girls and Y_{ai} represents the value of the a^{th} covariate for girl i. Each covariate described above was assessed for evidence of effect modification and confounding using the modeling methods outlined by Kleinbaum.[220] Association was first assessed in bivariate logistic models (including the main exposure, plus the potential effect modifier), with an alpha=0.30. If an association was found at this stage, effect modification was assessed using appropriate interaction terms. Variables (including interaction terms) found to be associated with case status in bivariate analyses were then entered into a multivariate logistic model and assessed for inclusion, using an alpha of 0.05. Potential confounders and effect modifiers were selected using a theory based approach, as outlined above.

(2) Effect of POP exposure on age at menarche

(a) Study objective

The second component of the second objective of this dissertation is to describe exposure to polyfluoroalkyl chemicals, polychlorinated biphenyls, dioxins and brominated flame retardants, among reproductive age women in the United States, and the effect of these exposures on age at menarche. This analysis was performed using information from the 2003-2004 National Health and Nutrition Examination Survey.

(b) Study design

Self-reported age at menarche in integer years was collected in the Reproductive Health Questionnaire, also administered during the household interview to participants aged ≥12 years. Women were asked "How old were you when you had your first menstrual period?" Demographic information was collected during the same household interview, including age, race/ethnicity, education, marital status, and household income and size.

In addition to demographic information, biological samples were also collected.[204] For participants aged ≥12 years, serum samples were analyzed for different groups of POPs, including coplanar compounds (dioxins, furans, and coplanar PCBs), non-dioxin like PCBs, PBDEs, 2,2',4,4',5,5'-hexabromobiphenyl (PBB 153), and PFCs. Each group of chemicals was analyzed in a 1/3 subsample of the NHANES cohort, with non-overlapping subsample assignment. Since some of these compounds were not produced until the 1970's, the analysis was restricted to women aged 12-40 years at the time of screening. For analytes detectable in at least 30% of serum samples, values below the limit of detection (LOD) were replaced by (LOD/√2). Analytes detectable in fewer than

30% of serum samples were not included in further congener-specific analyses; in analyses of summed congeners, values below the LOD were treated as missing. In addition, toxic equivalents (TEQs) were calculated for mono-ortho substituted PCBs, dibenzo-p-dioxins, and chlorinated dibenzofurans, using the 2005 World Health Organizations categorization and toxic equivalency factor (TEF) values.[221] PCB 114 and 123 were not included in the mono-ortho substituted PCB TEQ analysis because they were not measured in the NHANES cohort.

(c) Analysis

Analyses were performed in SAS and SAS-callable SUDAAN to account for the complex sample design, utilizing appropriate weights provided by NHANES.[222] Level of each POP was examined overall, as well as by race/ethnicity, age at screening and poverty income ratio. Medians, quartiles and associated confidence intervals were calculated using SAS, while geometric means and associated confidence intervals were calculated using SAS-callable SUDAAN. To determine association with age at menarche, survival analysis was used where self-reported age at menarche is the outcome, and each POP considered as a separate exposure. Survival analysis models yield hazard rates and hazard ratios as the main effect measure, as in the following model:

$$h(t) = h_0(t)^{(\beta_1 z_1 + \ldots + \beta_m z_m)}$$

Where h(t) denotes the hazard rate for a given time, or the probability of failure conditional upon survival up until the given time, $h_0(t)$ is the baseline hazard, β the effect of each covariate included in the model, and z the value of these covariates for an

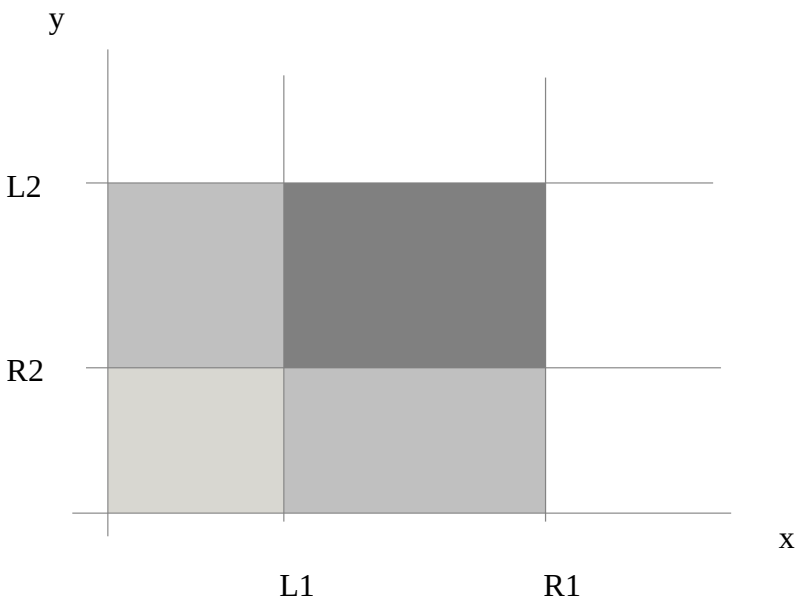
individual. Hazard ratios are obtained by dividing the hazard in one group (i.e. the exposed) by the hazard rate in another group (i.e. the unexposed).

Lipid-adjusted serum concentration of each POP (except for PFCs and TEQs, which are not lipid-adjusted) were modeled first as continuous (after taking a natural log transformation), then as binary (above or below the median serum level) exposures. Race/ethnicity, PIR, and age at screening were considered as potential confounders and effect modifiers. Each of these factors is associated with age at menarche, the outcome.[30, 39, 223] These variables are also likely to be associated with exposure to environmental chemicals, and are not likely to be in the causal pathway between such exposures and age at menarche. Association with the exposure was determined using t-tests, comparing the mean lipid-adjusted serum level of each analyte among the race, age and income groups. If any two groups had mean values that were significantly different at the $p<0.05$ level, the variable was considered to be associated with the exposure, and therefore a potential confounder. Appropriate confounders were then included in analyte specific models. Effect modification was assessed using interaction terms; if an interaction term was significant in the multivariate model at the $p<0.05$ level, effect modification was considered to be present, and stratified results were presented.

Table 4.1. Power calculations for nested case-control study

P_0	Odds Ratio	Power
0.20	1.70	0.67
0.20	2.00	0.89
0.30	1.70	0.77
0.30	2.00	0.94
0.40	1.70	0.80
0.40	2.00	0.96

Figure 4.1. The most darkly shaded area indicates the region of support for the likelihood function



Chapter 5. Initiation of puberty in girls enrolled in a contemporary British cohort

I. Introduction

In females, the first visible signs of pubertal development are breast and pubic hair development.[5, 6] These processes are governed by two separate physiologic systems (the hypothalamic pituitary gonadal axis and hypothalamic pituitary adrenal axis, respectively), so breast and pubic hair development do not necessarily occur at the same time, or at the same rate of progression. A majority of girls are thought to experience relatively synchronous pubarche and thelarche, with the first appearance of pubic hair and breast budding occurring within a few months of each other (or observed at the same clinic visit); however, some girls will begin pubarche without corresponding thelarche and vice versa;[4, 59, 62, 64] this is often referred to as asynchronous development. Different initiation pathways may reflect differential exposures, both external environmental exposures and exposure to endogenous hormones, and timing of pubertal milestones may impact future health outcomes including risk for overweight/obesity and breast cancer.[59, 224-226] Despite the importance of the initiation of puberty, the process and factors which impact pathway and timing, have not been well described in a contemporary cohort.

Among participants in the Fels longitudinal study in the United States (1948), the majority of girls (85.8%) experienced asynchronous development, with over half of girls entering breast development before pubarche (Table 5.1).[56] A Swedish study (1976) found that while 47% of girls experienced synchronous breast and pubic hair development, nearly half (45%) of girls had thelarche preceding pubarche.[64] An opposite pattern was found in a Swiss study (1983), where over half of girls (53%) were reported to have experienced pubarche without breast development.[62] A more recent (2003) study in the US using clinical assessments of pubertal stage found that 51.6% of eligible girls experienced asynchronous development.[59] Further, those girls who experienced thelarche as the initial visible marker of

pubertal development had an earlier age at menarche compared to girls who experienced pubarche as the initial marker (12.6 years and 13.1 years, respectively), and were also more likely to have higher BMIs before and throughout puberty. Some reasons for the differences between studies with regards to pathway distribution may include a biased sampling frame, sample size, and method for pubertal staging. However, these studies suggest that asynchronous initiation of secondary sexual characteristic development may be relatively common, and an important predictor of age at menarche and future health outcomes. Puberty is occurring earlier than in the past, based on data from the late 1800's to present.[39, 40, 227, 228] Timing of maturation is important, as accelerated development has psychological and physiological consequences, including earlier age at first sexual activity, increased risk-taking behavior (including smoking, drinking, and use of illicit drugs), increased depression, and decreased physical activity.[42-46] Early age at menarche is also associated with increased risk of adult obesity[224, 225] and of breast cancer.[48] In this study, data from a contemporary, longitudinal cohort is used to describe initiation of secondary sexual characteristic development.

II. Methods

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective cohort study of ~14,000 pregnant women residing in Avon who had an expected delivery date between April 1, 1991 and December 31, 1992.[196] Details of the study design have been published and are available online.[196, 197] A 'Growing and Changing' questionnaire was developed to collect information on pubertal development; the questionnaire was distributed to participants at the ages of 8, 9, 10, 11, 13 and 14 years (1999-2005).[196, 229] Questionnaires were not distributed at age 12. For all ages, parents or guardians could choose to complete the questionnaire

themselves, complete it with their child or allow the child to complete it herself. Menarche status (and if appropriate, age at menarche) was determined via self-report. Physical development was assessed using diagrams of Tanner stages;[4] these diagrams were developed at the University of North Carolina's Population Center, and have been previously validated and successfully used in other cohort studies.[113, 198, 230] Each characteristic (breast and pubic hair development) has 5 possible stages, ranging from pre-pubertal (stage 1) to fully developed (stage 5). The respondent then selected the stage which most closely aligns with the girl's current stage of development.

For these analyses, the cohort consisted of female singletons completing at least one puberty questionnaire in the appropriate age range (i.e. only girls 8 years of age included for the 8 year old questionnaire). Girls who reported a certain stage of breast or pubic hair development on one questionnaire, then reported a lower stage of development on a subsequent questionnaire, were excluded from these analyses. Girls who reported achieving menarche on a questionnaire, but reported not yet having their first period on a subsequent questionnaire, were also excluded. If more than one age at menarche was given, the first reported age at menarche was used in analysis on the assumption that it was the most accurate report. If reported age at menarche was greater than age at questionnaire completion, age at menarche was treated as missing.

Initiation pathway was determined by identifying the first instance in which a girl reported being in stage 2 or higher for breast and/or for pubic hair development. If the initial report of being in a stage ≥ 2 was reported for only breast development or for only pubic hair development, the girl was categorized as being in the thelarche or

the pubarche pathway, respectively. If the initial report of being in stage≥2 was for both breast and pubic hair development, the girl was categorized as being in the synchronous pathway.

Median ages at entry into breast stage 2 and pubic hair stage 2 were determined using parametric survival analysis, assuming a normal distribution. Various other distributions were used for comparison, and similar results were found; therefore, only the normal distribution results are reported here. Since the ALSPAC data were collected on an annual basis, the exact age at which a girl entered a stage of breast of pubic hair development is not known. Rather, it is known what stage a girl is in at a particular point in time (i.e. at the time each questionnaire is completed), leading to interval censoring. If the start of the interval is not known, data are considered left censored, and if the end of the interval is not known (i.e. the event is not observed), data are considered right censored. The LIFEREG procedure in SAS accommodates right, left and interval censoring, and was used to perform all parametric survival analyses. Age at menarche was compared between initiation pathways using a regression model with initiation pathway included as a predictor, while proportion of girls reporting menarche by year 14 was compared using a chi-square test.

Polytomous logistic regression models were used to identify maternal and child characteristics associated with initiation pathway (thelarche, pubarche or synchronous). The candidate variables were identified from the literature as being associated with pubertal development [i.e. breast and/or pubic hair development, and/or menarche], and included mother's pre-pregnancy BMI, mother's age at delivery, mother's age at menarche, mother's educational level, mother's social class,

child's birthweight, child's race/ethnicity, child's birth order, and child's height and BMI at 8 years of age. Social class was derived using the 1991 Office of Population Censuses and Surveys,[231] Upper class consisted of classes I (professional occupations) or II (managerial and technical occupations); middle class of classes IIINM (non-manual skilled occupations) or IIIM (manual skilled occupations); and lower class of classes IV (partly skilled occupations) or V (unskilled occupations). Height and BMI at 8 years of age was obtained from clinic visit data when available (71.3% and 66.9% of girls, respectively), and from self-report at the 8-year Growing and Changing questionnaire where clinic data was not available (3.4% and 5.6% of girls, respectively). Percentiles of height and BMI for month of age were defined using female-specific growth standards from a representative sample from the UK.[202] The association of each candidate variable with initiation pathway was assessed using a polytomous logistic regression model, and assessed for association with pathway using a cutoff of $p<0.30$. Then, each variable that met this criterion in the univariate analysis was assessed for effect modification, by testing all 2-way interaction terms with each other covariate separately in polytomous logistic regression models. All variables that were associated with pathway in the univariate analysis, and any relevant interaction terms were then entered into a multivariate logistic regression model. A p-value of 0.05 was used to identify characteristics that remained associated with pathway in this model, using a stepwise selection method. Finally, the analysis was repeated excluding BMI as a covariate, due to the large number of girls missing information on BMI at 8 years of age (27.5%). We did not adjust for multiple comparisons.

Human subject protection was assessed and approved by the ALSPAC Law and Ethics Committee, the Local Research Ethics Committees, and the CDC Institutional Review Board.

III. Results

In the ALSPAC cohort, 4462 singleton girls completed and returned at least one Growing and Changing questionnaire in the correct age range. Of these, 4434 had submitted information on breast development, and 4427 on pubic hair development, for at least one questionnaire. A small number of girls returned inconsistent reports of menarche status (n=17). More girls had inconsistent reports of breast development (n=320, 7.2%) or pubic hair development (n=223, 5.0%), for a final sample size of 3938 girls (Figure 5.1). Initiation pathway could not be determined for some individuals, who did not progress beyond Tanner stage 1 for breast and pubic hair development (n=417); these participants were excluded from pathway-specific analyses.

Nearly all (96%) of girls were white, and the majority of girls were born to a mother with an O-level education (roughly equivalent to a United States high school diploma; 35.2%) or higher (40.1%; Table 5.2). Just over two thirds of the girls were the first (34.6%) or second (33.3%) born. The mean maternal age at delivery was 28.6 (SD=4.6), and ranged from 15 to 44. Most of the girls were in the normal height and BMI ranges for their age in months at age 8 (78.2% and 74.7%, respectively). Approximately one quarter were either overweight (12.4%) or obese (10.4%) at age 8.

Synchronous initiation was the most commonly reported pathway, with 46.3% of girls reporting stage 2 or higher for both breast and pubic hair. The thelarche initiation pathway was reported by nearly the same proportion of girls, 42.1%, and the remaining girls (11.6%) reported pubic hair development preceding breast development (pubarche pathway). The median age at entry into stage 2 of breast development was 10.19 (95% CI: 10.14, 10.24) years, while the median age at entry into stage 2 of pubic hair development was 10.95 (95% CI: 10.90, 11.00) years. Girls in the synchronous pathway had a median age at entry into stage 2 of 10.55 (95% CI: 10.48, 10.62) years for breast and for pubic hair development (Table 5.3). The median age at initiation for the thelarche and pubarche pathways were similar (9.24 [95% CI: 9.16, 9.32] years and 9.24 [95% CI: 9.09, 9.39] years, respectively).

In this cohort, 62.7% of girls had achieved menarche by the time of the 14 year questionnaire; age at menarche was missing for 208 (8.4%) of these girls. The median age at menarche estimated from the parametric survival analysis model was 12.87 (95% CI: 12.82, 12.91) years, and reported age at menarche ranged from 7.6 years to 14.9 years (Table 5.3). Using a life-table approach, the median age at menarche was 12.92 (inter-quartile range: 12.08, 13.67), and the mean was 12.86 (SE=0.02). Age at menarche differed by initiation pathway. For girls in the synchronous pathway, median age at menarche was 12.84 (95% CI: 12.78, 12.91) years; girls in the thelarche pathway had a similar median of 12.78 (95% CI: 12.71, 12.85) years, while girls in the pubarche pathway had a slightly later median age of 13.13 (95% CI: 13.00, 13.26) years.

Factors associated with initiation pathway in univariate logistic regression analysis were girl's BMI at 8 years of age, girl's ethnic background, girl's birth order, mother's educational attainment, mother's age at delivery, and mother's pre-pregnancy BMI. In the multivariate polytomous logistic regression model, variables which remained associated with initiation pathway were girl's BMI at 8 years of age, girl's ethnic background, mother's pre-pregnancy BMI, and birth order; no interaction terms remained in the final model (Table 5.4). Girls in the pubarche pathway were less likely to be obese at age 8, compared to girls in the synchronous and thelarche pathways (OR=0.29 [95% CI: 0.12, 0.67] and OR=0.11 [95% CI: 0.05, 0.25], respectively); they were also less likely to be overweight at age 8 compared to girls in the thelarche pathway (OR=0.37 [95% CI: 0.23, 0.58]). Girls in the thelarche pathway were more likely to be overweight or obese at age 8 compared to girls in the synchronous pathway (OR=1.82 [95% CI: 1.39, 2.38] and OR=2.65 [95% CI: 1.95, 3.60], respectively). They were also less likely to be of non-white race compared to girls in the synchronous or pubarche pathways (OR=0.47 [95% CI: 0.27, 0.80] and OR=0.31 [95% CI: 0.16, 0.58], respectively). Similarly to results for girl's own BMI, girls in the pubarche pathway were less likely to have overweight mothers compared to girls in the synchronous pathway (OR=0.60 [95% CI: 0.39-0.94]), and less likely to have obese mothers compared to girls in the thelarche pathway (OR=0.31 [95% CI: 0.11, 0.89]); in addition, girls in the thelarche pathway were less likely to have underweight mothers compared to girls in the synchronous pathway (OR=0.64 [95% CI: 0.42, 0.99]). Finally, girls in the thelarche pathway were less likely to be the third

born or later child, compared to girls in the synchronous pathway (OR=0.72 [95% CI: 0.57, 0.90]).

In the model excluding girl's BMI as a covariate, variables which remained associated with initiation pathway included mother's pre-pregnancy BMI, mother's age at delivery, girl's ethnic background, and birth order. Results were similar to those for the full model, with the addition that older maternal age at delivery, 25-29 years of age or ≥30 of age, was more common for girls in the pubarche pathway compared to girls in either the synchronous (OR=1.72 [95% CI: 1.14, 2.60] and OR=1.62 [95% CI: 1.06, 2.46], respectively) or thelarche pathway (OR=1.97 [95% CI: 1.31, 2.98 and OR=1.71 [95% CI: 1.12, 2.60], respectively).

IV. Discussion

Initiation of secondary sexual characteristic developmentwas assessed for girls participating in the ALSPAC study. Nearly half (46.3%) of girls reported synchronous initiation; this is similar to a recent US study,[59] and to an earlier Swedish study (Table 5.1).[64] However, among girls who reported asynchronous initiation, the proportion of girls entering puberty through the thelarche pathway in the US cohort was slightly lower compared to the present study (65.7% vs. 78.4%). Compared to an earlier Swiss study[62] and findings from the Fels longitudinal study in the US,[56] our findings are somewhat different, with a smaller proportion of girls experiencing synchronous initiation. This may be due to differences in socio-demographic and physiological characteristics (including body composition), as well as the use of a different rating system. In the Swiss cohort, the authors stated that their method of

visual inspection may have missed early signs of breast development, although the reasons for this were not given.[62]

We estimated median ages at entry into breast and pubic hair stage 2 to be 10.19 and 10.95 years. Girls in the pubarche and thelarche pathways entered puberty somewhat earlier (9.24 years), compared to girls in the synchronous pathway (10.55 years). However, this is likely due to the method of categorizing girls, in that girls who are classified as asynchronous are more likely to have early development of one marker (breast for girls in the thelarche group, and pubic hair for girls in the pubarche group) compared to the average age at development for the whole cohort. Similarly, synchronous girls are more likely to have later ages at initiation, because on average for the whole cohort, breast development occurs before pubic hair development. However, there is a biological basis for later onset of breast development among girls in the pubarche group, since adrenal androgens may have an inhibitory effect on ovarian estrogen production.

The estimated ages at initiation in this study are similar to or slightly younger than those reported by Biro et al (10.7 years for both thelarche and pubarche pathways)[59], which may be due to the differences in age range (9-20 vs. 8-14), and the exclusion of girls who had already begun pubertal development at the time of enrollment in the Biro study.[59] The median age at menarche in this cohort was 12.87 years, similar to earlier reports.[4, 74] However, age at menarche varied by puberty pathway; girls in the pubarche pathway had an older age at menarche compared to girls in the synchronous and thelarche pathways. In addition, a girl's BMI at 8 years of age, ethnic background, maternal pre-pregnancy BMI, and birth order were associated with

initiation pathway. These findings may relate to differences in the actions of the endocrine and central nervous system, which differentially regulate breast and pubic hair development. That is, some of these factors may act on the hypothalamic pituitary gonadal axis (and subsequent breast development), the hypothalamic pituitary adrenal axis (and subsequent pubic/axillary hair development), or both. Girls in the thelarche pathway were more likely to be white, and to have higher BMIs at age 8, and less likely to have an underweight mother or to have an older maternal age at delivery. Girls in the pubarche pathway were more likely to be non-white, have lower BMIs at age 8, and older maternal age at delivery, and were less likely to have overweight or obese mothers. This is similar to findings by Biro et al, who reported that girls in the thelarche pathway had, on average, higher BMIs and proportion body fat throughout puberty, compared to girls in the pubarche pathway.[59] Other studies have also reported an association between increased weight and accelerated pubertal development,[19] and noted that breast development may be more sensitive to body composition than pubic hair development due to differences in the regulatory pathways for the two processes. Race and ethnicity are also known to be associated with timing of puberty;[5, 6, 30, 59, 61] however, the small number of non-white girls and the heterogeneity of this group limit comparisons by race. The relationship of initiation pathway with maternal pre-pregnancy BMI may be due to the correlation between maternal and child BMI,[232, 233] as seen by the attenuation of the effect of maternal BMI in the model including both maternal and child BMI variables supports this possibility. The association between maternal weight, and child weight and pubertal development, may be due to a combination of shared genetic and

environmental factors. Finally, birth order has been reported to be associated with age at menarche, with first born girls generally having their first period at a younger age compared to later born girls.[72, 216] Although the reasons for this pattern are not known, they may relate to differences in birth intervals, and changing parental care practices.

There are some limitations in this analysis. Questionnaires were sent on a yearly basis; the long interval between assessments means that critical stages and transitions may not have been captured for each individual. Consequently, some girls may have been misclassified with regards to initiation pathway (i.e. entry into breast or pubic hair stage 2 may have occurred shortly after the completion of a questionnaire). However, with our large sample size and use of interval-censored survival analysis, we were able to estimate ages at transition for the cohort with good precision. We did not adjust for multiple comparisons in our modeling procedure, although the variables remaining in the multivariate model would have been eligible even using more stringent inclusion criteria. Not all of the eligible ALSPAC participants provided information on pubertal development, and most did not provide information every year the questionnaire was administered. To assess the potential for selection bias, we compared selected socio-demographic characteristics of participants who returned at least one growing and changing questionnaire, to those who did not. Respondents were more likely to have mothers with higher education (74.5% with an O-level or higher, compared to 59.5%), and mothers with an 'upper' social class (39.7% compared to 28.6%). In addition, respondents were less likely to be of non-white race/ethnicity (4.0% compared to 5.9%). These differences may have impacted our

findings; based on observed differences, respondents may be more likely to experience later maturation compared to the whole cohort, leading to overestimates of age at pubertal milestones and underestimation of association between socio-demographic characteristics and pubertal characteristics.

The Growing and Changing questionnaire could be completed by the parent, child or both. For earlier ages, the proportion of questionnaires completed by the parent alone was higher, while for later ages the proportion completed by the child alone was higher. However, a previous study reported good correlation between maternal and child assessments of breast and pubic hair stage.[234] Finally, developmental data were self-reported and may not accurately reflect a girl's stage of breast and pubic hair development. One possibility is that girls categorized as having synchronous initiation of breast and pubic hair development had more subtle physical manifestations, which were not noticed until a more advanced stage. Also, adipose tissue may be mistaken for breast development upon visual inspection. Thus, some overweight or obese girls may have been misclassified in terms of breast development stage, and therefore initiation pathway. Among the girls who regressed in breast stage, a higher proportion were categorized as obese or overweight at 8 years of age, compared to the entire cohort (20% vs. 10.4%, and 15.9% vs. 12.4%, respectively). This may indicate that some girls reported too high a stage of breast development, and reported 'regressing' in stage after a weight loss or re-distribution of adipose tissue. However, among obese girls included in these analyses, reported breast development progressed over time, which suggests true development of breast tissue as opposed to adipose tissue. Further, when stratifying by BMI group, girls who were

overweight or obese had an earlier age at entry into both breast and pubic hair development compared to girls who were underweight or normal weight. Since pubic hair stage is not likely to be affected by adiposity, an advancement in the age at beginning pubic hair development reinforces the possibility that heavier girls are indeed maturing more quickly.

Strengths of this analysis include the longitudinal design, large sample size, and representative nature of the cohort. Repeated assessments of girls' development allowed the estimation of initiation pathway, and age at initiation and at menarche. Our findings confirm that girls experience different initiation pathways to development of secondary sexual characteristics, and that pathway may be related to various maternal and child characteristics. Our findings also support a general trend towards earlier maturation, which may be due to factors including increasing prevalence of overweight and obesity, and environmental exposures. Further research will be needed to determine causal relationships for observed associations, as well as impact on future health outcomes.

Table 5.1. Age at entry into breast and pubic hair development and pathway of puberty initiation.

Study	Year	Population (n; age range)	Age B2 (SD)	Age PH2 (SD)	Asynchronous† (%)	Synchronous (%)
Reynolds [56]	1948	US (49; 8-18)	10.8 (1.1)	11.0 (1.1)	85.8 (53.1 T, 32.7 P)	14.3
Taranger [64]*	1976	Swedish (90; 8-17)	10.99 (0.04)	11.48 (0.04)	53 (45 T, 8 P)	47
Largo[62]	1983	Swiss (142; 8-18)	10.9 (1.2)	10.4 (1.2)	71 (18 T, 53 P)	29
Biro[59]*	2003	US (859; 9-20)	10.7 (0.7) thelarche group only	10.7 (0.9) pubarche group only	56.8 (37.3 T, 19.5 P)	43.3
Present*	2009	ALSPAC (3938; 8-14)	10.19 (0.03)	10.95 (0.03)	53.7 (42.1 T, 11.6 P)	46.3

*Proportions are based on 77 (Taranger), 781 (Biro), and 3521 (present study) girls with valid pathway information.
†T represents thelarche, P represents pubarche

Table 5.2. Characteristics of study participants, overall and by initiation pathway.

Characteristic[a]	**All girls**	**Thelarche**	**Pubarche**	**Synchronous**
	N (%)	N (%)	N (%)	N (%)
Total	3938 (100)	1482 (42.1)	408 (11.6)	1631 (46.3)
Mother's highest education[c]				
CSE/none	583 (15.4)	212 (14.8)	43 (10.7)	243 (15.7)
Vocational	351 (9.3)	116 (8.1)	29 (7.2)	158 (10.2)
O-level	1331 (35.2)	515 (35.9)	147 (36.7)	539 (34.8)
A-level	936 (24.8)	374 (26.1)	103 (25.7)	372 (24.0)
Degree	579 (15.3)	217 (15.1)	79 (19.7)	236 (15.3)
Mother's social class[d]				
Lower	355 (11.1)	133 (11.0)	30 (8.5)	143 (10.9)
Middle	1565 (48.9)	565 (46.5)	163 (46.2)	659 (50.0)
Upper	1280 (40.0)	516 (42.5)	160 (45.3)	514 (39.0)
Sugar in urine[d]				
1st trimester	67 (1.9)	23 (1.7)	7 (1.9)	31 (2.1)
2nd/3rd trimester	49 (1.4)	20 (1.5)	5 (1.3)	18 (1.2)
1st and 2nd/3rd trimester	6 (0.2)	2 (0.2)	2 (0.5)	2 (0.1)
Not at all	3454 (96.6)	1322 (96.7)	358 (96.2)	1417 (96.5)
Mother's pre-pregnancy BMI[b]				
<18.5	186 (5.2)	56 (4.1)	15 (4.0)	94 (6.4)
18.5-24.9	2671 (75.1)	1007 (74.4)	314 (84.2)	1075 (73.5)
25.0-29.9	516 (14.5)	208 (15.4)	37 (9.9)	217 (14.8)
≥30.0	183 (5.2)	83 (6.1)	7 (1.9)	76 (5.2)
Mother's age at menarche[d]				

8-11 years	615 (18.1)	253 (19.4)	61 (17.4)	250 (18.0)
12-14 years	2332 (68.6)	899 (69.1)	236 (67.4)	955 (68.6)
≥15 years	453 (13.3)	150 (11.5)	53 (15.1)	188 (13.5)
Mother's age at delivery[c]				
<20 years	103 (2.6)	37 (2.5)	4 (1.0)	50 (3.1)
20-24 years	620 (15.7)	240 (16.2)	43 (10.5)	252 (15.5)
25-29 years	1587 (40.3)	585 (39.5)	187 (45.8)	174 (39.1)
≥30 years	1628 (41.3)	620 (41.8)	174 (42.7)	692 (42.4)
Birthweight[d]				
<2500 grams	149 (3.8)	52 (3.6)	17 (4.2)	60 (3.7)
2500-2999 grams	3339 (86.0)	1250 (85.7)	353 (87.4)	1388 (86.2)
≥4000 grams	397 (10.2)	157 (10.8)	34 (8.4)	162 (10.1)
Birth order[c]				
First born	1326 (34.6)	540 (37.1)	155 (38.8)	515 (32.6)
Second born	1277 (33.3)	485 (33.4)	137 (34.3)	531 (33.6)
Third born or later	1235 (32.2)	429 (29.5)	108 (27.0)	535 (33.8)
Breastfeeding[d]				
Any	3095 (81.5)	1181 (81.8)	339 (84.8)	1275 (81.8)
None	702 (18.5)	263 (18.2)	61 (15.3)	284 (18.2)
Child ethnic background[b]				
White	3572 (96.0)	1377 (97.6)	374 (94.4)	1447 (95.0)
Non-white	148 (4.0)	34 (2.4)	22 (5.6)	77 (5.0)
Height at age 8[d]				
<5th percentile	124 (4.3)	50 (4.3)	11 (3.3)	41 (3.6)
5th-85th percentile	2268 (78.2)	891 (76.5)	266 (79.6)	892 (78.8)
85th-95th percentile	317 (10.9)	135 (11.6)	37 (11.1)	126 (11.1)

≥95th percentile	190 (6.6)	89 (7.6)	20 (6.0)	73 (6.5)
BMI at age 8[b]				
<5th percentile	69 (2.5)	20 (1.8)	12 (3.7)	29 (2.6)
5th-85th percentile	2096 (74.7)	740 (65.8)	285 (86.9)	868 (79.0)
85th-95th percentile	348 (12.4)	182 (16.2)	23 (7.0)	121 (11.0)
≥95th percentile	293 (10.4)	183 (16.3)	8 (2.4)	81 (7.4)

[a]Information was missing for some girls, including information on mother's education (n=158, 4.0%), mother's age at menarche (n=538, 13.7%), mother's social class (n=738, 18.7%), mother's pre-pregnancy BMI (n=382, 9.7%), sugar in urine at any point during pregnancy (n=362, 9.2%), breastfeeding (n=141, 3.6%), girl's birth order (n=100, 2.5%), girl's birthweight (n=53, 1.4%), girl's race/ethnicity (n=218, 5.5%), girl's height at age 8 (n=1039, 26.4%), and girl's BMI at age 8 (n=1132, 28.8%).

P-value is from bivariate logistic regression models:

[b] $p < 0.01$

[c] $0.01 \leq p < 0.05$

[d] $p \geq .05$

Table 5.3. Median age* at entry into Tanner stage ≥2 of breast and pubic hair development and at menarche, overall and by initiation pathway.

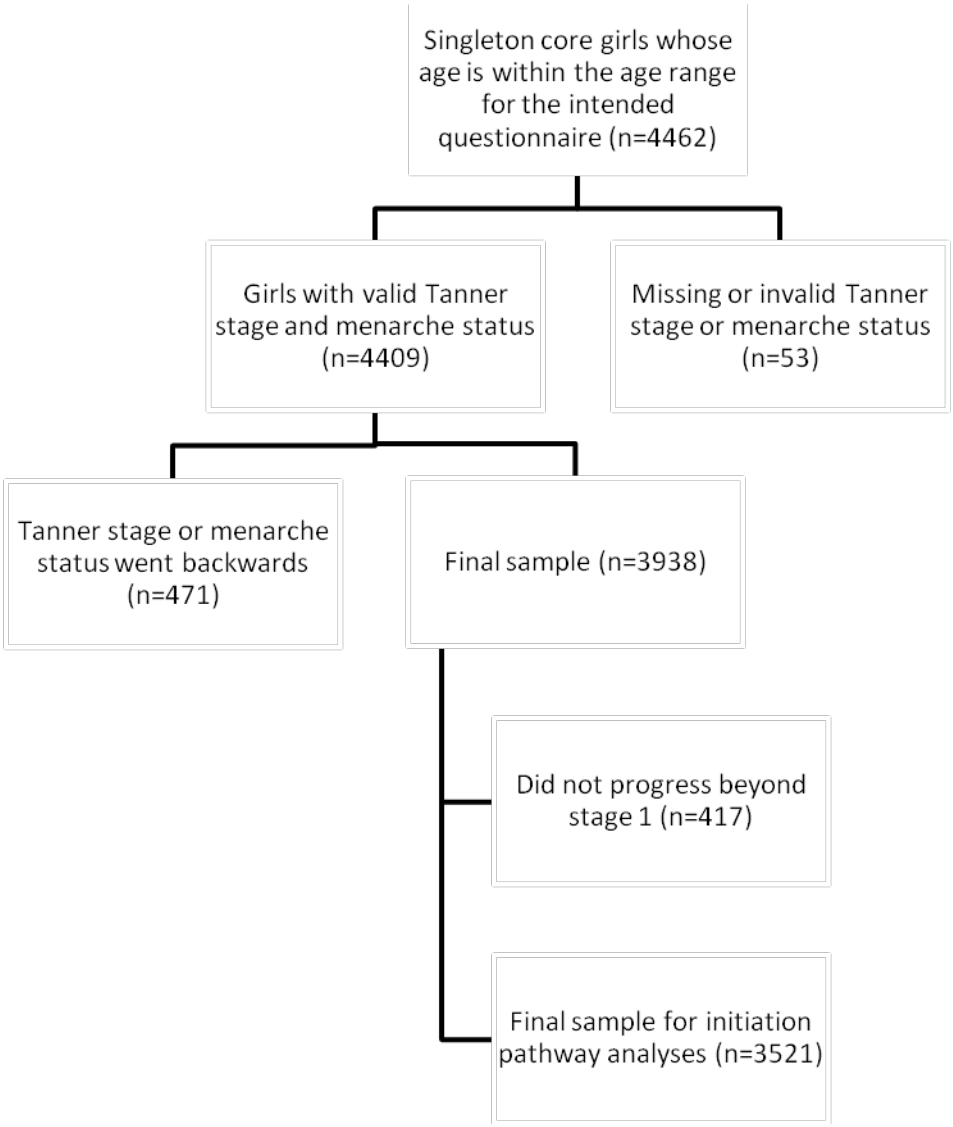
	Breast	
Group (n)	**Age (95% CI)**	**Quartiles**
All girls (3938)	10.19 (10.14, 10.24)	9.16-11.22
Synchronous (1631)	10.55 (10.48, 10.62)	9.72-11.38
Thelarche (1482)	9.24 (9.16, 9.32)	8.23-10.19
Pubarche (408)	11.24 (11.12, 11.37)	10.46-12.02
	Pubic Hair	
All girls (3938)	10.95 (10.90, 11.00)	9.99-11.91
Synchronous (1631)	10.55 (10.48, 10.62)	9.72-11.38
Thelarche (1482)	11.59 (11.53, 11.66)	10.82-12.37
Pubarche (408)	9.24 (9.09, 9.39)	8.32-10.17
	Menarche	
All girls (3938)	12.87 (10.82, 12.91)	12.08-13.65
Synchronous (1631)	12.84 (12.78, 12.91)	12.06-13.63
Thelarche (1482)	12.78 (12.71, 12.85)	12.00-13.57
Pubarche (408)	13.13 (13.00, 13.26)	12.34-13.92

*Normal distribution specified

Table 5.4. Polytomous logistic regression model for initiation pathway.

Characteristic	**Thelarche vs. Synchronous OR (95% CI)**	**Pubarche vs. Synchronous OR (95% CI)**	**Pubarche vs. Thelarche OR (95% CI)**
BMI at age 8			
<5th percentile	0.79 (0.41, 1.49)	1.24 (0.58, 2.64)	1.58 (0.71, 3.55)
5th-85th percentile	Reference	Reference	Reference
85th-95th percentile	**1.82 (1.39, 2.38)**	0.67 (0.41, 1.07)	**0.37 (0.23, 0.58)**
≥95th percentile	**2.65 (1.95, 3.60)**	**0.29 (0.12, 0.67)**	**0.11 (0.05, 0.25)**
Child ethnic background			
White	Reference	Reference	Reference
Non-white	**0.47 (0.27, 0.80)**	1.55 (0.87, 2.77)	**3.23 (1.71, 6.45)**
Mother's pre-pregnancy BMI			
<18.5	**0.64 (0.42, 0.99)**	0.68 (0.37, 1.24)	1.05 (0.55, 1.99)
18.5-24.9	Reference	Reference	Reference
25.0-29.9	0.90 (0.68, 1.15)	**0.60 (0.39, 0.94)**	0.68 (0.44, 1.06)
≥30.0	1.13 (0.74, 1.73)	0.35 (0.13, 1.01)	**0.31 (0.11, 0.89)**
Birth order			
First born	Reference	Reference	Reference
Second born	0.82 (0.66, 1.01)	0.95 (0.69, 1.29)	1.16 (0.85, 1.58)
Third born or later	**0.72 (0.57, 0.90)**	0.74 (0.53, 1.03)	1.03 (0.74, 1.44)

Figure 5.1. Flowchart of eligibility and exclusions.



Chapter 6. Characterization of the correlation between ages at entry into breast and pubic hair development

I. Introduction

Although average ages at attainment of female secondary sexual characteristics have been estimated for various populations, the interrelationship of the timing of these events is not well characterized. One reason is that many studies of puberty are cross-sectional in nature, utilizing 'status quo' assessments of developmental stage at the current age for the individual level, to generate population estimates of timing of initiation of various stages. However, individual-level estimates are not possible in a cross-sectional setting. Consequently, if individuals are not followed over time, it is difficult to estimate the correlation between the ages at beginning breast development and pubic hair development, since both of these events are not likely to be observed in the same girl. Even in longitudinal studies, exact timing of events may not be known, since the transition generally occurs between study assessments or clinic visits. This phenomenon of interval censored data, where the event is only known to occur within some interval, must be accounted for in estimating age at event. In the few longitudinal studies that have attempted to estimate the correlation between beginning breast and pubic hair development, two main approaches have been used. These are to (1) estimate age at event as the age at the first 'post-event' study assessment, or (2) estimate age at event as the midpoint between the last 'pre-event' assessment, and the first 'post-event' assessment. The first method is likely to skew the age distribution toward older ages; the second method is more likely to estimate age at event correctly, but could also present problems if there are few assessments, or long intervals between assessments.

These methodological considerations may explain some of the variation in estimated correlation between age at beginning breast and pubic hair development among studies. For example, Largo et al estimated a correlation of 0.34 among girls participating in the First Zurich Longitudinal study,[62] while Nicholson et al report a correlation of 0.74 among girls participating in the Guidance Study (Table 6.1).[235] Estimates from the Swedish Growth study[64] and the Fels study[56] are intermediate (0.70 and 0.66, respectively). Notably, these studies were reported between 1948 and 1983; there are no estimates from a contemporary longitudinal cohort. Further, it is not known which demographic or physiological characteristics might impact the relationship between timing of breast and pubic hair development.

We used the Avon Longitudinal Study of Parents and Children (ALSPAC) to estimate the correlation between ages at beginning breast and pubic hair development, using a maximum likelihood approach adapted to accommodate interval censored data.

II. Methods

When looking at age at breast development and age at pubic hair development separately, age at occurrence may be treated as a survival time, perhaps following a univariate normal distribution. To characterize the relationship between ages at the two events, a natural extension is the bivariate normal distribution. Parametric survival analyses allow the estimation of the mean (and standard deviation) age at event. However, when using these methods, the correlation between ages at occurrence of the two events is not easily obtained. Most extensions of survival analysis for multiple outcomes use stratification or competing risk scenarios, in which the correlation is either accounted for but not

estimated, or only one outcome is observed per individual. A further difficulty is introduced by interval censoring of event times. In order to estimate the correlation between ages at occurrence of two distinct and observed events, a maximum likelihood approach will be used, with modification to accommodate the interval censored nature of the available data.

A pair of event times, defined as X for the first event and Y for the second, follow some joint probability density distribution $f_{X,Y}(x, y)$. The corresponding cumulative distribution function of the paired variables, $F_{X,Y}(x, y) = P(X \leq x, Y \leq y)$. If X and Y follow a bivariate normal distribution, $f_{X,Y}(x, y)$ takes the following form:

$$f(x,y) = \frac{1}{2\pi\sigma_x\sigma_y\sqrt{(1-\rho^2}} \exp\left[\frac{-1}{2(1-\rho^2)}\left[\left(\frac{x-\mu_x}{\sigma_x}\right)^2 + \left(\frac{y-\mu_y}{\sigma_y}\right)^2 - 2\rho\left(\frac{x-\mu_x}{\sigma_x}\right)\left(\frac{y-\mu_y}{\sigma_y}\right)\right]\right]$$

while F(x, y) takes the following form:

$$F_{X,Y}(x,y) = \int_{-\infty}^{x}\int_{-\infty}^{y} f(s,t)dsdt$$

As there is no closed expression for $F_{X,Y}(x, y)$, the double integrand is approximated using iterative numerical techniques.

In the case of interval censoring, X and Y are not observed directly, but are known to fall within a certain interval; in the case of left and right censoring, the endpoint of the interval is negative or positive infinity, respectively. These intervals are indicated as (L_x, R_x) for the interval containing x, and (L_y, R_y) for the interval containing y (Figure 6.1). Given these intervals, a region of support can be obtained which contains the pair of event times (x, y) as follows: $[F_{X,Y}(R_x, R_y) - F_{X,Y}(R_x, L_y) - F_{X,Y}(L_x, R_y) + F_{X,Y}(L_x,$

$L_y)]$.[206-208] Evaluating $F_{X,Y}$ over this region approximates the likelihood contribution for each individual. To obtain a likelihood function for the cohort, the individual likelihood contributions are multiplied together, and the resulting function maximized with respect to ρ. To increase efficiency without loss of specificity, an alternative is to minimize the negative of the natural log of the likelihood.

This method was used to estimate the correlation between age at entry into Tanner stage 2 (or higher, if stage 2 is not observed) of breast and of pubic hair development among girls participating in the ALSPAC.[196] The physical development of this cohort has been assessed using annual questionnaires which ascertain Tanner stage of breast and pubic hair development (self-assessed at time of questionnaire completion), mailed to participants from the ages of 8-14. Since data were collected annually, entry into breast and pubic hair development is known to occur within intervals defined by timing of questionnaire completion. The median age at entry for each marker and accompanying standard deviation were calculated using the LIFEREG procedure in SAS®,[236] with interval censoring and the normal distribution specified. This median age is equivalent to the mean, due to the normal distribution assumption and the fact that the event occurs within a very specific timeframe where there are no outliers or skewness. Then, the likelihood procedure was implemented in SAS, utilizing the PROBBNRM function to estimate the CDF of the bivariate normal distribution. In order to determine whether the correlation between ages at entry into breast and pubic hair development is a function of some underlying variable, the analysis was repeated for selected maternal and child characteristics which are not intermediate between breast and pubic hair development, and are known to be associated with pubertal development. These included mother's pre-

pregnancy BMI, birth order, child's BMI at age 8, and child's race. Human subject protection was assessed and approved by the ALSPAC Law and Ethics Committee, the Local Research Ethics Committees, and the CDC Institutional Review Board.

III. Results

There were 3938 participants with information on breast and pubic hair development. Among these girls, the mean ages at entry into breast stage 2 and pubic hair stage 2 were estimated to be 10.19 (SD=1.52) and 10.95 (SD=1.42). There were 1000 girls who were left censored for age at entry into breast stage 2, and 608 who were left censored for age at entry into pubic hair stage 2; 469 and 712 girls were right censored for age at entry into breast and pubic hair stage 2, respectively. For these girls, the left interval endpoint was set at ($\mu - 4*\sigma$) and the right interval endpoint at ($\mu+4*\sigma$) as appropriate, where μ and σ represent the estimated mean and standard deviation of age at entry. The likelihood was evaluated over the range of the correlation coefficient ρ, from -0.999 to 0.999. The negative of the natural log of the likelihood was minimized when ρ reached a value of 0.503 – 0.506 (same value for this range of ρ). The correlation between ages at entry into breast and pubic hair stage 2 were similar between subgroups based on maternal and child characteristics associated with pubertal development (Table 6.2). The greatest difference was seen by BMI. The correlation was greatest among girls who were underweight (0.59) or whose mothers were underweight (0.57), and lowest among girls who were overweight or obese (0.31-0.41) or whose mothers were classified as obese (0.41). Correlations ranged from 0.46-0.57 when looking at birth order, and from 0.50-0.53 depending on child's race.

To evaluate the performance of the maximum likelihood approach, the correlation was also estimated using either the end of the interval (i.e. age at completing questionnaire where event is first recorded), or the midpoint of the interval (i.e. age intermediate between questionnaires immediately preceding the event, and where event is first recorded. These approaches did not substantially change the estimate of ρ (ρ=0.56 and 0.50, respectively).

IV. Discussion

We used data from a contemporary, representative cohort of girls to estimate the correlation between ages at entry into stage 2 of breast and of pubic hair development. Using a novel application of a likelihood maximization technique, we found that approximately half of the variation in timing of breast development is explained by the variation in timing of pubic hair development, an estimate which is intermediate between (and somewhat lower than most) of previously reported correlations. Reasons for the difference between the present and previously reported studies include use of a different estimation method, different populations and timing, and differences in frequency of study assessments.

It is expected that the timing of breast and pubic hair development will be related, since the cascade of events for both is ultimately regulated by the central nervous system. However, the moderate degree of association estimated in this study is consistent with the biology of breast and pubic hair development, which is governed by the hypothalamic pituitary gonadal axis and hypothalamic pituitary adrenal axis, respectively, and thus may not be expected to display a high degree of dependence. Environmental exposures or

genetic characteristics which alter production of or response to androgens would be expected to impact timing of pubic hair development, while those factors affecting estrogen would impact timing of breast development. In either case, such disruption could lower the correlation between ages at entry into these developmental milestones.

The magnitude of the association between timing of the two markers was similar within strata of characteristics associated with pubertal development, suggesting that timing of breast and pubic hair development are not independent. That is, differences in the relative timing of breast and pubic hair development do not appear to be caused by the demographic characteristics explored in this analysis. There was some heterogeneity by both child and maternal BMI, however, and the correlation was greatest among underweight girls and girls whose mothers were underweight, and lowest among heavier girls and girls whose mothers were heavier. The similarity in pattern by child and maternal BMI may be due to the fact that maternal BMI acts as a proxy for girl's BMI.[232, 233] Girls who are more overweight may mistake adipose tissue for more advanced breast development, creating spurious differences in timing of maturation for breast and pubic hair development.[19, 59]

There were some limitations to these analyses. Correlation represents a linear relationship between variables, and thus does not capture more complex dependence that may exist between timing of breast and pubic hair development. Entry into stage 2 was not observed for some girls, only transition from stage 1 to stage ≥ 3. Since data were collected on an annual basis, events occurring months apart are likely to be reported in the same questionnaire year, which could artificially inflate our estimate of correlation between event times. Finally, correlation is influenced by variance of the individual

random variables and covariance between them,[237] which may affect subgroup analyses. However, the decreased correlation with higher BMI shows a dose-response relationship not explained by within-strata variance alone.

We considered various distributions in the univariate survival analysis. Mean and median time at entry into breast and pubic hair development were similar under varying distributional assumptions, so the normal distribution was chosen for ease of interpretation. However, this method could be extended to other bivariate distributions, and to higher order distributions, maximizing the likelihood with respect to the covariance matrix. This application of maximum likelihood analysis to interval censored puberty data provides a novel approach for assessing the relationship between timing of milestones in growth and development.

Table 6.1. Estimated correlation coefficients

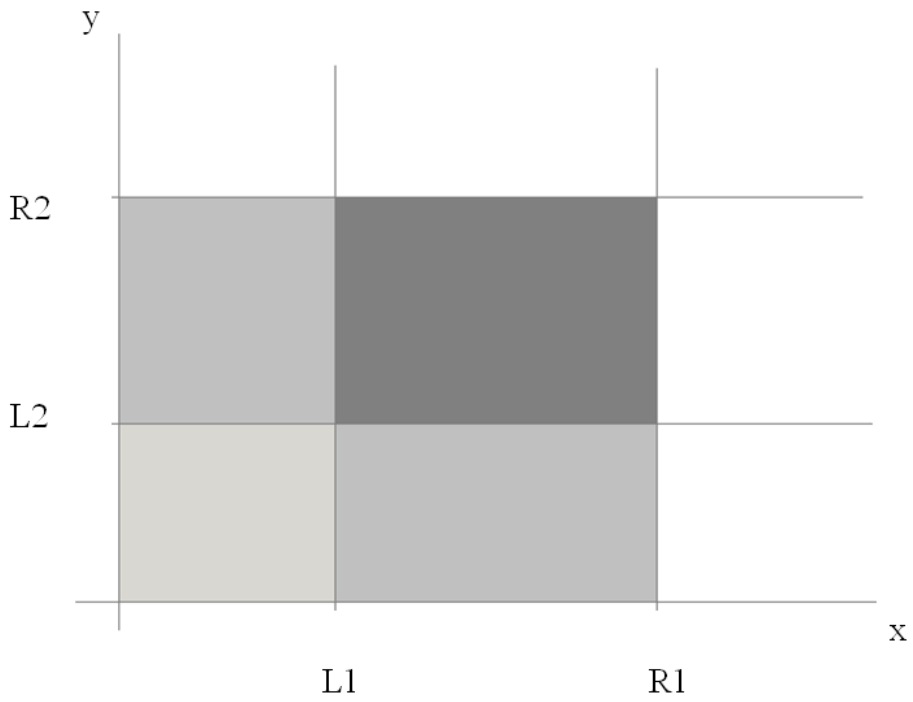
Source	Study	Method	Estimate of ρ
Largo and Prader[62]	First Zurich Longitudinal Study (Switzerland)	Age at observation	0.34
Taranger and Prader[64]	Growth study (Sweden)	Midpoint	0.70
Nicholson and Hanley[235]	Guidance Study (US, CA)	Midpoint	0.74
Reynolds and Wines[56]	Fels Study (US, OH)	Not stated; probably age at observation	0.66

Table 6.2. Maximum likelihood estimate of the correlation coefficients, overall and by selected covariates

Characteristic	**N (%)**	**Correlation**
Total	3938 (100)	0.503-0.506
Girl's BMI at 8 years of age		
<5th percentile	69 (2.5)	0.59
5th-85th percentile	2096 (74.7)	0.55-0.56
85th-95th percentile	348 (12.4)	0.31
≥95th percentile	293 (10.4)	0.41
Mother's pre-pregnancy BMI		
<18.5	186 (5.2)	0.57
18.5-24.9	2671 (75.1)	0.51-0.52
25.0-29.9	516 (14.5)	0.48
≥30.0	183 (5.2)	0.41
Birth order		
First born	1326 (34.6)	0.49
Second born	1277 (33.3)	0.46-0.47
Third born or later	1235 (32.2)	0.57
Child ethnic background		
White	3572 (96.0)	0.50
Non-white	148 (4.0)	0.53

* Information was missing for some girls, including information on girl's BMI at 8 years of age (n=1132, 28.8%), mother's pre-pregnancy BMI (n=382, 9.7%), girl's birth order (n=100, 2.5%), and girl's race/ethnicity (n=218, 5.5%).

Figure 6.1. The most darkly shaded area indicates the region of support for the likelihood function.



Chapter 7. Progression of puberty in girls enrolled in a contemporary British cohort

I. Introduction

Puberty is a critical time of growth and development. Timing and pattern of milestones in adolescence are related to endocrine function, overall health status, previous exposures, and may predict future health outcomes.[41, 60] Marshall and Tanner reported in the 1940's that girls participating in the Harpenden Growth Study (United Kingdom) took approximately 4.5 years to complete breast development, and 2.7 years to complete pubic hair development; the estimated duration of puberty (time elapsed between breast development and menarche) was 2.3 years.[4] In a more contemporary (1988-1994) group of non-Hispanic white girls participating in the cross-sectional United States study NHANES III, the average duration of breast and pubic hair development were 5.1 and 5.7 years, respectively.[6] Most recently (1986-1997), Biro et al report that among white girls participating in the longitudinal National Heart, Lung and Blood Institute Growth and Health Study, duration of puberty was 2.5 years.[61] On average, 4.03 years elapsed between onset of puberty and completion of secondary sexual characteristic development. This apparent lengthening of the duration of pubertal development may indicate that puberty is starting earlier but is progressing more slowly in contemporary girls, or could reflect differences in the cohort population, measurement techniques, and exposures.

There is evidence that age at menarche, and age at beginning breast development decreased between 1940 and 1994 among girls in the United States;[39] however, age at entry into subsequent stages of breast and pubic hair development, and duration of puberty, have not been reported in many contemporary, longitudinal studies. Therefore, it is not known whether advanced age at entry into puberty is associated with a

subsequent advancement in age at attainment of adult breast and pubic hair stage, or shortened duration of puberty. It is important to understand the overall pacing of development as well as initiation of puberty, as multiple studies have shown that accelerated puberty is associated with adverse physiological[14, 35, 48, 50] and psychological[42-46] outcomes. We used data from the Avon Longitudinal Study of Parents and Children (ALSPAC) to describe timing of pubertal milestones, and factors associated with progression of pubertal development.

II. Methods

(1) Study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective cohort study of approximately 14,000 pregnant women residing in southern England who had an expected delivery date between April 1, 1991 and December 31, 1992.[196] The study area and population have been described in detail elsewhere.[196, 197]

(2) Pubertal assessment

A self-assessment instrument was mailed to participants at the ages of 8, 9, 10, 11, 13 and 14 (1999-2005) to collect information on pubertal development. For all ages, parents or guardians could choose to complete the questionnaire themselves, complete it with their child or allow the child to complete it herself. Timing of menarche was determined via reported age at first period, and secondary sexual characteristics were self-assessed using Tanner stages;[4] respondents were sent a series of line drawings showing different stages of breast and pubic hair development, along with written descriptions of each stage. These diagrams were developed at the University of North Carolina's Population Center,

and have been previously validated (correlation with physician assessments of 0.63 for breast development, and 0.81 for pubic hair distribution[198]) and successfully used in other cohort studies.[113, 230] Each characteristic (breast and pubic hair development) has 5 possible stages, ranging from pre-pubertal (stage 1) to fully developed (stage 5). The respondent then selected the stage which most closely aligned with the girl's current stage of development. For these analyses, the cohort was restricted to female singletons completing at least one puberty questionnaire with valid information. In addition, respondents had to be in the appropriate age range at the time of completing the questionnaire (i.e. only girls 8 years of age were included for the 8 year old questionnaire).

(3) Determinants of secondary sexual characteristic development

To identify factors that may influence attainment of secondary sexual characteristics, ordinal probit models were used to model the progression through Tanner stages of breast and pubic hair development. Probit analysis is used when the observed outcome variable—in this case, Tanner stage—is a discrete variable representing or characterizing an underlying continuous outcome variable. It is reasonable to assume that breast and pubic hair development are both continuous processes; once a 'threshold' level of development is achieved, it is recognized as entry into the next Tanner stage.

Using the ordinal probit model, probability of being in each breast and pubic hair stage, for a given set of covariates, is calculated as follows:

$$P(Y_j = k \mid \beta_0, \beta_1) = \Phi[(y_k - z_j)/\beta_0] - \Phi[(y_k - 1 - z_j)/\beta_0$$

Intercept values estimated through this modeling procedure (y_k) represent the threshold for the underlying continuous outcome (breast or pubic hair development) to be recognized as the next ordinal stage; in this case there are 4 intercepts estimated, representing the thresholds for entry into Tanner stages 2-5. Beta coefficients for covariates represent the change in the Z-score, for each one-unit increase in the covariate. Thus, a positive coefficient value indicates an increased probability of being in higher stages, while negative coefficient values indicate increased probability of being in lower stages. In order to account for the within-subject correlation resulting from repeated assessments, we included a random effect using the method of Hedeker and Giddons, which accommodates clustering of data.[75] The GLIMMIX procedure in SAS was used to fit these random-effects ordinal probit models.

Maternal and child characteristics potentially associated with breast and pubic hair development (described below) were included individually in repeated measures ordinal probit models along with age in months. Any variable associated with breast or pubic hair stage with a $p<0.30$ were retained for additional analyses. Next, potential interaction of variables with age was assessed by including the relevant interaction terms in the model.; if the interaction term was not associated with pubertal stage with a $p<0.05$, it was dropped from further analyses. Finally, a multivariate model with all factors which were associated with Tanner stage in univariate analysis, and relevant interaction terms, was constructed, and variables assessed for association with Tanner stage using a backward elimination strategy, with a $p<0.05$ required for retention in the final model.

(4) Estimation of length of time between pubertal milestones

Parametric survival analyses were used to estimate the timing of pubertal milestones, as well as the time elapsed between initiation of puberty and menarche (denoted as duration of puberty). Median age at menarche was determined from self-reported age at first menses, using parametric survival analysis and assuming a normal distribution. Median ages at entry into Tanner stages 2 through 4 of breast and pubic hair development were also calculated using a similar model; stage 5 was not assessed due to the small number of girls who had reached stage 5 of breast or pubic hair development by the time of the 14 year questionnaire (16.8% and 27.6% of girls, respectively. Since the ALSPAC data were collected on an annual basis, the exact age at which a girl entered a stage of breast of pubic hair development is not known. Rather, it is known what stage a girl reports at different points in time (i.e. at the time each questionnaire is completed), leading to interval censoring. If the start of the interval is not known, data are considered left censored, and if the end of the interval is not known (i.e. the event is not observed), data are considered right censored. The LIFEREG procedure in SAS accommodates right, left and interval censoring, and was used to perform all parametric survival analyses.

We also categorized girls according to whether they experienced breast or pubic hair development alone, or both, as the first sign of puberty. This was determined by identifying the first instance in which a girl is reported to be in stage 2 or higher for breast and for pubic hair development. If the initial report of being in a stage ≥ 2 is reported for only breast development or for only pubic hair development, the girl is categorized as being in the thelarche or the pubarche pathway of pubertal initiation,

respectively. If the initial report of being in stage ≥2 is for both breast and pubic hair development, the girl is categorized as being in the synchronous pathway.

(5) Covariates

Maternal and child characteristics associated with pubertal development were identified from the literature and assessed for association with Tanner stage. These variables include mother's pre-pregnancy BMI (<18.5 [underweight], 18.5-24.9 [normal], 25-29.9 [overweight], and ≥30 [obese]), mother's age at delivery (<20, 20-29, ≥30), mother's age at menarche (8-11, 12-14, 15+), mother's educational level (CSE/none, vocational, O-level, A-level, degree), mother's social class (lower, middle, upper), child's birthweight (<2500 g, 2500-3999 g, ≥4000 g), child's ethnic background (white [both parents report white race/ethnicity], non-white [otherwise]), birth order (first born, second born, third born or later), and child's BMI at time of completing the Growing and Changing questionnaire (<5^{th} percentile for month of age [underweight], 5^{th}-85^{th} percentile for month of age [normal], 85^{th}-95^{th} percentile for month of age [overweight], and ≥95^{th} percentile for month of age [obese]). Social class was derived using the 1991 Office of Population Censuses and Surveys.[231] Upper class consisted of classes I (professional occupations) or II (managerial and technical occupations); middle class of classes IIINM (non-manual skilled occupations) or IIIM (manual skilled occupations); and lower class of classes IV (partly skilled occupations) or V (unskilled occupations). Percentiles of BMI were defined using female-specific growth standards from a representative sample from the UK.[202] Initiation pathway was not included as an independent predictor due to the lack of independence between pathway definition and age at stage 2 transition.

III. Results

(1) Study population

In ALSPAC, 4462 singleton girls completed and returned at least one growing and changing questionnaire in the correct age range. Of these, 4434 submitted information on breast development, and 4427 on pubic hair development, for at least one questionnaire. A small number of girls returned inconsistent reports of menarche status (i.e. reported achieving menarche on a questionnaire, but reported not yet having their first period on a subsequent questionnaire; n=17). If more than one age at menarche was given, the first reported age at menarche was used in analysis. If age at menarche was not given or was greater than age at questionnaire completion, age at menarche was set to missing (n=208). More girls had inconsistent reports (i.e. reported a certain stage of development on one questionnaire, then reported a lower stage on a subsequent questionnaire) of breast development (n=320) or pubic hair development (n=223). These participants were excluded, for a final sample size of 3938 girls (Figure 7.1). There were 417 girls who did not report progress beyond stage 1 of breast and pubic hair development; therefore, initiation pathway could not be determined for these girls.

Nearly all (96%) of girls were white (Table 7.1). The majority of girls were born to a mother with an O-level education (35.2%) or higher (40.1%). Just over two thirds of the girls were the first (37.1%) or second (33.4%) born child. The mean maternal age at delivery was 28.6 (SD=4.6), and ranged from 15 to 44. The synchronous and thelarche initiation pathways were the most commonly reported (46.3% and 42.1% of girls, respectively), while the pubarche pathway was reported for the smallest proportion of

girls (11.6%). Breast and pubic hair stages were relatively concordant for each questionnaire year. Only one girl reported being in stage 1 for breast, and stage 5 for pubic hair development (in the 13-year questionnaire), and fewer than 1% reported a discrepancy of 3 stages in any given year. The majority of girls reported no more than a one or two stage discrepancy between breast and pubic hair stage; a discrepancy of 2 stages was reported most often in the 10 and 11 year questionnaire, where approximately 5% of girls reported being in breast stage 3 and pubic hair stage 1. Over half of girls reached stage 4 of breast (54.8%) and pubic hair (61.2%) development by the age 14 questionnaire (Table 7.2).

(2) Determinants of secondary sexual characteristic development

Results from the repeated measures ordinal probit analysis identified several variables associated with breast and pubic hair stage after adjusting for age. Factors associated with breast stage were: girl's BMI, girl's ethnic background, birth order, mother's pre-pregnancy BMI, mother's age at menarche, and an interaction term between age and BMI (Table 7.3). These same factors, with the exception of mother's pre-pregnancy BMI and the interaction term, were also associated with pubic hair stage. Increasing age, being overweight or obese, and mother's age at menarche of 8-11 years were associated with higher breast and pubic hair stages (Figure 7.2, 7.3). Mother's age at menarche of 15+ years, white race, and being the second or later birth were associated with lower breast and pubic hair stages. In addition, having an overweight or obese mother was associated with higher breast stages. Similar results were seen in the models excluding girl's BMI as a variable; additional factors associated with breast development were maternal education and an interaction between girl's age and maternal BMI (increasing age

dampened the effect of overweight maternal BMI), while an interaction between age and birth order was noted in the model for pubic hair stage (increasing age increased the effect of being third born or later).

(3) Estimation of length of time between pubertal milestones

For all girls, median age at entry into stage 2 of breast and pubic hair development were 10.2 (95% CI: 10.1-10.2), and 11.0 (95% CI: 10.9-11.0), respectively (Table 7.4a). Approximately 1.5 years lapsed between entry into breast stages 2 and 3, and 3 and 4 (Table 7.4b). Pubic hair development appeared to progress more quickly, with about 1 year between entry into stages 2 and 3, and 3 and 4.

Girls who reported breast development before pubic hair development (thelarche pathway) spent, on average, 3.7 years in breast stages 2-3, and 1.5 years in pubic hair stages 2-3. Girls in the pubarche pathway (pubic hair development before breast development) spent a shorter time in stages 2-3 of breast development compared to girls in the thelarche pathway (2.4 years), while time spent in stages 2-3 of pubic hair development was longer (3.3 years). Those girls in the synchronous pathway were somewhat intermediate, spending on average 2.7 years in breast stages 2-3, and 2.2 years in pubic hair stages 2-3. The median age at menarche for girls in the ALSPAC cohort was 12.9 (95% CI: 12.8-12.9) years, and varied by initiation pathway (Table 7.4a). Consequently duration of puberty was 2.7 years for the whole cohort, but varied by initiation pathway. The longest duration was seen for girls in the thelarche and pubarche pathways (3.5 and 3.9 years, respectively), and the shortest for girls in the synchronous pathway (2.3 years).

IV. Discussion

We used data from a contemporary, longitudinal cohort to describe the pacing of pubertal development, and describe factors associated with being in Tanner stages of breast and pubic hair development. Compared to an earlier British study, we found earlier estimated ages at entry into breast and pubic hair stages 2 and 3.[4] Compared to data for non-Hispanic white girls in the Third National Health and Nutrition Examination Survey (NHANES III), we found similar ages at entry into breast and pubic hair Tanner stages 2 and 3 for the ALSPAC cohort, although girls in the present study tended to be slightly older at entry into pubic hair stage 2.[6] Duration of puberty, as measured by time from entry into breast or pubic hair stage 2, and menarche, was longer in the ALSPAC girls compared to the findings from the Harpenden Growth Study (2.7 years compared to 2.3 years).[4] This may be the result of a greater shift in timing of pubertal initiation, compared to the lesser shift in age at menarche. As a sensitivity analysis, the maximum and minimum possible intervals between entry into breast stage 2 and menarche were considered, among girls who achieved both markers during the study period. As an estimate of the maximum time interval between the two markers, age at entry into breast stage 2 was taken to be the age at which a girl last reported being in breast stage 1 (on the assumption that she entered breast stage 2 immediately after returning the questionnaire); conversely, the minimum interval was constructed by taking age at entry into breast stage 2 to be the age at which a girl first reported being in breast stage 2 (on the assumption that she entered breast stage 2 immediately before returning the questionnaire). Using this method, the median of the maximum interval was 2.9 years (IQR: 2.2-3.7 years),

while the mean was 3.0 (SD=1.1) years. The median of the minimum interval was 1.4 years (IQR: 0.5-2.3 years), while the mean was 1.4 (SD=1.7) years.

Ordinal probit analysis was used to assess characteristics associated with secondary sexual characteristics, treating Tanner stages as discrete markers characterizing an underlying continuous process of development. To our knowledge, this is the first application of repeated measures ordinal probit analysis to pubertal development. This technique provides further insight into pubertal development, and the assumption that breast and pubic hair development are continuous rather than discrete processes provides an appropriate alternative to other analytical methods. Our analysis identified several variables associated with breast and pubic hair stage, and found that risk factors varied by initiation pathway. However, BMI and maternal age at menarche were associated with both developmental markers in all analyses. Increased weight has previously been reported to be associated with accelerated pubertal development,[19, 238] in particular breast development, which is more sensitive to body composition than pubic hair development due to differences in the regulatory pathways for the two processes. Approximately half of the variability in timing of menarche among girls in developed countries is thought to be genetic,[239] which supports our findings that maternal age at menarche and maternal pre-pregnancy BMI are associated with girl's pubertal development.

There are some limitations in this analysis. Not all of the eligible ALSPAC participants provided information on pubertal development, and most did not provide information every year the questionnaire was administered. There is potential for selection bias, if timing and progression of pubertal development differed between respondents and non-respondents. To assess the potential for selection bias, we

compared selected socio-demographic characteristics of participants who returned at least one Growing and Changing questionnaire, to those who did not. Respondents were more likely to have mothers with higher education (74.5% with an O-level or higher, compared to 59.5%), and mothers with an 'upper' social class (defined as social class I and II; 39.7% compared to 28.6%). In addition, respondents were less likely to be of non-white race/ethnicity (4.0% compared to 5.9%), and were born to mothers who were less likely to be overweight (14.8% compared to 16.7%) or obese (5.3% compared to 7.1%). Mothers of respondents were also less likely to have had an early age at menarche (8-11 years; 18.7% compared to 22.7%). Data were available for respondents up to age 14; however, since there were incomplete data in some cases, age at initiation and initiation type could not be determined for all girls, and not every stage was observed for each girl (i.e. she may have reported stages 1 and 3 on subsequent questionnaires, but not stage 2). Multiple analyses were performed without correction for multiple testing; however, in most cases the associations were strong enough that they would have remained even using a more stringent criteria. Finally, all developmental data were self-reported and may not accurately reflect a girl's true stage of breast and pubic hair development.

Strengths of this analysis include the longitudinal design, large sample size, and representative nature of the cohort. Repeated assessments of girls' development allowed the examination of progression of pubertal markers, and estimation of age at entry into each stage of development. Ordinal probit analysis was used to determine factors associated with breast and pubic hair stage, and allows for sub-group comparison while accommodating repeated measures.

In summary, we found that girls in the ALSPAC cohort had a similar or slightly increased interval of time between initiation of puberty and menarche to that reported in an earlier British cohort, but a slightly earlier age of initiation of puberty and of menarche. The duration of puberty, as well as the timing, is important since puberty represents a vulnerable period of intense and rapid growth. Any increase in the length of this period of vulnerability could have implications for environmental exposures, particularly endocrine disruptors. Maternal age at menarche and girl's BMI were consistently associated with probability of being in each breast and pubic hair stages. Girl's race/ethnicity, birth order, and mother's pre-pregnancy BMI were also associated with stage in certain subgroups. Puberty is an important marker of health, and is sensitive to individual exposures; further, there may be secular trends in the timing and pattern of pubertal development. This study provides a description of pubertal development in a contemporary, representative cohort, and identifies factors which may influence progression of puberty.

Table 7.1. Characteristics of study participants.

	N	Percent
Total	3938	100
Initiation Pathway		
Synchronous	1631	46.3
Thelarche	1482	42.1
Pubarche	408	11.6
Missing	*417*	*10.6*
Mother's highest education		
CSE/none	583	15.4
Vocational	351	9.3
O-level	1331	35.2
A-level	936	24.8
Degree	579	15.3
Missing	*158*	*4.0*
Mother's social class		
Lower	355	11.1
Middle	1565	48.9
Upper	1280	40.0
Missing	*738*	*18.7*
Child ethnic background		
White	3572	96.0
Non-white	148	4.0
Missing	*218*	*5.5*
Mother's age at menarche		
8-11 years	615	18.1
12-14 years	2332	68.6
≥15 years	453	13.3
Missing	*538*	*13.7*
Mother's age at delivery		
<20 years	103	2.6
20-24 years	620	15.7
25-29 years	1587	40.3
≥30 years	1628	41.3
Birthweight		
<2500 grams	149	3.8
2500-2999 grams	3339	86.0
≥4000 grams	397	10.2
Missing	*54*	*1.4*
Birth order		
First born	1326	34.6
Second born	1277	33.3
Third born or later	1235	32.2
Missing	*100*	*2.5*

Mother's pre-pregnancy BMI		
<18.5	186	5.2
18.5-24.9	2671	75.1
25.0-29.9	516	14.5
≥30.0	183	5.2
Missing	*382*	*9.7*

Table 7.2. Proportion of girls in each breast and pubic hair stage by age, and proportion reaching each stage by the time of the 14 year questionnaire

Age	8	9	10	11	13	14	Reached by age 14
Tanner stage of breast development							
Stage 1	88.1	66.4	41.9	14.1	1.8	0.1	--
Stage 2	11.1	28.5	36.2	34.2	10.7	1.1	88.1
Stage 3	0.8	4.6	18.3	36.5	36.1	16.9	76.3
Stage 4	0.04	0.5	3.5	13.6	40.2	55.1	54.8
Stage 5	--	0.1	0.2	1.6	11.3	26.9	16.8
Tanner stage of pubic hair development							
Stage 1	95.4	83.3	60.8	29.4	4.8	0.2	--
Stage 2	4.2	13.7	26.3	30.9	11.9	0.6	81.8
Stage 3	0.5	2.4	9.4	22.6	23.6	8.4	72.5
Stage 4	--	0.5	2.6	13.1	40.7	47.4	61.2
Stage 5	--	0.1	0.9	3.9	19.1	43.6	27.6

Table 7.3. Coefficients from ordinal probit models for progression through breast and pubic hair stages.

	Breast		Pubic Hair	
Characteristic	Beta coefficient (SE)	p-value	Beta coefficient (SE)	p-value
Age	0.78 (0.01)	<0.0001	0.75 (0.01)	<0.0001
BMI				
<5th percentile	0.68 (0.40)	0.09	-0.30 (0.07)	<0.0001
5th-85th percentile	Reference	--	Reference	--
85th-95th percentile	1.40 (0.26)	<0.0001	0.19 (0.05)	<0.0001
≥95th percentile	2.31 (0.28)	<0.0001	0.20 (0.06)	0.0004
Mother's pre-pregnancy BMI				
<18.5	-0.03 (0.09)	0.65	--	--
18.5-24.9	Reference	--	--	--
25.0-29.9	0.11 (0.06)	0.05	--	--
≥30.0	0.26 (0.09)	0.004	--	--
Mother's age at menarche				
8-11 years	0.28 (0.05)	<0.0001	0.34 (0.05)	<0.0001
12-14 years	Reference	--	Reference	--
≥15 years	-0.30 (0.06)	<0.0001	-0.31 (0.06)	<0.0001
Child ethnic background				
White	-0.29 (0.10)	0.008	-0.56 (0.10)	<0.0001
Non-white	Reference	--	Reference	--
Birth order				
First born	Reference	--	Reference	--
Second born	-0.21 (0.05)	<0.0001	-0.13 (0.05)	0.007
Third born or later	-0.23 (0.05)	<0.0001	-0.20 (0.05)	<0.0001
Interaction: Age and BMI <5th percentile	-0.10 (0.03)	0.004	--	--

Interaction: Age and BMI in the 85th-95th percentile	-0.08 (0.02)	0.0004	--	--
Interaction: Age and BMI ≥95th percentile	-0.15 (0.02)	<0.0001	--	--

Table 7.4a. Median age* at entry in Tanner stage 2-4 of breast and pubic hair development and at menarche, overall and by initiation pathway.

	Breast Development			
Group (n)	**Stage 2**	**Stage 3**	**Stage 4**	**Menarche**
	Median age (25th and 75th percentiles)			
All girls (3938)	10.19 (9.16-11.22)	11.66 (10.80-12.52)	13.19 (12.20-14.18)	12.87 (12.08-13.65)
Thelarche (1482)	9.24 (8.23-10.19)	11.41 (10.57-12.26)	12.97 (12.05-13.89)	12.78 (12.00-13.57)
Pubarche (408)	11.24 (10.46-12.02)	12.19 (11.40-12.99)	13.61 (12.63-14.60)	13.13 (12.34-13.92)
Synchronous (1631)	10.55 (9.72-11.38)	11.64 (10.78-12.51)	13.23 (12.18-14.28)	12.84 (12.06-13.63)
	Pubic Hair Development			
Group (n)	**Stage 2**	**Stage 3**	**Stage 4**	**Menarche**
	Median age (25th and 75th percentiles)			
All girls (3938)	10.95 (9.99-11.91)	11.99 (11.19-12.80)	12.88 (12.04-13.73)	12.87 (12.08-13.65)
Thelarche (1482)	11.59 (10.82-12.37)	12.26 (11.54-12.98)	13.07 (12.29-13.84)	12.78 (12.00-13.57)
Pubarche (408)	9.24 (8.32-10.17)	11.42 (10.50-12.33)	12.57 (11.69-13.44)	13.13 (12.34-13.92)
Synchronous (1631)	10.55 (9.72-11.38)	11.81 (11.00-12.62)	12.76 (11.88-13.65)	12.84 (12.06-13.63)

*Calculated using parametric survival models, with the normal distribution specified

Table 7.4b. Time spent in Tanner stages 2-4 of breast and pubic hair development, and time from initiation of puberty to menarche, overall and by initiation pathway*

	Breast Development			
Group (n)	Stage 2	Stage 3	Stage 4	Stage 2 of initial marker, to menarche
All girls (3938)	1.47	1.53	2.59	2.68
Thelarche (1482)	2.17	1.56	2.54	3.54
Pubarche (408)	0.95	1.42	2.57	--
Synchronous (1631)	1.09	1.59	2.68	2.29
Pubic Hair Development				
Group	Stage 2	Stage 3	Stage 4	Stage 2 of initial marker, to menarche
All girls	1.04	0.89	1.97	--
Thelarche	0.67	0.81	2.07	--
Pubarche	2.18	1.15	1.83	3.89
Synchronous	1.26	0.95	1.95	2.29

*Calculated as difference in median age at entry into subsequent stages. Breast stage 2 is considered the initial marker for all groups except pubarche, where pubic hair stage 2 is considered the initial marker

Figure 7.1. Flowchart of eligibility and exclusions.

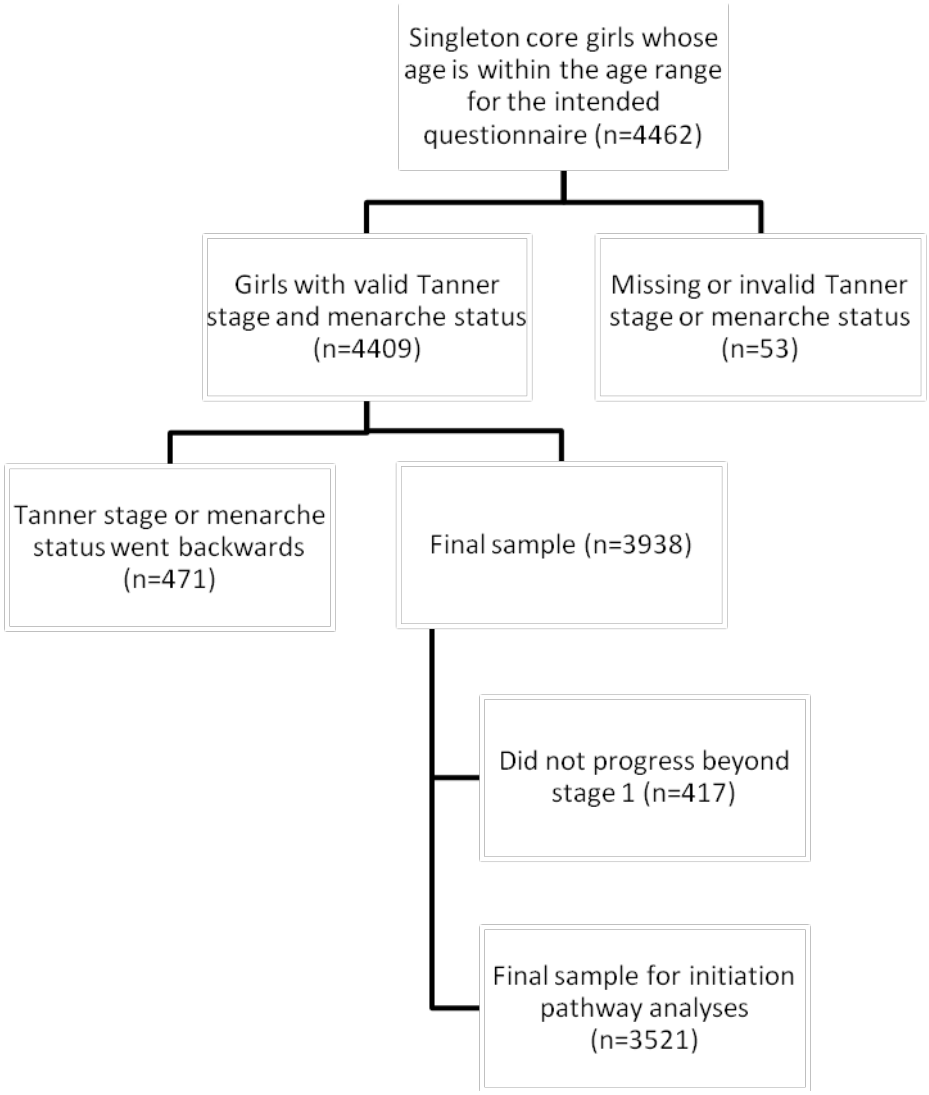


Figure 7.2. Cumulative probability of being in each breast stage by maternal age at menarche, at age 11.

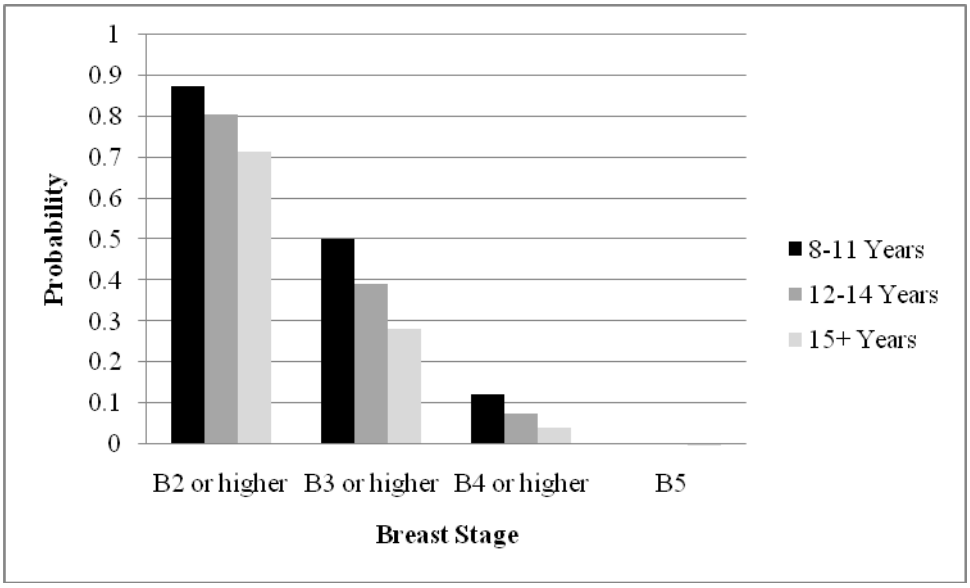
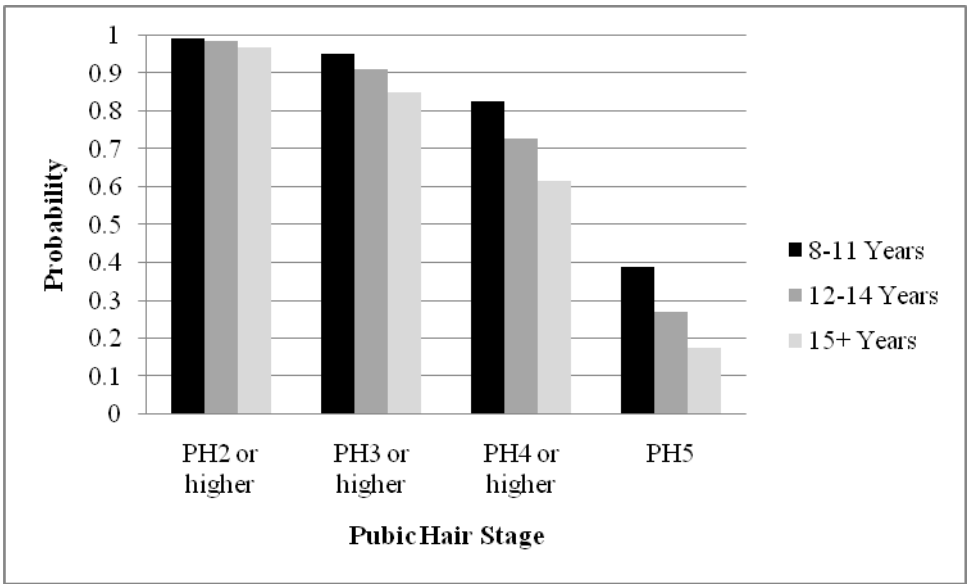


Figure 7.3. Cumulative probability of being in each pubic hair stage by maternal age at menarche, at age 13.



Chapter 8. Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary British cohort

I. Introduction

Puberty is a critical time of growth and development. Timing and pattern of developmental milestones yield information on overall health status, past exposures, and may predict future health outcomes.[41, 60] Age at menarche has decreased from the late 19th century to present,[24, 228] and a secular trend towards earlier development of secondary sexual characteristics has been reported among girls in the United Kingdom.[240] While improvements in nutritional status may be responsible in part, exposure to environmental chemicals may also contribute to altered timing and patterns of pubertal development.

Polyfluoroalkyl chemicals (PFCs) are a class of commercially synthesized chemicals, used as surfactants, surface coatings, and in other applications to decrease staining and sticking. The manufacture of PFCs began in the 1950's, and although there are many different PFCs, the two most studied compounds are perfluorooctanoate (PFOA; also called C8) and perfluorooctane sulfonate (PFOS). Exposure to PFCs can occur through inhalation, ingestion, and dermal absorption; in addition, PFCs are able to cross the placental barrier in both humans[241] and animals,[242, 243] leading to potential fetal exposure. The estimated half-lives for some PFCs are on the order of several years.[244, 245] PFCs act by a variety of mechanisms, many of which could impact timing and progression of pubertal development and reproductive function. Exposure to PFCs can alter the expression of estrogen-responsive genes, including estrogen receptor β.[138, 246-249] PFC exposure may also alter endogenous hormone production; numerous rat studies have shown that exposure decreases testosterone in male rats, and increases estradiol in serum

in both male and female rats.[137, 143, 144, 250, 251] This is thought to occur by induction of hepatic aromatase activity, an enzyme which is key in converting androgens to estrogens. Among female rats exposed to PFOA, increased corticosterone and norepinephrine levels, decreased leptin levels, and disruptions in estrous cycles were all observed.[136] Studies in mice have also demonstrated an effect of PFOA exposure on mammary glands; F0 and F1 mice exposed to PFOA during pregnancy both showed stunted mammary epithelial branching and growth,[140] and incidence of mammary fibroadenomas was increased in rats exposed during adulthood.[141] Some PFCs act as peroxisome proliferator-activated receptor (PPAR) agonists,[252] and thus impact cell differentiation, development and metabolism. In addition, PFCs can bind liver proteins,[253] serum albumin,[253] and thyroid hormone transport proteins.[254, 255] PFCs may increase fatty acid metabolism, and have been shown to decrease lipids and cholesterol in non-human primates,[256] rats and mice.[243] This disruption in fatty acid metabolism and thyroid hormone production can affect development of the fetus and infant, in part by creating an undernourished fetal environment. Fetal nutrient deprivation can, in the phenomenon of fetal programming, impact future risk of developing cardiovascular disease, diabetes, stroke, and obesity.[257, 258] This has implications for pubertal development, as overweight and obese girls experience accelerated puberty compared to their peers,[19, 59, 259] possibly due to increased estrogenic compounds by adipose tissue. Further, fetal nutrition affects the development of the reproductive axis.[260]

We used data from the Avon Longitudinal Study of Parents and Children (ALSPAC) conducted in the United Kingdom to perform a nested case-control study examining the association between age at menarche and gestational exposure to PFCs.

II. Methods

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective cohort study of approximately 14,000 pregnant women residing in Avon (UK) who had an expected delivery date between April 1, 1991 and December 31, 1992.[196] Information has been collected on these women and their offspring through interviews, mailed questionnaires, and clinic visits; details on recruitment and study methods have been described elsewhere.[196, 197] A 'Growing and Changing' questionnaire was developed to collect information on pubertal development and distributed to participants at the ages of 8, 9, 10, 11, 13 and 14 years (1999-2005).[240] Menarche was determined via self-report of menarche status and, if appropriate, age at menarche. Case and control series were selected from the 3682 girls who had completed at least two puberty staging questionnaires (including self-assessed Tanner stage of pubic hair and breast development, and reported age at menarche) between the ages of 8 and 13 (5 possible questionnaires returned; Figure 8.1). Girls meeting eligibility criteria were ordered according to reported age at menarche, at the time the 13 year old data became available. A cut-off of 11.5 years was established as defining 'earlier' menarche. Of girls reporting menarche before the age of 11.5 (n=338), 71% (n=240) of these had at least one prenatal maternal serum sample available, and were considered potential cases. Among girls who reported menarche at or after the age of 11.5, a random sample of 394 was chosen; of these, 71.6% (n=282) had at least one maternal serum sample available, and were considered potential controls. After evaluating the integrity of the maternal serum samples, 90.8% (n=218) of potential cases and 81.6% (n=230) of potential controls had

analyzable samples. Serum samples were collected from mothers during pregnancy; since serum concentrations of PFCs are relatively stable throughout pregnancy,[156] the earliest available serum sample was chosen in the event that multiple samples were available. These analyses were designed to detect an odds ratio of 1.7 or greater with a power of 0.80 (based on 225 cases and 225 controls).

The following PFCs were included in these analyses: perfluorooctane sulfonamide (PFOSA), 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH), 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), perfluorononanoate (PFNA), and perfluorodecanoate (PFDeA). Maternal serum samples were collected from storage facilities at the University of Bristol, and sent to the National Center for Environmental Health at the Centers for Disease Control and Prevention in Atlanta, GA, where they were analyzed by on-line solid-phase extraction coupled to isotope dilution high-performance liquid chromatography-tandem mass spectrometry.[261]

For analytes which were detectable in at least 30% of samples, values below the limit of detection (LOD) were replaced with $\sqrt{LOD} / 2$. For anlaytes detectable in fewer than 30% of samples, no substitution was made for values below the LOD (value set to missing). PFCs concentrations were assessed both individually, and in summation. The following categories were used to group PFCs, based on chemical classes: sulfonamides (PFOSA), sulfonamide esters (Et-PFOSA-AcOH, Me-PFOSA-AcOH), sulfonates (PFOS, PFHxS), and carboxylates (PFDeA, PFNA, PFOA). As with many environmental exposures, distribution of the PFC concentrations was skewed, and a natural log

transformation was used to approximate normality in continuous analyses. In addition, each PFC concentration was treated as a binary exposure, with values categorized as being either at or above the median of values among cases, or below the median.

Potential confounders were identified from the literature on characteristics associated with pubertal development. These included mother's pre-pregnancy BMI (<18.5 [underweight], 18.5-24.9 [normal], 25-29.9 [overweight], and ≥30 [obese]), mother's age at delivery (<20, 20-29, and ≥30 years), mother's age at menarche (8-11, 12-14, and 15+ years), mother's educational level (CSE/none, vocational, O-level, A-level, degree), mother's social class (lower, middle, upper), child's ethnic background (white, non-white), and birth order (first born, second born, third born or later). Social class was derived using the United Kingdom's 1991 Office of Population Censuses and Surveys.[231] Upper class consisted of classes I (professional occupations) or II (managerial and technical occupations); middle class of classes IIINM (non-manual skilled occupations) or IIIM (manual skilled occupations); and lower class of classes IV (partly skilled occupations) or V (unskilled occupations).

In order to assess the association between potential confounders and earlier age at menarche, logistic regression models were used, with a criterion of $p \leq 0.30$. Next, association of potential confounders with total PFC concentration (after natural log transformation) was assessed by a linear model, again using a criterion of $p \leq 0.30$. Those variables associated with both outcome (earlier age at menarche) and exposure (PFC concentration) using these guidelines were considered potential confounders, and included in multivariate logistic models to determine association of maternal PFC concentration with earlier age at menarche. Interaction terms were also included to

evaluate effect modification; these were retained if they had a p-value <0.05 in the multivariate model. Human subject protection was assessed and approved by the ALSPAC Law and Ethics Committee, the Local Research Ethics Committees, and CDC Institutional Review Board.

III. Results

In the ALSPAC cohort, girls were born to mothers who had relatively high education and social class (Table 8.1). Cases were more likely to have mothers with an earlier age at menarche (32.5% reporting menarche between 8 and 11 years of age, compared to 15.2% of controls; p=0.0004). They were also more likely to have mothers who had an overweight or obese pre-pregnancy BMI (29.4% compared to 14.9% of controls; p=0.01), and less likely to have mothers in either the youngest or the oldest age groups at delivery (p=0.29). Cases were more likely to be the first born child (38.9% compared to 33.0% of controls; p=0.23). Although there was little racial/ethnic diversity in the overall population, there were more non-white girls among the cases (5.7% compared to 1.4% of controls; p=0.03). At the time of selection, 51.3% of controls had achieved menarche (median age of 12.42 years); the median age at menarche among cases was 11.08 years. After adding the 14-year data, which had not been available at the time of subject selection, an additional 39 controls (14.2%) had achieved menarche.

PFOS and PFOA were found at the highest median concentrations of 19.8 ng/mL and 3.7 ng/mL, respectively (Table 8.2). All PFCs were detectable in most samples, with the exception of PFDeA, which was only detected in 2.7% of samples. The median total

PFC concentration was 27.3 ng/mL, but this varied somewhat by maternal and child characteristics (Figure 8.2). There was an inverse relationship of total PFC concentration with birth order (p-value<0.0001), with the highest concentrations among first-time mothers (median of 30.8 ng/mL) and the lowest among women who had three or more children (median of 24.4 ng/mL). Also, white girls' mothers had higher total PFC concentrations compared to non-white girls' mothers (medians of 27.3 ng/mL and 23.4 ng/mL, respectively; p-value=0.03). However, due to the small number of non-white children, race/ethnicity was not included in further analyses. Median total PFC concentrations was higher for older compared to younger mothers (median of 28.6 ng/mL for mothers aged 25-29 years, compared to 23.3 ng/mL for mothers aged <20 years; p-value=0.27), and among mothers with CSE or no education (24.7 ng/mL), compared to mothers with an A-level education (29.6 ng/mL, p-value=0.31). There was little variation in PFC concentrations by maternal age at menarche, pre-pregnancy BMI, or social class (p-values of 0.40, 0.46 and 0.92, respectively).

In univariate analysis, there was no difference between cases and controls with respect to the mothers' total PFC concentrations, whether as a continuous or as a binary variable (Table 8.3, Figure 8.3). Only the mothers' total serum concentrations of carboxylates were associated with increased odds of earlier age at menarche of their offspring; mothers' serum concentrations of PFOSA, the sulfonamide esters and sulfonates were all associated with decreased odds of earlier age at menarche. In multivariate analysis, birth order and maternal age at delivery were included as potential confounders based on association with both outcome and PFC serum concentrations (Table 8.3). Results were similar to univariate analysis, with most effect measures

attenuated by the inclusion of these covariates. However, the association of mothers' total sulfonates serum concentrations with their girls' age at menarche was accentuated in the multivariate model, with an OR=0.66 (95% CI: 0.40-1.08) when treated as a continuous, and an OR=0.74 (95% CI: 0.50-1.09) when treated as a binary outcome.

As a sensitivity analysis, the models were run including race/ethnicity as a covariate, and excluding girls of non-white race/ethnicity (n=15; Table 8.4a). This analysis did not substantially change the results found in the original multivariate models. As a second sensitivity analysis, exposure was redefined using the first and third total PFC maternal concentration quartiles among cases; girls born from mothers with serum concentrations during pregnancy at or above the 75^{th} percentile were considered 'exposed' while girls born from mothers with serum concentrations at or below the 25^{th} percentile were considered 'unexposed' (Table 8.4b). Again, this did not substantially alter the original results. The direction of association did switch from below to above the null for the sulfonates and the odds ratio was increased for the carboxylates using these more extreme cut-offs; however, all of the confidence intervals included the null value of 1.0. In the case of the sulfonates, the change in the direction of association was due to a larger proportion of cases (57%) in the second quartile compared to quartiles 1 (43%), 3 (45.5%) and 4 (49%). Finally, the analysis was performed using ordinal logistic regression, where the girls' age at menarche was categorized as 8-10 years, 11-12 years, or ≥13 years (Table 8.4c). Using this ordinal outcome, all associations were in the direction of higher maternal PFC concentrations associated with later age at menarche of the girls, although again all the confidence intervals included the null.

IV. Discussion

We used data from the ALSPAC cohort to explore the association between gestational PFC exposure and age at menarche. Although study participants had nearly ubiquitous exposure to all PFCs except PFDeA, gestational PFC exposure as estimated from the pregnant women's PFCs serum concentrations, did not appear to be strongly associated with age at menarche. The strongest effect was seen for the sulfonates, which were associated with reduced odds of earlier age at menarche (i.e. higher maternal serum concentrations of sulfonates were associated with the girls' later age at menarche). The direction of effect did depend on the class of PFC examined, with differing results seen for the sulfonates compared to PFOSA, sulfonamido esters, and carboxylates. This may be due to differences in the mechanism of action resulting from the physicochemical properties of the PFCs studied, or may be due to chance given the low level of effect.

Serum concentrations of PFCs during 1991-1992 among mothers of girls participating in the ALSPAC were similar to or slightly lower compared to other studies.[262] A Danish study of pregnant women participating in the Danish National Birth Cohort from March 1996 to November 2002 reported mean PFOS serum concentrations of 35.3 (+/- 13.0) ng/mL during the first trimester, and mean PFOA concentrations of 5.6 (+/- 2.5) ng/mL.[156] Among female participants in the 2003-2004 National Health and Nutrition and Examination Survey (NHANES; a nationally representative sample of the general US population) aged 12 years and over, the geometric mean serum concentration of PFOS was 18.4 μg/L, and was higher for the older age groups, and for non-Hispanic white and black participants compared to Mexican American participants.[167] Serum concentrations of PFOA were lower compared to PFOS (geometric mean of 3.5 μg/L),

and there was less variability by age. However, serum concentrations were still elevated among non-Hispanic whites compared to non-Hispanic blacks and Mexican-Americans. The composition of the ALSPAC cohort limited our ability to compare PFC exposure by race, but similarly to the United States findings, white girls had a higher serum concentration of PFCs compared to non-white girls. Variation was also seen by socio-economic indicators, maternal pre-pregnancy BMI and age at delivery, and birth order. Specifically, PFC concentrations during pregnancy were higher for older moms, and who were giving birth to their first-born child. These differences may reflect decreasing exposure to PFCs over time, as well as decreased maternal body burden with successive pregnancies.

Limitations of this study include lack of complete information on age at menarche among controls and some missing information on covariates. Further, due to a relatively small sample size, the study may have been underpowered to detect an association between gestational PFC exposure and age at menarche. Strengths of this study are the inclusion of multiple PFC biomarkers, inclusion of information on important maternal and child characteristics, and the representative nature of the cohort. Finally, we were able to assess gestational exposure to PFCs; chemical exposures during pregnancy are biologically relevant, since this represents the period of organ and brain development. Further, the fetus is more susceptible to such exposures due to smaller size, lack of a complete blood-brain barrier, and absence of metabolizing enzymes.

We compared exposure to PFCs during pregnancy among mothers of girls who did and did not have earlier age at menarche in the ALSPAC cohort. PFC serum concentrations, both total and for individual compounds, varied by maternal

characteristics. However, gestational PFC exposure during pregnancy did not appear to be associated with age at menarche in this cohort.

Table 8.1. Characteristics of study participants.

	Early Girls (N=218)		**Not Early Girls (N=230)**		
Characteristic*	N	%	N	%	P-value for difference**
Mother's highest education					0.88
CSE/none	31	14.8	26	11.9	
Vocational	17	8.1	15	6.9	
O-level	67	31.9	73	33.3	
A-level	61	29.1	66	30.1	
Degree	34	16.2	39	17.8	
Mother's social class					0.99
Lower	18	10.5	19	10.0	
Middle	75	43.6	84	44.0	
Upper	79	45.9	88	46.1	
Mother's age at menarche					0.0004
8-11 years	63	32.5	30	15.2	
12-14 years	119	61.3	153	77.3	
≥15 years	12	6.2	15	7.6	
Mother's pre-pregnancy BMI					0.01
<18.5	7	3.6	11	5.4	
18.5-24.9	132	67.0	163	79.5	
25.0-29.9	39	19.8	19	9.3	
≥30.0	19	9.6	12	5.6	
Mother's age at delivery					0.29
<20 years	1	0.2	7	3.1	
20-24 years	43	19.9	41	17.9	
25-29 years	83	38.4	81	35.4	
≥30 years	89	41.2	100	43.7	
Birth order					0.23
First born	81	38.9	73	33.0	
Second born	66	31.7	87	39.4	
Third born or later	61	29.3	61	27.6	
Child ethnic background					0.03
White	199	94.3	214	98.6	
Non-white	12	5.7	3	1.4	

Menarche status and age at menarche					
Number achieving menarche by age 13 (%)	218	100	118	51.3	--
	Median	Quartiles	Median	Quartiles	--
Age at menarche	11.08	10.75-11.33	12.58	12.17-(NA)	--

*Information was missing for some girls, including information on mother's education (n=19, 4.2%), mother's social class (n=85, 18.9%), mother's age at menarche (n=56, 12.5%), mother's pre-pregnancy BMI (n=46, 10.3%), mother's age at delivery (n=3, 0.7%), girl's birth order (n=19, 4.2%), and girl's race/ethnicity (n=20, 4.5%).

**Compared using logistic regression

Table 8.2a. Gestational serum concentrations among mothers of girls in the case-control study.

Analyte*	N (%) above LOD	Median (quartiles)
PFOSA	311 (69.4)	0.2 (0.2-0.3)
Sulfonamido esters	--	1.1 (0.8-1.8)
Et-PFOSA-AcOH	437 (97.5)	0.6 (0.4-0.9)
Me-PFOSA-AcOH	383 (85.5)	0.4 (0.3-0.8)
Sulfonates	--	21.7 (16.6-27.2)
PFOS	448 (100)	19.8 (15.1-24.9)
PFHxS	447 (99.8)	1.6 (1.2-2.2)
Carboxylates	--	4.4 (3.4-5.7)
PFOA	448 (100)	3.7 (2.8-4.8)
PFNA	447 (99.8)	0.6 (0.5-0.8)
PFDeA	12 (2.7)	--
ΣPFC	--	27.3 (21.6-34.5)

*The limits of detection (LOD) was 0.1 ng/mL (PFOSA, PFHxS, PFOA, PFNA) and 0.2 ng/mL (Et-PFOSA-AcOH, Me-PFOSA-AcOH, PFOS and PFDeA).

Table 8.2b. Gestational serum concentrations among mothers of girls with and without earlier age at menarche.

	Early Girls (N=218)		**Not Early Girls (N=230)**		
Analyte*	N (%) above LOD	Median (quartiles)	N (%) above LOD	Median (quartiles)	P†
PFOSA	153 (70.2)	0.2 (0.2-0.3)	158 (68.7)	0.2 (0.2-0.3)	0.23
Sulfonamido esters	--	1.1 (0.8-1.7)	--	1.2 (0.8-1.9)	0.53
Et-PFOSA-AcOH	212 (97.3)	0.7 (0.4-1.0)	225 (97.8)	0.6 (0.4-0.9)	0.75
Me-PFOSA-AcOH	179 (82.1)	0.4 (0.3-0.7)	204 (88.7)	0.4 (0.3-0.8)	0.26
Sulfonates	--	21.0 (17.0-27.2)	--	21.9 (16.1-26.8)	0.95
PFOS	218 (100)	19.5 (15.4-24.8)	230 (100)	20.0 (14.6-24.9)	0.99
PFHxS	218 (100)	1.7 (1.3-2.2)	229 (99.6)	1.6 (1.2-2.2)	0.47
Carboxylates	--	4.6 (3.4-5.8)	--	4.3 (3.3-5.4)	0.15
PFOA	218 (100)	3.9 (2.9-5.0)	230 (100)	3.6 (2.7-4.7)	0.15
PFNA	217 (99.5)	0.7 (0.5-0.8)	230 (100)	0.6 (0.5-0.8)	0.67
PFDeA	5 (2.3)	--	7 (3.0)	--	--
ΣPFC	--	27.3 (22.3-34.8)	--	27.3 (20.9-34.2)	0.78

*The limits of detection (LOD) was 0.1 ng/mL (PFOSA, PFHxS, PFOA, PFNA) and 0.2 ng/mL (Et-PFOSA-AcOH, Me-PFOSA-AcOH, PFOS and PFDeA).

†P-value for difference between cases and controls

Table 8.3. Regression analysis association between maternal PFC serum concentrations and earlier age at menarche, unadjusted and controlling for birth order and maternal age at delivery*

	Continuous		**Binary**	
Analyte	Unadjusted	Adjusted	Unadjusted	Adjusted
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
PFOSA	0.89 (0.66-1.20)	0.91 (0.67-1.24)	0.82 (0.57-1.19)	0.85 (0.58-1.25)
Sulfonamido esters	0.90 (0.64-1.25)	0.91 (0.65-1.28)	0.76 (0.52-1.11)	0.78 (0.53-1.14)
Et-PFOSA-AcOH	1.02 (0.75-1.40)	1.03 (0.75-1.43)	1.17 (0.81-1.70)	1.17 (0.80-1.72)
Me-PFOSA-AcOH	0.86 (0.67-1.11)	0.86 (0.66-1.12)	0.85 (0.58-1.24)	0.85 (0.57-1.25)
Sulfonates	0.82 (0.52-1.30)	0.66 (0.40-1.08)	0.83 (0.57-1.20)	0.74 (0.50-1.09)
PFOS	0.85 (0.53-1.36)	0.68 (0.40-1.13)	0.92 (0.63-1.33)	0.83 (0.56-1.23)
PFHxS	1.00 (0.74-1.33)	0.89 (0.65-1.22)	1.17 (0.81-1.70)	1.11 (0.76-1.64)
Carboxylates	1.21 (0.76-1.95)	0.98 (0.58-1.66)	1.35 (0.93-1.96)	1.25 (0.84-1.88)
PFOA	1.25 (0.79-1.95)	1.01 (0.61-1.68)	1.35 (0.93-1.96)	1.29 (0.86-1.93)
PFNA	1.00 (0.66-1.52)	0.91 (0.59-1.40)	1.28 (0.88-1.86)	1.15 (0.78-1.69)

*Continuous represents natural log transformed values (values below the LOD are substituted with √LOD/2), while binary represents at or above the median value among cases, versus below the median value among cases. PFDeA is not included in the summed value if it was below the LOD.

Table 8.4a. Multivariate analysis for association between maternal PFC serum concentrations and earlier age at menarche.

	Continuous		**Binary**	
Analyte	Controlling for maternal age at delivery, birth order and race	Removing non-white girls (n=15)	Controlling for maternal age at delivery, birth order and race	Removing non-white girls (n=15)
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
PFOSA	0.92 (0.67-1.26)	0.89 (0.64-1.22)	0.87 (0.59-1.29)	0.85 (0.57-1.27)
Sulfonamido esters	0.91 (0.64-1.28)	0.94 (0.66-1.33)	0.78 (0.52-1.16)	0.79 (0.53-1.18)
Et-PFOSA-AcOH	1.03 (0.74-1.43)	1.08 (0.77-1.51)	1.23 (0.83-1.82)	1.29 (0.87-1.92)
Me-PFOSA-AcOH	0.86 (0.66-1.13)	0.87 (0.67-1.15)	0.90 (0.61-1.34)	0.92 (0.62-1.38)
Sulfonates	0.74 (0.44-1.25)	0.77 (0.45-1.32)	0.77 (0.51-1.15)	0.78 (0.52-1.17)
PFOS	0.77 (0.45-1.31)	0.79 (0.46-1.38)	0.90 (0.58-1.28)	0.88 (0.59-1.32)
PFHxS	0.96 (0.69-1.34)	0.99 (0.71-1.39)	1.11 (0.75-1.65)	1.13 (0.76-1.68)
Carboxylates	1.12 (0.65-1.94)	1.16 (0.67-2.05)	1.32 (0.87-2.00)	1.37 (0.90-2.08)
PFOA	1.14 (0.68-1.94)	1.20 (0.70-2.05)	1.36 (0.90-2.06)	1.41 (0.93-2.15)
PFNA	0.99 (0.64-1.56)	0.98 (0.62-1.55)	1.19 (0.80-1.76)	1.13 (0.76-1.69)

Table 8.4b. Univariate and multivariate analysis for association between maternal PFC serum concentrations and earlier age at menarche.

	Using 25th and 75th percentiles among cases to define 'exposed' and 'unexposed'	
Analyte	Unadjusted	Adjusted
	OR (95% CI)	OR (95% CI)
PFOSA	0.87 (0.55-1.35)	0.89 (0.56-1.41)
Sulfonamido esters	1.01 (0.61-1.67)	1.08 (0.64-1.85)
Et-PFOSA-AcOH	1.04 (0.62-1.74)	1.04 (0.61-1.79)
Me-PFOSA-AcOH	0.79 (0.50-1.23)	0.82 (0.51-1.30)
Sulfonates	1.15 (0.69-1.92)	1.17 (0.67-2.04)
PFOS	1.21 (0.73-2.01)	1.13 (0.65-1.96)
PFHxS	1.13 (0.70-1.81)	1.04 (0.63-1.71)
Carboxylates	1.58 (0.93-2.69)	1.41 (0.76-2.62)
PFOA	1.61 (0.95-2.72)	1.48 (0.80-2.76)
PFNA	1.02 (0.65-1.60)	0.92 (0.57-1.49)

Table 8.4c. Ordinal logistic regression analysis for association between maternal PFC serum concentrations and age at menarche, categorized as 8-10 years, 11-12 years, or 13+years.

	Continuous		**Binary**	
Analyte	Unadjusted	Adjusted	Unadjusted	Adjusted
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
PFOSA	0.83 (0.61-1.13)	0.83 (0.61-1.13)	0.79 (0.54-1.14)	0.82 (0.56-1.21)
Sulfonamido esters	0.82 (0.58-1.14)	0.79 (0.56-1.12)	0.73 (0.50-1.06)	0.73 (0.49-1.08)
Et-PFOSA-AcOH	0.90 (0.65-1.24)	0.89 (0.64-1.24)	1.02 (0.70-1.47)	0.95 (0.65-1.39)
Me-PFOSA-AcOH	0.85 (0.66-1.10)	0.84 (0.64-1.09)	0.87 (0.59-1.27)	0.88 (0.59-1.30)
Sulfonates	0.88 (0.55-1.39)	0.75 (0.46-1.23)	0.77 (0.53-1.12)	0.72 (0.48-1.06)
PFOS	0.89 (0.56-1.43)	0.76 (0.46-1.26)	0.88 (0.61-1.28)	0.84 (0.57-1.25)
PFHxS	0.95 (0.71-1.27)	0.88 (0.64-1.20)	0.93 (0.64-1.35)	0.92 (0.63-1.36)
Carboxylates	0.95 (0.59-1.53)	0.83 (0.49-1.40)	0.90 (0.62-1.31)	0.87 (0.58-1.31)
PFOA	0.98 (0.64-1.53)	0.84 (0.59-1.54)	0.90 (0.62-1.31)	0.89 (0.59-1.34)
PFNA	0.85 (0.56-1.30)	0.82 (0.53-1.27)	1.10 (0.76-1.60)	1.03 (0.70-1.52)

Figure 8.1. Flowchart of eligibility and exclusions.

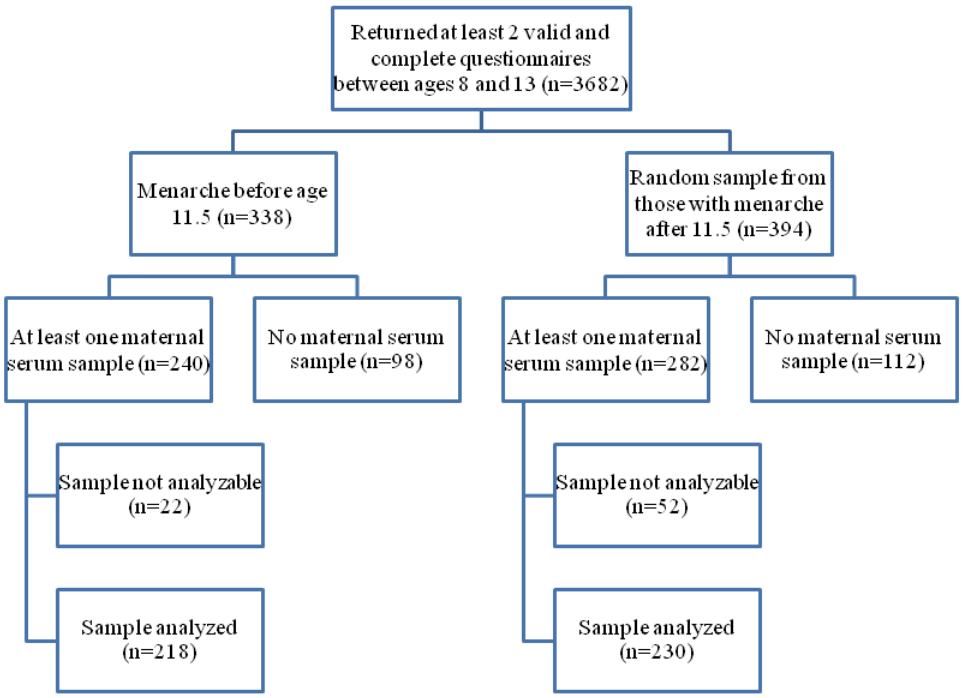


Figure 8.2. Median and quartiles of total maternal PFC serum concentrations by maternal and child characteristics.

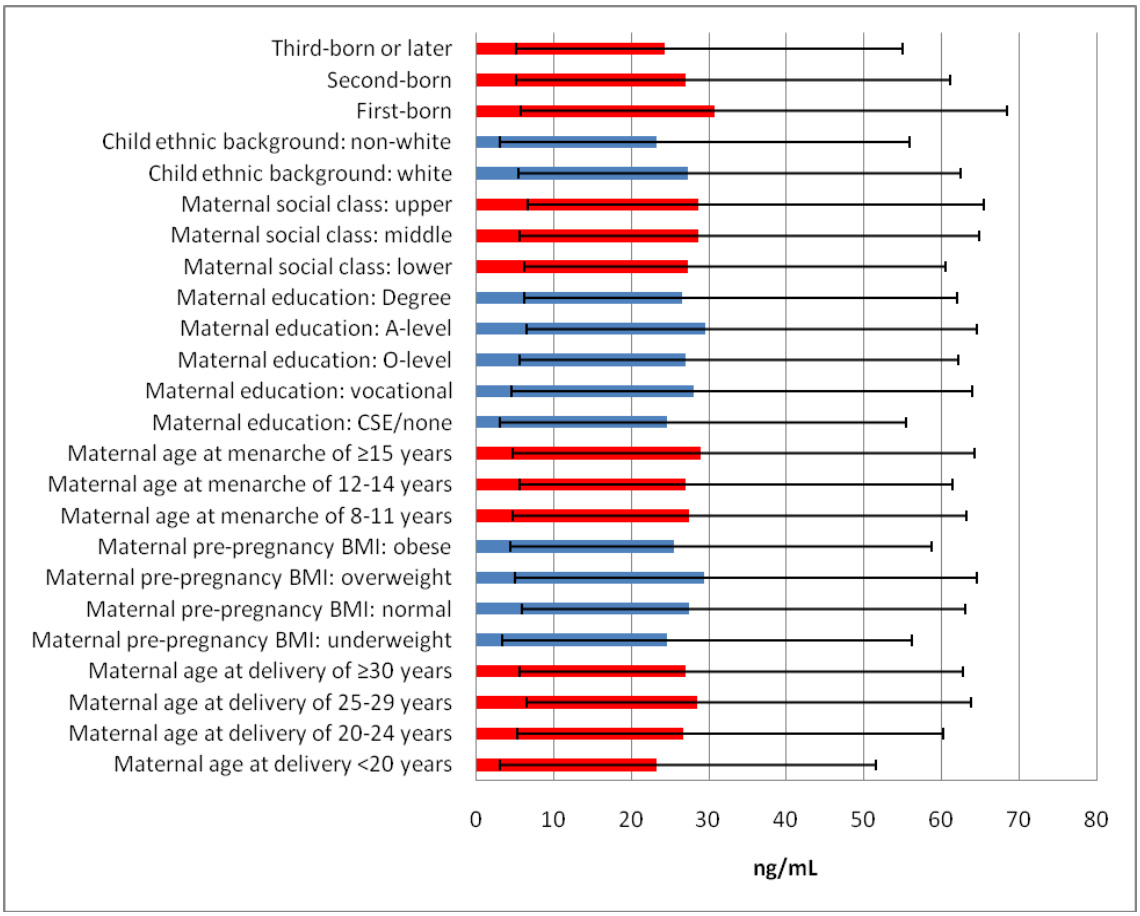
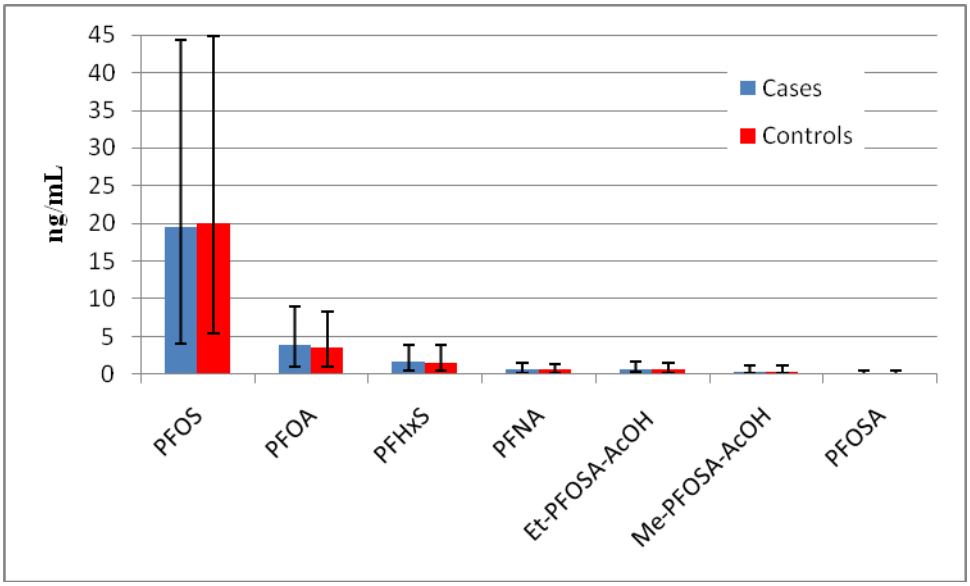


Figure 8.3. Median and quartiles of maternal PFC concentration for cases and controls.



Chapter 9. Differences in the pacing of pubertal development by race and environmental exposures

I. Introduction

Puberty is a critical time of growth and development. Timing and pattern of developmental milestones yield information on overall health status and past exposures, and may impact future health outcomes.[41, 60] Age at menarche has decreased from 1940-1994 among United States girls;[39] however, the reasons for this secular trend are not fully known. While changing nutritional status may be responsible in part, differences by race/ethnicity and exposure to endocrine disrupting chemicals may also contribute to altered timing and patterns of pubertal development.

Timing of puberty varies by race/ethnicity. In the US, Black girls experience earlier initiation of puberty than white girls, and Latina girls experience slightly delayed pubic hair development compared to white girls.[30] At every stage of development, Black girls tend to be at more advanced stages of breast and pubic hair development compared to white girls, and the age at menarche is significantly earlier, as well. However, this divergence in timing of puberty among racial/ethnic groups is fairly recent. Using nationally representative US data, McDowell et al report that among women born before 1950, the age at menarche was similar among Mexican American and non-Hispanic White women, and later among non-Hispanic Black women.[31] However, non-Hispanic Black women had a more rapid decline in the age at menarche compared to the other two groups, so that for women born in later years, they had an earlier age at menarche (12.2 in 1980-1984) compared to non-Hispanic White women (12.5 in 1980-1984).[31] The authors speculate that

this secular pattern could be attributable to differences in nutritional status and different patterns of exposure to endocrine disrupting chemicals.

There is substantial evidence both from animal and from human studies, that exposure to environmental chemicals such as persistent organic pollutants (POPs) may disrupt normal endocrine system development and function, with subsequent consequences for reproductive outcomes. The first evidence that POPs can impact timing of puberty came from the polybrominated biphenyl (PBB) contamination incident in Michigan in the 1970's. Girls who were exposed to PBBs in utero and through breastfeeding began menarche approximately a year earlier than non-exposed girls, and also had earlier ages at pubic hair development.[88] Subsequent studies have shown that polychlorinated biphenyls (PCBs), a similar halogenated compound, can also impact pubertal development. A US study of North Carolina girls found that PCB exposure was associated with earlier breast and pubic hair development, although these were not statistically significant associations.[113] Similarly, earlier age at menarche was reported among Mohawk Nation girls exposed to PCBs.[129] Conversely, a study among girls in New York City found that elevated PCB levels were associated with delayed breast development, but only among girls with BMI below the median for the group.[114] Another group of halogenated compounds, polybrominated diphenyl ethers (PBDEs), are also thought to impact pubertal development. PBDEs are brominated flame retardants which are used in both industry and consumer products; they are closely related to PBBs in structure and function. Animal studies have shown that PBDEs can impact sexual development,[87, 192, 263] possibly through both anti-androgenic and estrogenic action. Due to their widespread use, over 90% of the US population has detectable serum levels of PBDEs,[165] and serum levels appear to be

increasing over time.[264] Finally, polyfluoroalkyl chemicals (PFCs) may also have endocrine disrupting activity. PFCs are commercially synthesized chemicals used as surfactants, surface coatings, and in other applications to decrease staining and sticking. As with PBDEs, the presence of PFCs in consumer products has led to widespread human exposure. However, there is little information on differences in exposure to these environmental chemicals, by race/ethnicity. Further, it is not known whether health effects of such exposures varies by race/ethnicity. We used data from a nationally representative cohort to examine distribution of exposure to certain POPs, and potential association with age at menarche.

II. Methods

Data from the 2003-2004 cycle of the National Health and Nutrition Examination Survey (NHANES)[265] were used to describe exposure to POPS among reproductive-age women, and association of POPs with age at menarche. The NHANES is a nationally representative, sample survey of the civilian, non-institutionalized US population.[203] Certain groups are over-sampled, including adolescents and those ≥60 years of age, African Americans and Mexican Americans.

Biological samples are collected from NHANES participants.[204] For participants aged ≥12 years, serum samples were analyzed for different groups of POPs, including coplanar compounds (dioxins, furans, and coplanar PCBs); non-dioxin like PCBs; PBDEs; 2,2',4,4',5,5'-hexabromobiphenyl (PBB 153); and PFCs. Each group of chemicals was analyzed in a 1/3 subsample of the NHANES cohort, with non-overlapping subsample assignment. Since some of these compounds were not produced

until the 1970's, we restricted our analysis to women aged 12-40 years at the time of screening. For analytes detectable in at least 30% of serum samples, values below the limit of detection (LOD) were replaced by (LOD/√2). Analytes detectable in fewer than 30% of serum samples were not included in further congener-specific analyses; in analyses of summed congeners, values below the LOD were treated as missing. In addition, toxic equivalents (TEQs) were calculated for mono-ortho substituted PCBs, dibenzo-p-dioxins, and chlorinated dibenzofurans, using the 2005 World Health Organizations categorization and toxic equivalency factor (TEF) values.[221] PCB 114 and 123 were not included in the mono-ortho substituted PCB TEQ analysis because they were not measured in the NHANES cohort.

Information on age, sex, race/ethnicity, and other demographic characteristics were collected in the household interview. To investigate variation in exposure to POPs and in age at menarche, certain demographic characteristics were included in the analysis, including age at screening (12-25 or 26-40 years of age), household poverty income ratio (PIR; categorized as low [<1], medium [1 to <2], or high [≥2]), and race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, other/mixed/missing, and other Hispanic). Poverty income ratio is calculated as the ratio of family income to the federal poverty threshold, which is adjusted for household size. The 'other/mixed' race/ethnicity category also included those with unknown race, and was not included in analyses of menarche and POP exposure due to the small size and heterogeneity in this group. Self-reported age at menarche in integer years was collected in the Reproductive Health Questionnaire, also administered during the household interview to participants aged ≥12

years. Women were asked "How old were you when you had your first menstrual period?"

All analyses were performed in SAS and SAS-callable SUDAAN to account for the complex sample design, utilizing appropriate weights provided by NHANES.[222] Estimates based on fewer than 30 unweighted records are considered unreliable, and are not presented. Serum concentrations of each compound are described overall and by demographic characteristics using the estimated geometric mean (and associated standard error) and quartiles. The association between serum concentration of each POP and age at menarche was assessed using survival analysis. Lipid-adjusted serum concentration of each POP was modeled as continuous (after taking a natural log transformation), and as above or below the median. Race/ethnicity, PIR, and age at screening were considered as potential confounders of the association between POP exposure and age at menarche, since each is known to be associated with age at menarche (the outcome). Association with exposure was determined using t-tests, comparing the mean lipid-adjusted serum concentration of each analyte (after taking a natural log transformation) among the race, age and income groups. If any of these covariates were associated with a given analyte, they were included in a multivariate survival analysis model along with the appropriate interaction terms. Effect modification was considered to be present if the interaction term reached a level of significance <0.05; in this case, stratified results are presented. If no effect modification was present, adjusted results are presented.

As a sensitivity analysis, the variability in exposure to POPs, and association with age at menarche, was also examined restricting the study group to women aged 12-18 years, in order to reduce potential recall bias of age at menarche. In addition to the

demographic characteristics already described, body mass index (BMI) was also considered a potential confounder, since BMI at time of measurement would more closely represent BMI at time of menarche. Among older women, changes in BMI over time limit the ability to assess the relationship with POP exposure and age at menarche. Body burden of POPs is likely to change with BMI, since many are stored in adipose tissue and are therefore mobilized during pregnancy, breastfeeding and weight loss.

III. Results

Out of 10,122 persons screened for the 2003-2004 NHANES cohort, 5152 (50.9%) were female (Figure 9.1). Of these, 2088 (40.5%) were between 12-40 years of age, and 2019 (96.7%) of these were interviewed and had a medical exam. The final weighted sample size was 58,840,778 (SD=4,251,374) women (9.1). Most of the women were US citizens (89.5% [SE=1.4%]), and nearly two-thirds were of non-Hispanic White race/ethnicity (66.2% [SE=3.9%]). Of the remaining women, 14.4% (SE=2.2%) were non-Hispanic Black, and 10.8% (SE=2.2%) were Mexican American; fewer than 5% were of other Hispanic, or other/mixed/missing race/ethnicity. Most women had a higher income (family PIR of 2 or greater times the poverty threshold; 55.6% [SE=2.4%]), while approximately one fifth had low income (PIR<1; 22.0% [SE=2.2%]) or medium income (PIR between 1 and 2 times the poverty threshold; 22.4% [SE=1.2%]). Distribution of income varied significantly by race/ethnicity (chi-square p-value<0.0001); non-Hispanic White women were more likely to have a high income (65.9% [SE=3.4%]), compared to non-Hispanic Black women (33.8% [SE=2.9%]) and Mexican American women (28.0% [SE=1.9%]), and less likely to have low income (Table 9.1). The majority of women

aged 20 or older at the time of interview had either some college or an associate's degree (35.7% [SE=1.9%]), or a college degree or above (27.5% [SE=2.2$]). Approximately 15% had less than a high school education/GED. Approximately 45% of women aged 14 or older were never married, and over one third were married (39.7% [SE=1.8%]). The median age at screening was 25.6 years (95% CI: 24.0-27.1), and nearly all (97.9% [SE=0.5%]) of women had had their first period at the time of interview.

The mean age at menarche was 12.45 (SE=0.05) years (Table 9.2). However, when stratifying by age at interview, women who were older (26-40 years old) at interview had a later mean age at menarche compared to women who were younger (12-25 years) at interview (12.64 [SE=0.08] vs. 12.26 [SE=0.04], respectively). Age at menarche also varied by race/ethnicity, with women of other Hispanic, Mexican American and non-Hispanic Black race/ethnicity reporting the earliest mean ages at menarche (12.14 [SE=0.20], 12.16 [SE=0.11], and 12.28 [SE=0.12] respectively) and women of non-Hispanic White race/ethnicity reporting the oldest age (12.54 [SE=0.07]). Mean age at menarche also varied by income; the mean age at menarche for women with a low income was 12.18 (SE=0.11), significantly lower compared to women with a medium income (12.51 [SE=0.08]) or high income (12.53 [SE=0.07]).

Median lipid-adjusted serum concentration of coplanar compounds (dioxins, furans, coplanar PCBs) ranged from non-detectable in over 70% of samples (PCB 189, 1,2,3, 4,7,8- hxcdd, 1,2,3,7,8,9-hxcdd, 2,3,7,8,9-tcdf, 1,2,3,7,8-pncdf, 1,2,3,7,8,9-hxcdf, 2,3,4,6,7,8-hxcdf, 1,2,3,4,7,8,9-hpcdf, 1,2,3,4,6,7,8,9-ocdf, 2,3,7,8-tcdd), to detectable in all samples (Table 9.3). Among the coplanar PCBs, median lipid-adjusted serum concentration ranged from 0.10 (PCB 167) to 4.80 (PCB 28) ng/g lipid (Figure 9.2a).

When summing all coplanar PCBs together, serum concentration were highest among persons reporting non-Hispanic White race (median=16.60), and lowest among persons reporting Mexican American and non-Hispanic Black race (medians of 12.93 and 15.71, respectively) (Figure 9.2b). Serum concentrations were also higher among older women (median of 18.35 for women aged 26-40 years, vs. 14.47 for women aged 12-25 years), and among women with higher income. All non-dioxin like PCBs except PCB 128 were detected in at least 30% of samples, and median lipid-adjusted serum concentrations ranged from 0.20 (PCB 151 and 172) to 8.30 ng/g lipid (PCB 153) (Figure 9.3a). Serum concentrations of summed non-dioxin-like PCBs were highest among persons reporting non-Hispanic White and non-Hispanic Black race (medians of 55.47 and 53.08 ng/g lipid, respectively), and lowest among persons reporting Mexican American race (34.64 ng/g lipid) (Figure 9.3b). As with coplanar PCBs, serum concentrations were higher for older women compared to younger women (67.40 and 37.56 ng/g lipid, respectively) and among women with higher income. PBDE 17, 85, 183 and 66 were detected in fewer than 30% of samples. PBDE median serum concentrations ranged from 0.44 (BDE 154) to 22.85 ng/g lipid (BDE 47) (Figure 9.4a). The highest serum concentrations of summed PBDEs were among persons reporting non-Hispanic Black and non-Hispanic White race (42.79 and 42.19 ng/g lipid, respectively) (Figure 9.4b). In contrast to the PCBs, PBDE serum concentrations were higher for younger women (47.41 and 40.18 ng/g lipid, respectively) and for women with lower income. The only PBB analyzed, PBB 153, was detected in 65.7% of samples, with a median level of 0.97 ng/g lipid. Non-Hispanic White women had the highest serum concentrations of PBB 153, followed by non-Hispanic Black women (1.27 and 0.85 ng/g lipid, respectively), as did women with

higher income (Figure 9.4c). Similar to PCBs, serum concentrations were higher among older women compared to younger women (1.69 vs. 0.40 ng/g lipid, respectively). Toxic equivalent levels were very low for mono-ortho substituted PCBs (median: $1.09x10^{-6}$ [IQR: 0.69-1.66 $x10^{-6}$]) ng/g lipid). Median TEQ for dibenzo-p-dioxins was higher (11.29 [IQR: 10.89-11.70]) ng/g, and the highest TEQ was chlorinated dibenzofurans at 23.24 (IQR: 16.49-38.75) ng/g. Finally, several PFCs were detected in nearly all samples, with PFOS (median: 15.93 ng/mL) and PFOA (median: 3.16 ng/mL) the most prominent PFC congeners (Figure 9.5a). PFC serum concentrations were highest among non-Hispanic Black and White women (23.73 and 22.80 ng/mL, respectively) and lower among Mexican American women (17.60 ng/mL) (Figure 9.5b). PFC serum concentrations increased with income, with a median level of 20.90 ng/mL in the lowest income group compared to 23.08 ng/mL in the highest income group.

In survival analysis models, the TEQ for mono-ortho substituted PCBs, coplanar analytes (PCB 66, 74, 118, 156; 2,3,4,7,8-pncdf) and non-dioxin like PCBs (PCB 99, 138, 146, 153, 170, 172, 177, 178, 180, 183, 187, 194, 196&203, 199, 206) led to hazard ratios significantly less than one, indicating decreased 'risk' of menarche for a given age and therefore later average age at menarche (Table 9.4). PBDEs, PBB 153 and PFCs were not significantly associated with age at menarche, and no analyte was associated with a younger age at menarche. In multivariate survival models, confounding by age, income and race/ethnicity was present for various analytes (Table 9.4). Effect modification was also noted in some instances; for dioxin-like compounds, association with age at menarche differed by race/ethnicity in many instances, while for non-dioxin-like PCBs, effect sometimes differed by age group.

In the subgroup analysis of women aged 12-18 years, a similar distribution of race/ethnicity and of income was observed compared to the overall group of women aged 12-40 years (Table 9.5). Among these younger women, the majority were of normal BMI (18.5 to 24.9; 55.94% [SE=3.13%]). The remaining women were relatively equally divided among the underweight (<18.5; 14.86% [SE=1.96]), overweight (25 to <30; 16.83% [SE=1.95%]) and obese (30 and above; 12.37% [SE=1.94%]) categories. The median serum concentration of PCBs was 13.17 ng/g lipid for coplanar PCBs, and 35.14 for non-dioxin-like PCBs (Table 9.6). The median serum concentration of PBDEs was 44.74 ng/g lipid; since PBB 153 was detected in fewer than 30% of women, it was not considered in further analysis. The median serum concentration of PFCs was 26.80 ng/mL. There was no difference in serum concentrations of either coplanar or non-dioxin-like PCBs by race/ethnicity. However, serum concentrations of non-dioxin like PCBs were higher among underweight women compared to obese women (median serum levels of 40.44 and 25.81 ng.g lipid, respectively), and among medium income women compared to low income women (40.06 and 29.43 ng/g lipid, respectively). Serum concentrations of PBDEs also varied by BMI (higher concentrations among normal weight women compared to obese women; median serum concentrations of 49.71 and 20.20 ng/g lipid, respectively); no difference was seen by income group. PFC serum concentrations were highest among non-Hispanic White women (28.06 ng/mL) and lowest among Mexican American women (20.69 ng/mL). In survival analysis models, non-dioxin-like PCBs were associated with older age at menarche among normal-weight women (adjusted HR=0.49 [95% CI: 0.31-0.79]) (Table 9.7). The TEQ for mono-ortho substituted PCBs was also associated with older age at menarche among non-Hispanic

White women (adjusted HR=0.47 [95% CI: 0.32-0.71]) Higher PBDE serum concentrations were associated with younger age at menarche, among non-Hispanic Black and Mexican American women (adjusted HR=1.28 [95% CI: 1.05-1.55] and 1.30 [95% CI: 1.05-1.62], respectively).

Finally, the survival analysis models for summed congeners and TEQs were repeated, using quartiles of serum concentration to assess dose-reponse (Table 9.8). In most cases, the strongest associations were seen when comparing women with the highest serum concentrations (quartile 4) to women with the lowest serum concentrations (quartile 1). However, significant differences were seen when comparing quartiles 3 and 1 of serum coplanar PCB concentration (HR=0.56 [95% CI: 0.38-0.84) and the TEQ for dioxin-like PCBs (HR=0.61 [95% CI: 0.41-0.90]), as well as when comparing quartiles 4 and 1 of the TEQ for chlorinated dibenzofurans (HR=0.770: 95% CI: 0.50-0.99]).

IV. Discussion

Serum concentrations of PCBs, PBDEs, PBB 153, and PFCs were detectable in most female 2003-2004 NHANES participants. Exposure varied by age, family income, and by race/ethnicity. For PCBs, serum concentrations tended to be higher among older women and women with higher income; this is likely due to the restrictions of PCB production in the 1970s, as older women would have a longer period of exposure (younger women would receive most exposure through transplacental and translactational routes). Also, since PCBs were widely used in transformers and capacitors, persons living in households with multiple electric appliances (and presumably higher income) would have higher exposure. In contrast, PBDE serum concentrations were higher

among younger participants and those with lower family income. Exposure to PBDE appears to be increasing over time in the United States, reflecting the continued widespread use of these chemicals in consumer products.[264, 266] Since PBDEs are not covalently bonded to the materials they are applied to, they are released into the environment through abrasion and weathering, and can be identified in household air and dust.[267] PFC serum concentrations were generally low, with the exception of PFOS, but did tend to be higher among those with higher family income.

We found that certain PCBs and PBDEs are associated with altered age at menarche in the cohort, but no association was found with PBB 153 or PFCs. In some cases, there was confounding or effect modification by age, race/ethnicity and income. Both coplanar and non-dioxin-like PCBs were associated with a later age at menarche, adjusting for these demographic characteristics. This pattern is consistent with the higher serum concentrations of PCBs among women with higher household income, who also had a later age at menarche compared to women with lower household income. The association between PCB serum concentration and age at menarche was attenuated after adjustment for age, race and income, and income remained significantly associated with age at menarche only among non-Hispanic Black women in the model for coplanar PCB serum concentration (race was not significantly associated with age at menarche in any of the multivariate models for PCB serum concentration). The association with a later age at menarche is in contrast to some earlier studies,[113, 129] which found that PCB exposure is associated with accelerated pubertal development. One difference between those analyses and the current study is the classification of PCB congeners; rather than consider total PCB exposure, we separated the effects of non-dioxin like and coplanar PCBs. We

also considered a different classification of PCBs, based on activity (estrogenic, anti-estrogenic or enzyme-inducing) proposed by Wolff et al.[268] In this grouping, estrogenic PCBs were not associated with age at menarche (HR for continuous serum concentration=0.83 [95% CI: 0.63-1.10]), but anti-estrogenic and enzyme inducing PCBs were associated with later age at menarche (HR=0.77 [95% CI: 0.64-0.92] and HR=0.78 [95% CI: 0.67-0.91], respectively), similar to findings for coplanar and non-dioxin like PCBs.

PBDE serum concentration was associated with an earlier age at menarche among younger non-Hispanic Black and Mexican American women, after adjustment for BMI. Although PBDE serum concentration was not significantly different between race/ethnicity and income groups, there was a trend toward higher serum concentrations among the younger non-Hispanic Black and Mexican American women (compared to non-Hispanic White women). A previous study of exposure to brominated flame retardants after an industrial accident also found that highly exposed women experienced an earlier age at menarche, as well as earlier age at pubic hair development.[88] Although this earlier finding focused on polybrominated biphenyl exposure, PBBs and PBDEs have nearly identical chemical structures and may have similar mechanisms of action.

There were some limitations in this analysis. Most importantly, due to the cross-sectional nature of the data, it is not possible to determine a causal effect of POP serum concentration on age at menarche. In some subgroups (for example 'other Hispanic' and 'other/mixed/missing' participants and for some analytes) there were a relatively small number of unweighted observations. Age at menarche was reported in whole years, which could lead to a downward bias in self reported age at first period. If this is the

case, the average age at menarche for this cohort is likely somewhat higher than that estimated from these data. Also, a small number of women had not had their first period by the time of survey; this could also bias the estimated mean and median downward. However, when we substituted 16 as the age at menarche for these women and recalculated the median, the difference was small (11.94 vs. 11.90 years; a difference of roughly 15 days). Some women were missing information on age at menarche (10.51% [SE=1.45%]) or reported not knowing/remembering when they had their first period (0.56% [SE=.26%]), and were not included in survival analysis. When an analyte was below the LOD, the value was replaced with LOD/√2 rather than attempting to assign a value based on a distributional approach. However, such replacement methods have been shown to perform well when the number of non-detects is moderate.[269] We were not able to include PCBs 114 and 123 in the analysis of the toxic equivalent for mono-ortho substituted PCBs, since these analytes were not included in the lab component of NHANES 2003-2004. Finally, we were also not able to evaluate PBB exposure among younger women due to low levels of exposure.

Strengths of this analysis include the use of the NHANES to generate robust, nationally representative estimates of exposure to POPs, and potential association wtih age at menarche in the United States. We were able to include in our analyses important covariates, including a measure of SES, race/ethnicity, age, and BMI. Further, we assessed the effect of both specific congeners and total congener groups. In conclusion, we found that serum concentrations of certain POPs vary by demographic characteristics, and may be associated with altered age at menarche. The variation in serum concentrations among the race/ethnicity and income groups may explain, in part, the

younger age at menarche among non-white and lower income women. Since these chemicals are nearly ubiquitous; it is critical that we understand how these exposures may affect human health, and identify populations at risk for adverse health outcomes through high exposure.

Table 9.1. Characteristics of study participants.

	N (SD)	Percent (SE)
Total	58840778 (4251374)	100 (-)
Country of birth		
US	50407647 (4537304)	85.67 (2.03)
Mexico	3096869 (308352)	5.26 (0.72)
Elsewhere	5336262 (883759)	9.07 (1.72)
Citizenship status		
Citizen	52630506 (4371755)	89.45 (1.37)
Not a citizen	6210272 (619820)	10.55 (1.37)
Age at screening		
12-25 Years	28615840 (2261927)	48.63 (2.23)
26-40 Years	30224938 (2724214)	51.37 (2.23)
Race/ethnicity		
Non-Hispanic White	38967283 (4956183)	66.23 (3.92)
Non-Hispanic Black	8478444 (1120599)	14.41 (2.16)
Mexican American	6330208 (1016942)	10.76 (2.24)
Other/mixed	2528451 (585860)	4.30 (1.01)
Other Hispanic	2536391 (293562)	4.31 (0.69)
Poverty income ratio		
<1	12302193 (1515295)	21.97 (2.24)
Non-Hispanic White	6246092 (1432323)	16.76 (2.83)
Non-Hispanic Black	2951880 (504575)	36.43 (2.50)
Mexican American	2192529 (230036)	36.63 (1.97)
1 to <2	12563380 (1139891)	22.45 (1.15)
Non-Hispanic White	6455732 (1088521)	17.32 (1.78)
Non-Hispanic Black	2409134 (265762)	29.73 (2.95)
Mexican American	2118888 (272181)	35.40 (2.34)
≥2	31108104 (2618740)	55.6 (2.37)
Non-Hispanic White	24575306 (2884763)	65.93 (3.44)
Non-Hispanic Black	2741414 (423673)	33.93 (2.92)
Mexican American	1674170 (277535)	27.97 (1.93)
Education (adults 20+ years)		
<9th grade	1347057 (215104)	3.16 (0.61)
9th-11th grade	5091655 (477709)	11.95 (1.14)
High school/GED	9222436 (1454199)	21.65 (1.99)
Some college/AA	15226999 (1834480)	35.74 (1.93)
College graduate or above	11713045 (743066)	27.49 (2.17)
Education (children <20 years)		
4th grade	45529 (34781)	0.28 (0.21)
5th grade	598739 (158102)	3.69 (.86)
6th grade	1813425 (151387)	11.17 (0.74)
7th grade	1892023 (239803)	11.65 (1.58)
8th grade	2496730 (199022)	15.37 (1.03)
9th grade	1732833 (226137)	10.67 (1.26)

10th grade	2274106 (382202)	14.00 (2.10)
11th grade	2237738 (200981)	13.78 (1.27)
12th grade, no diploma	305270 (85761)	1.88 (0.50)
High school graduate	1194852 (258934)	7.36 (1.46)
GED/Equivalent	37175 (9840)	0.23 (0.07)
More than high school	1364983 (361944)	8.41 (1.97)
Less than 5th grade	15836 (11375)	0.10 (0.07)
Less than 9th grade	223705 (90567)	1.38 (0.58)
Don't know	6640 (6640)	0.04 (0.04)
Marital status (14+ years)		
Married	21199473 (2166427)	39.67 (1.75)
Widowed	148301 (81075)	0.27 (0.14)
Divorced	2826178 (731617)	5.16 (1.15)
Separated	1372121 (273409)	2.50 (0.42)
Never married	24554763 (1547919)	44.79 (2.31)
Living with partner	4722416 (618055)	8.61 (0.89)
Menarche status (based on AAM)		
No	1084086 (237251)	2.06 (0.47)
Yes	51572493 (4102398)	97.94 (0.47)
	Mean (SE)	**Median (95% CI)**
Age at screening	26.16 (0.42)	25.55 (23.98-27.12)

*Information was missing for some covariates, including marital status (6.83% [SE=0.64%]), poverty income ratio (4.87% [SE=0.89%]), and menarcheal status (11.07% [SE=1.54%]).

Table 9.2. Age at menarche*, overall, by age at screening, and by race/ethnicity

	Age at menarche			
Group	Mean	SE	Median (95% CI)	Quartiles
Total	12.45	0.05	11.90 (11.77-12.03)	11.02-12.83
Age at screening				
12-25 Years	12.26	0.04	11.72 (11.58-11.86)	10.81-12.66
26-40 Years	12.64	0.08	12.09 (11.82-12.37)	11.17-12.98
Race/ethnicity				
Non-Hispanic White	12.54	0.07	11.98 (11.78-12.19)	11.15-12.84
Non-Hispanic Black	12.28	0.12	11.66 (11.37-11.95)	10.59-12.77
Mexican American	12.16	0.11	11.61 (11.26-11.96)	10.45-12.79
Other/mixed	12.72	0.31	12.27 (11.56-12.97)	10.64-13.26
Other Hispanic	12.14	0.20	11.72 (11.16-12.28)	10.78-12.55
Poverty income ratio				
<1	12.18	0.11	11.68 (11.32-12.04)	10.57-12.74
1 to <2	12.51	0.08	12.00 (11.82-12.19)	11.13-12.87
≥2	12.53	0.07	11.92 (11.76-12.07)	11.08-12.83

*Among women who achieved menarche by the time of interview (97.9% [SE=0.48%])

Table 9.3. Distribution of analytes.

Analyte	Proportion above LOD (SE)*	Median serum level	Quartiles of serum level	Geometric mean serum level (SE)
Dioxins, furans, coplanar PCBs (ng/g lipid)				
PCB28	100 (--)	4.80	3.19-6.28	4.64 (0.24)
PCB66	99.96 (0.04)	1.29	0.88-1.70	1.28 (0.06)
PCB74	100 (--)	2.90	1.85-4.32	02.90 (0.12)
PCB105	98.11 (0.55)	0.87	0.58-1.29	0.88 (0.06)
PCB118	100 (--)	3.90	2.58-5.88	3.99 (0.22)
PCB156	81.18 (2.26)	1.09	0.50-2.01	0.86 (0.08)
PCB157	53.27 (3.42)	0.19	0.08-0.59	0.22 (0.02)
PCB167	43.34 (3.80)	0.10	0.07-0.57	0.20 (0.02)
PCB189	**14.64 (3.60)**	--	--	--
ΣPCB	--	16.05	11.37-22.59	16.40 (0.79)
Non-Hispanic White	--	16.60	11.67-24.05	16.73 (1.13)
Non-Hispanic Black	--	15.71	11.32-22.25	16.56 (0.90)
Mexican American	--	12.93	10.07-16.40	13.40 (0.85)
ΣPCB				
12-25 Years	--	14.47	10.37-19.42	14.26 (0.67)
26-40 Years	--	18.35	12.67-27.17	18.68 (1.30)
ΣPCB				
PIR <1	--	13.79	10.65-18.01	13.70 (0.80)
1≤PIR<2	--	19.25	10.79-23.11	18.01 (1.54)
PIR≥2	--	16.07	11.81-24.69	17.12 (0.88)
1,2,3,7,8-pncdd	38.80 (3.33)	1.09	0.74-3.30	1.54 (0.10)
1,2,3, 4,7,8-hxcdd	**10.21 (1.92)**	--	--	--
1,2,3,6,7,8-hxcdd	71.68 (2.86)	10.58	4.41-16.64	9.16 (0.60)
1,2,3,7,8,9-hxcdd	**18.84 (3.76)**	--	--	--
1,2,3,4,6,7,8-hpcdd	96.45 (1.40)	19.91	13.03-29.03	19.41 (0.71)
1,2,3,4,6,7,8,9-ocdd	87.68 (1.19)	145.47	97.12-225.88	153.68 (6.24)
2,3,7,8,9-tcdf	**1.35 (0.80)**	--	--	--
1,2,3,7,8-pncdf	**1.62 (0.78)**	--	--	--
2,3,4,7,8-pncdf	57.44 (2.38)	2.72	1.76-4.19	2.78 (0.08)
2,3,4,7,8-pncdfm	49.48 (4.19)	2.54	1.78-3.69	2.63 (0.10)
1,2,3,6,7,8-	39.33 (1.99)	2.41	1.74-3.30	2.47 (0.07)

hxcdf				
1,2,3,7,8,9-hxcdf	**0.63 (0.63)**	--	--	--
2,3,4,6,7,8-hxcdf	**2.87 (0.99)**	--	--	--
1,2,3,4,6,7,8-hpcdf	88.30 (2.26)	7.12	5.08-9.86	6.94 (0.35)
1,2,3,4,7,8,9-hpcdf	**4.75 (1.19)**	--	--	--
1,2,3,4,6,7,8,9-ocdf	**27.97 (2.45)**	--	--	--
3,3',4,4',5-pncb	90.07 (2.32)	11.50	8.35-16.87	11.79 (0.67)
3,4,4',5-tcb	33.89 (5.01)	4.26	3.44-6.44	4.84 (0.23)
3,3',4,4',5,5'-hxcb	34.84 (2.94)	--	--	4.84 (0.23)
2,3,7,8-tcdd	**21.51 (2.83)**	--	--	--
Non-dioxin-like PCBs (ng/g lipid)				
PCB44	100 (--)	1.99	1.34-3.03	2.08 (0.10)
PCB49	98.48 (0.74)	1.33	0.89-1.89	1.28 (0.07)
PCB52	100 (--)	2.69	1.70-3.95	2.63 (0.15)
PCB87	79.40 (3.04)	0.79	0.44-1.20	0.59 (0.06)
PCB99	100 (--)	2.59	1.80-3.87	2.78 (0.17)
PCB101	99.00 (0.66)	1.58	1.05-2.56	1.63 (0.10)
PCB110	98.79 (0.55)	1.14	0.70-1.87	1.19 (0.08)
PCB128	**21.07 (2.95)**	--	--	--
PCB138	100 (--)	7.17	4.29-11.55	7.35 (0.44)
PCB146	98.59 (0.61)	0.90	0.55-1.62	0.96 (0.05)
PCB149	95.30 (1.03)	0.54	0.37-0.88	0.56 (0.03)
PCB151	71.52 (3.50)	0.20	0.09-0.39	0.21 (0.02)
PCB153	100 (--)	8.30	5.14-14.97	8.98 (0.52)
PCB170	96.61 (1.00)	2.00	1.14-4.00	2.04 (0.14)
PCB172	53.16 (2.56)	0.20	0.08-0.50	0.22 (0.02)
PCB177	77.98 (2.23)	0.54	0.27-0.99	0.47 (0.04)
PCB178	67.80 (3.26)	0.36	0.10-0.78	0.32 (0.02)
PCB180	99.53 (0.28)	5.30	2.91-10.34	5.58 (0.34)
PCB183	84.46 (2.54)	0.70	0.39-1.20	0.62 (0.05)
PCB187	97.50 (0.45)	1.74	0.89-3.10	1.71 (0.10)
PCB194	75.16 (3.00)	1.03	0.27-2.09	0.76 (0.07)
PCB195	39.55 (3.38)	0.19	0.15-0.66	0.32 (0.03)
PCB196&203	83.42 (2.39)	1.00	0.50-2.06	0.89 (0.07)
PCB199	82.10 (2.51)	--	--	0.89 (0.07)

PCB206	91.56 (1.70)	0.70	0.40-1.43	0.80 (0.06)
PCB209	90.70 (2.10)	0.50	0.32-0.89	0.57 (0.05)
ΣPCB	--	49.04	33.17-82.95	52.56 (2.64)
Non-Hispanic White	--	55.47	35.41-85.20	54.43 (3.70)
Non-Hispanic Black	--	53.08	34.26-82.78	55.23 (3.92)
Mexican American	--	34.64	26.15-48.22	36.22 (2.39)
ΣPCB				
12-25 Years	--	37.56	25.66-55.49	38.94 (1.71)
26-40 Years	--	67.40	41.95-96.49	68.80 (5.23)
ΣPCB				
PIR <1	--	39.00	26.35-62.72	40.68 (3.31)
1≤PIR<2	--	55.13	31.78-98.69	59.11 (6.64)
PIR≥2	--	55.06	35.59-86.73	55.85 (2.61)
Toxic Equivalents (ng/mL)				
Mono-ortho substituted PCBs	--	$1.09x10^{-6}$	$0.69\ x10^{-6}$-$1.66\ x10^{-6}$	--
Dibenzo-p-dioxins	--	23.24	16.49-38.75	26.15 (1.10)
Chlorinated dibenzofurans	--	11.29	10.89-11.70	11.80 (0.25)
PBDEs (ng/g lipid)				
PBDE 17	**5.21 (1.54)**	--	--	--
PBDE 28	82.14 (2.79)	1.15	0.57-1.94	1.19 (0.10)
PBDE 47	98.16 (0.78)	22.85	11.83-39.31	22.72 (2.05)
PBDE 85	**23.74 (2.15)**	--	--	--
PBDE 99	72.29 (4.23)	4.76	2.30-8.91	5.38 (0.43)
PBDE 100	95.74 (1.06)	4.04	2.24-7.17	4.41 (0.42)
PBDE 153	93.80 (1.65)	5.17	2.93-11.62	6.02 (0.52)
PBDE 154	54.33 (3.87)	0.44	0.33-0.87	0.64 (0.04)
PBDE 183	**7.16 (1.80)**	--	--	--
PBDE 66	**18.15 (2.44)**	--	--	--
ΣPBDE	--	42.19	23.43-71.57	44.30 (3.71)
Non-Hispanic White	--	42.79	22.83-72.08	45.11 (4.75)
Non-Hispanic Black	--	39.42	21.86-75.90	43.24 (3.99)
Mexican American	--	41.21	23.49-68.44	44.20 (4.10)
ΣPBDE				
12-25 Years	--	47.41	23.98-77.72	47.82 (4.86)

26-40 Years	--	40.18	23.40-63.58	41.51 (3.71)
ΣPBDE				
PIR <1	--	45.59	24.81-66.06	43.50 (2.55)
1≤PIR<2	--	41.64	23.29-86.81	47.75 (6.12)
PIR≥2	--	40.42	21.90-71.66	43.25 (5.20)
PBB 153 (ng/g lipid)				
PBB 153	65.66 (4.20)	0.96	0.37-2.23	1.15 (0.13)
Non-Hispanic White	73.22 (3.63)	1.27	0.39-2.68	1.36 (0.17)
Non-Hispanic Black	67.43 (9.98)	0.85	0.38-1.64	1.16 (0.24)
Mexican American	46.31 (4.13)	0.40	0.31-1.26	0.72 (0.10)
PBB 153				
12-25 Years	45.78 (5.65)	0.40	0.33-1.12	0.68 (0.05)
26-40 Years	82.81 (4.10)	1.69	0.78-3.16	1.81 (0.28)
PBB 153				
PIR <1	70.90 (5.79)	0.86	0.38-1.79	1.13 (0.17)
1≤PIR<2	57.45 (7.02)	0.93	0.34-1.94	0.90 (0.13)
PIR≥2	68.82 (4.53)	1.13	0.38-2.92	1.32 (0.17)
PFCs (ng/mL)				
PFOA	99.62 (0.28)	3.16 (2.76-3.56)	2.30-4.65	3.15 (0.16)
PFOS	99.94 (0.06)	15.93 (14.09-17.77)	11.14-22.00	16.08 (0.77)
PFHS	97.07 (0.64)	1.46 (1.29-1.64)	0.77-2.80	1.62 (0.08)
EPAH	**2.55 (0.88)**	--	--	0.75 (0.10)
MPAH	**25.36 (4.15)**	--	(--) – 0.14	0.94 (0.02)
PFDE	**23.25 (5.05)**	--	--	0.53 (0.05)
PFBS	**0.12 (0.07)**	--	--	0.63 (0.17)
PFHP	**9.86 (1.56)**	--	--	0.55 (0.07)
PFNA	98.53 (0.69)	0.77 (0.65-0.88)	0.50-1.14	0.80 (0.07)
PFSA	**15.35 (3.61)**	--	--	0.26 (0.01)
PFUA	**4.40 (1.95)**	--	--	0.67 (0.10)
PFDO	**0 (--)**	--	--	--
ΣPFC	--	22.80 (20.08-25.52)	16.42-32.16	23.15 (1.12)
Non-Hispanic White	--	23.73 (21.00-26.46)	16.89-35.01	25.05 (1.21)
Non-Hispanic Black	--	22.10 (17.76-26.43)	15.19-33.14	22.45 (1.21)
Mexican American	--	17.60 (14.63-20.57)	12.44-24.34	17.26 (1.06)
ΣPFC				
12-25 Years	--	23.76 (21.11-06.42)	17.12-35.01	24.38 (1.18)

26-40 Years	--	21.69 (18.52-24.85)	15.40-30.73	22.11 (1.30)
ΣPFC				
PIR <1	--	20.09 (16.48-23.70)	14.14-28.42	20.49 (1.30)
1≤PIR<2	--	21.35 (16.39-26.32)	14.83-32.33	21.75 (1.29)
PIR≥2	--	23.08 (19.58-26.58)	16.78-33.60	24.82 (1.41)

*Bold font indicates analytes detected in fewer than 30% of samples

Table 9.4. Survival analysis results

	Unadjusted Hazard Ratio (95% CI)		Adjusted Hazard Ratio (95% CI)	
Analyte	**Continuous**	**Binary**	**Continuous**	**Binary**
Dioxins, furans, coplanar PCBs				
PCB28[a]	0.85 (0.71-1.02)	0.81 (0.64-1.02)	--	**0.78 (0.60-0.97)**
Non-Hispanic White			0.84 (0.66-1.08)	--
Non-Hispanic Black			0.83 (0.55-1.26)	--
Mexican American			0.70 (0.45-1.09)	--
PCB66	**0.82 (0.68-0.99)**	**0.73 (0.56-0.93)**	**0.82 (0.68-0.99)**	**0.73 (0.56-0.93)**
PCB74[b]	**0.75 (0.61-0.92)**	**0.65 (0.52-0.83)**		**0.63 (0.48-0.82)**
Non-Hispanic White	--	--	**0.67 (0.49-0.94)**	--
Non-Hispanic Black	--	--	0.88 (0.56-1.38)	--
Mexican American	--	--	0.96 (0.54-1.68)	--
PCB105[a]	0.92 (0.75-1.13)	0.81 (0.63-1.03)	0.94 (0.79-1.11)	0.80 (0.65-1.00)
PCB118 [b]	0.84 (0.70-1.02)	**0.66 (0.53-0.83)**	0.89 (0.72-1.10)	**0.69 (0.54-0.88)**
PCB156 [b]	**0.87 (0.80-0.95)**	**0.64 (0.51-0.79)**	**0.88 (0.78-0.99)**	**0.66 (0.47-0.93)**
PCB157 [b]	0.88 (0.77-1.01)	0.79 (0.62-1.00)		
Non-Hispanic White	--	--	0.88 (0.73-1.05)	0.78 (0.55-1.10)
Non-Hispanic Black	--	--	0.82 (0.60-1.12)	0.63 (0.35-1.15)
Mexican American	--	--	0.85 (0.53-1.37)	0.93 (0.42-2.03)
PCB167 [b]	0.94 (0.83-1.07)	0.95 (0.71-1.28)	0.97 (0.84-1.13)	1.02 (0.76-1.38)
ΣPCB [b]	**0.77 (0.59-1.01)**	**0.69 (0.51-0.92)**	--	0.66 (0.49-0.89)
Non-Hispanic White	--	--	0.76 (0.54-1.06)	--
Non-Hispanic Black	--	--	1.00 (0.52-1.94)	--
Mexican American	--	--	0.82 (0.43-1.59)	--
1,2,3,7,8-pncdd[d]	0.96 (0.83-1.11)	1.07 (0.89-1.29)	0.98 (0.85-1.12)	1.08 (0.90-1.30)
1,2,3,6,7,8-hxcdd [b]	0.95 (0.79-1.16)	0.87 (0.64-1.19)	0.95 (0.76-1.19)	0.84 (0.60-1.17)
1,2,3,4,6,7,8-hpcdd[a]	1.00 (0.82-1.22)	0.89 (0.72-1.11)	0.98 (0.80-1.21)	0.88 (0.71-1.09)
1,2,3,4,6,7,8,9-ocdd[d]	1.02 (0.83-1.25)	0.93 (0.70-1.25)	1.08 (0.87-1.35)	0.98 (0.73-1.31)
2,3,4,7,8-pncdf [b]	0.84 (0.64-1.10)	**0.78 (0.62-0.99)**		
Non-Hispanic White	--	--	0.90 (0.57-1.43)	0.84 (0.58-1.22)
Non-Hispanic	--	--	**0.45 (0.30-0.69)**	**0.44 (0.29-0.68)**

Black				
Mexican American	--	--	1.49 (0.88-2.52)	1.35 (1.00-1.84)
2,3,4,7,8-pncdfm[d]	0.92 (0.71-1.19)	1.03 (0.82-1.31)	0.94 (0.73-1.21)	1.05 (0.83-1.04)
1,2,3,6,7,8-hxcdf[c]	0.91 (0.70-1.20)	0.84 (0.67-1.07)	0.94 (0.70-1.26)	0.85 (0.67-1.07)
1,2,3,4,6,7,8-hpcdf[e]	1.05 (0.93-1.18)	1.00 (0.80-1.24)	1.03 (0.91-1.16)	0.99 (0.79-1.25)
3,3',4,4',5-pncb[b]	1.12 (0.90-1.39)	1.08 (0.82-1.41)		1.09 (0.83-1.42)
Non-Hispanic White	--	--	1.17 (0.86-1.59)	--
Non-Hispanic Black	--	--	1.01 (0.72-1.42)	--
Mexican American	--	--	1.19 (0.69-2.06)	--
3,4,4',5-tcb[a]	0.96 (0.78-1.17)	1.11 (0.95-1.29)	0.93 (0.74-1.15)	1.10 (0.95-1.27)
3,3',4,4',5,5'-hxcb[c]	0.81 (0.61-1.06)	0.87 (0.65-1.17)	0.81 (0.62-1.07)	--
Low income			--	**1.39 (1.01-1.93)**
Medium income			--	0.75 (0.50-1.11)
High income			--	0.78 (0.53-1.16)
Non-dioxin-like PCBs				
PCB44[a]	0.97 (0.80-1.16)	0.95 (0.74-1.22)	0.97 (0.77-1.14)	--
12-25 years	--	--	--	1.28 (0.98-1.74)
26-40 years	--	--	-	0.67 (0.44-1.03)
PCB49[d]	1.01 (0.83-1.23)	0.87 (0.66-1.14)	1.01 (0.82-1.24)	
12-25 years	--	--	--	1.23 (0.85-1.79)
26-40 years	--	--	--	**0.60 (0.41-0.87)**
PCB52[a]	0.98 (0.84-1.14)	1.03 (0.85-1.24)	--	--
Non-Hispanic White	--	--	0.98 (0.79-1.20)	1.05 (0.82-1.35)
Non-Hispanic Black	--	--	0.89 (0.60-1.32)	0.75 (0.41-1.38)
Mexican American	--	--	0.86 (0.59-1.25)	0.86 (0.53-1.38)
PCB87[d]	0.95 (0.85-1.06)	0.84 (0.61-1.15)		1.05 (0.69-1.44)
12-25 years	--	--	1.05 (0.91-1.21)	--
26-40 years	--	--	**0.85 (0.75-0.96)**	--
PCB99[a]	0.86 (0.74-1.00)	**0.78 (0.65-0.95)**	**0.88 (0.78-0.99)**	**0.79 (0.67-0.94)**
PCB10[a]	0.92 (0.78-1.07)	0.85 (0.65-1.11)	--	--
12-25 years	--	--	--	0.97 (0.69-1.35)
Non-Hispanic White	--	--	1.09 (0.88-1.35)	--
Non-Hispanic Black	--	--	1.00 (0.78-1.29)	--
Mexican American	--	--	0.92 (0.56-1.50)	--
26-40 years	--	--	**0.66 (0.47-0.93)**	--

Non-Hispanic White	--	--	0.90 (0.41-1.95)	--
Non-Hispanic Black	--	--	0.80 (0.50-1.27)	--
Mexican American	--	--		
PCB110[a]	0.96 (0.83-1.10)	0.90 (0.68-1.19)	--	--
Non-Hispanic White	--	--	0.92 (0.77-1.11)	0.83 (0.57-1.22)
Non-Hispanic Black	--	--	0.96 (0.72-1.27)	0.83 (0.48-1.45)
Mexican American	--	--	0.89 (0.63-1.25)	1.02 (0.69-1.50)
PCB138[b]	**0.83 (0.73-0.95)**	**0.64 (0.49-0.85)**	0.86 (0.73-1.02)	**0.66 (0.45-0.97)**
PCB146[b]	**0.78 (0.66-0.93)**	**0.67 (0.53-0.84)**	**0.78 (0.64-0.95)**	**0.69 (0.50-0.94)**
PCB149[a]	0.93 (0.77-1.12)	0.90 (0.66-1.23)	0.92 (0.76-1.10)	--
Non-Hispanic White	--	--	--	0.85 (0.57-1.26)
Non-Hispanic Black	--	--	--	0.79 (0.46-1.36)
Mexican American	--	--	--	0.92 (0.59-1.43)
PCB151	0.96 (0.81-1.13)	0.91 (0.68-1.23)	0.96 (0.81-1.13)	0.91 (0.68-1.23)
PCB153[b]	**0.80 (0.69-0.92)**	**0.63 (0.49-0.83)**	**0.80 (0.66-0.97)**	**0.63 (0.44-0.92)**
PCB170[b]	**0.79 (0.71-0.87)**	**0.65 (0.52-0.83)**	**0.77 (0.67-0.90)**	**0.66 (0.46-0.94)**
PCB172[b]	0.87 (0.76-1.00)	**0.72 (0.57-0.91)**	0.88 (0.75-1.03)	0.74 (0.55-1.01)
PCB177[b]	**0.85 (0.73-0.99)**	**0.70 (0.51-0.95)**	**0.86 (0.74-0.93)**	**0.69 (0.50-0.96)**
PCB178[b]	**0.84 (0.72-0.99)**	**0.70 (0.57-0.85)**	**0.83 (0.71-0.99)**	**0.70 (0.54-0.91)**
PCB180[b]	**0.81 (0.71-0.93)**	**0.65 (0.52-0.82)**	**0.81 (0.68-0.97)**	**0.65 (0.45-0.94)**
PCB183[b]	**0.86 (0.79-0.94)**	**0.66 (0.52-0.83)**	**0.87 (0.78-0.98)**	**0.65 (0.47-0.88)**
PCB187[b]	**0.86 (0.75-0.98)**	**0.76 (0.61-0.93)**	0.86 (0.75-1.00)	
12-25 years	--	--	--	**0.54 (0.37-0.78)**
26-40 years	--	--	--	1.02 (0.66-1.59)
PCB194[b]	**0.88 (0.79-0.98)**	**0.70 (0.54-0.91)**	0.89 (0.77-1.04)	
Low income	--	--	--	1.13 (0.64-2.00)
Medium income	--	--	--	0.98 (0.63-1.54)
High income	--	--	--	**0.59 (0.36-0.95)**
PCB195[b]	0.89 (0.77-1.04)	0.81 (0.63-1.06)	--	--
Non-Hispanic White	--	--	0.93 (0.78-1.10)	0.89 (0.63-1.25)
Non-Hispanic Black	--	--	**0.74 (0.56-0.98)**	**0.60 (0.39-0.92)**
Mexican American	--	--	0.79 (0.50-1.26)	0.64 (0.28-1.42)
PCB196&203[b]	**0.87 (0.78-0.97)**	0.82 (0.63-1.08)	0.87 (0.74-1.02)	0.84 (0.56-1.25)
PCB199[b]	0.92 (0.81-1.04)	**0.76 (0.62-0.93)**	0.92 (0.80-1.06)	0.75 (0.51-1.09)
PCB206[b]	0.84 (0.71-1.00)	**0.74 (0.58-0.93)**	0.86 (0.68-1.09)	0.75 (0.55-1.02)
PCB209[b]	0.94 (0.78-1.14)	0.86 (0.67-1.11)	0.86 (0.68-1.09)	0.75 (0.55-1.02)
ΣPCB[b]	**0.78 (0.62-0.98)**	**0.70 (0.55-0.90)**	0.80 (0.63-1.02)	**0.75 (0.57-0.98)**

Toxic Equivalents				
Mono-ortho substituted PCBs[b]	0.85 (0.69-1.05)	0.66 (0.49-0.89)	0.91 (0.73-1.14)	**0.68 (0.47-0.98)**
Dibenzo-p-dioxins[d]	0.92 (0.71-1.20)	0.87 (0.67-1.12)	0.96 (0.73-1.25)	0.89 (0.71-1.12)
Chlorinated dibenzofurans[b]	0.83 (0.59-1.19)	0.88 (0.68-1.13)	0.85 (0.57-1.26)	0.89 (0.67-1.18)
PBDEs				
PBDE 28	0.90 (0.77-1.05)	0.95 (0.72-1.26)	0.90 (0.77-1.05)	0.95 (0.72-1.26)
PBDE 47[e]	0.91 (0.80-1.03)	0.90 (0.76-1.08)	0.91 (0.81-1.03)	0.91 (0.77-1.09)
PBDE99	0.94 (0.83-1.06)	0.89 (0.72-1.08)	0.95 (0.84-1.07)	0.90 (0.73-1.11)
PBDE100[g]	0.91 (0.79-1.05)	0.88 (0.72-1.08)	0.92 (0.80-1.07)	0.89 (0.71-1.12)
PBDE 153[g]	0.94 (0.80-1.09)	1.03 (0.79-1.32)	0.95 (0.80-1.12)	1.04 (0.81-1.34)
PBDE 154	0.90 (0.76-1.07)	0.95 (0.73-1.23)	0.90 (0.76-1.07)	0.95 (0.73-1.23)
ΣPBDE	0.94 (0.80-1.11)	0.91 (0.69-1.20)	0.94 (0.80-1.11)	0.91 (0.69-1.20)
PBB 153				
PBB153[b]	1.00 (0.89-1.13)	1.03 (0.74-1.44)		
12-25 years	--	--	0.98 (0.92-1.04)	--
26-40 years	--	--	1.01 (1.00-1.02)	--
Non-Hispanic White	--	--	--	1.19 (0.70-2.02)
Non-Hispanic Black	--	--	--	1.04 (0.76-1.42)
Mexican American	--	--	--	1.02 (0.41-2.55)
PFCs				
PFOA[g]	0.95 (0.85-1.07)	1.03 (0.83-1.29)	0.94 (0.85-1.05)	0.97 (0.78-1.22)
PFOS[g]	0.95 (0.86-1.05)	1.14 (0.95-1.37)	--	1.16 (0.95-1.41)
Low income	--	--	0.88 (0.65-1.19)	--
Medium income	--	--	0.80 (0.61-1.05)	--
High income	--	--	1.00 (0.72-1.38)	--
PFHS[b]	1.00 (0.89-1.13)	1.01 (0.74-1.38)	0.94 (0.82-1.07)	0.96 (0.75-1.24)
PFNA[a]	0.99 (0.89-1.12)	1.08 (0.90-1.30)	1.03 (0.89-1.20)	1.11 (0.85-1.45)
ΣPFC[b]	0.93 (0.83-1.05)	1.10 (0.90-1.34)	0.87 (0.74-1.02)	1.03 (0.81-1.31)

[a]adjusted for age and race
[b]adjusted for age, race and income
[c]adjusted for age and income
[d]adjusted for age
[e]adjusted for race
[f]adjusted for income
[g]adjusted for race and income

Table 9.5. Characteristics of study participants aged 12-18 years.

	N (SD)	Percent (SE)
Total	14347161 (855814)	100 (-)
Race/ethnicity		
Non-Hispanic White	9309828 (1155204)	64.89 (4.78)
Non-Hispanic Black	2293704 (278606)	15.99 (2.25)
Mexican American	1587690 (321868)	11.07 (2.60)
Other/mixed	547766 (180240)	3.82 (1.26)
Other Hispanic	608172 (185330)	4.24 (1.37)
Poverty income ratio		
<1	3046275 (392155)	22.29 (3.03)
1 to <2	2874972 (223837)	21.04 (1.55)
≥2	7742526 (786141)	56.66 (3.45)
Body mass index		
Underweight (<18.5)	2120344 (285491)	14.86 (1.96)
Normal (18.5 to <25)	7985352 (738887)	55.94 (3.13)
Overweight (25 to <30)	2401793 (329772)	16.83 (1.95)
Obese (30 and above)	1766123 (258222)	12.37 (1.94)
Menarche status		
No	1080122 (237200)	7.96 (1.67)
Yes	12489451 (891746)	92.04 (1.67)

*Information was missing for some covariates, including poverty income ratio (4.76% [SE=1.10%]), BMI (0.51% [SE=0.18%]), and menarche status (5.4% [SE=1.25%]).

Table 9.6. Distribution of analytes among study participants aged 12-18 years.

Analyte	Median serum level	Quartiles of serum level	Geometric mean serum level (SE)
Coplanar PCBs (ng/g lipid)			
ΣPCB	13.12	9.52-17.68	13.17 (0.57)
Non-Hispanic White	13.18	10.01-17.89	13.71 (0.80)
Non-Hispanic Black	12.03	9.48-16.10	12.55 (0.56)
Mexican American	10.94	8.48-15.69	11.53 (0.85)
PIR <1	11.70	9.03-15.61	11.83 (0.73)
1≤PIR<2	13.87	10.00-19.77	15.40 (1.44)
PIR≥2	13.17	9.50-17.84	12.93 (1.08)
Underweight	--	--	--
Normal	13.59	9.99-17.57	13.40 (0.68)
Overweight	10.63	8.87-16.14	11.84 (1.48)
Obese	9.73	8.16-19.35	12.15 (2.19)
Non-dioxin-like PCBs (ng/g lipid)			
ΣPCB	35.59	24.48-45.78	35.14 (1.64)
Non-Hispanic White	35.80	24.34-52.01	36.17 (2.42)
Non-Hispanic Black	34.70	27.71-46.19	34.81 (2.00)
Mexican American	28.55	22.14-37.96	29.70 (2.11)
PIR <1	29.43	20.51-38.40	29.48 (2.69)
1≤PIR<2	40.06	29.94-47.43	38.83 (2.73)
PIR≥2	34.92	25.76-49.25	35.38 (2.99)
Underweight	40.44	33.92-47.39	41.69 (3.55)
Normal	35.24	24.53-54.85	36.78 (3.14)
Overweight	35.03	22.80-39.66	32.18 (4.41)
Obese	25.81	17.71-33.61	26.95 (4.39)
Toxic Equivalents (ng/mL)			
Mono-ortho substituted PCBs	6.55×10^{-7}	$5.48\text{-}7.61 \times 10^{-7}$	--
Dibenzo-p-dioxins	17.87	14.25-21.53	18.74 (0.63)
Chlorinated dibenzofurans	9.80	8.33-11.42	10.07 (0.22)
PBDEs (ng/g lipid)			
ΣPBDE	44.57	22.66-76.00	44.74 (5.74)

Non-Hispanic White	36.98	19.65-69.88	39.39 (7.12)
Non-Hispanic Black	42.48	27.05-72.61	48.63 (3.80)
Mexican American	57.77	34.05-100.28	57.56 (7.98)
PIR <1	60.71	33.39-82.88	50.40 (7.40)
1≤PIR<2	39.52	33.02-105.55	51.66 (10.77)
PIR≥2	35.31	21.93-66.68	39.09 (6.01)
Underweight	--	--	--
Normal	49.71	25.26-81.76	48.82 (7.38)
Overweight	37.76	25.71-58.95	42.23 (7.74)
Obese	20.20	19.01-33.43	24.69 (5.93)
PFCs (ng/mL)			
ΣPFC	26.45	19.45-36.28	26.80 (1.33)
Non-Hispanic White	28.06	21.33-37.57	28.71 (1.59)
Non-Hispanic Black	26.68	18.82-36.90	26.09 (2.11)
Mexican American	20.69	15.90-29.79	22.76 (0.97)
PIR <1	26.53	17.19-36.70	25.41 (2.49)
1≤PIR<2	26.43	17.68-35.09	25.51 (1.82)
PIR≥2	27.49	19.72-39.38	28.21 (2.14)
Underweight	--	--	--
Normal	26.41	19.61-35.30	26.43 (1.66)
Overweight	22.68	17.30-35.07	22.88 (2.61)
Obese	25.50	14.60-41.22	26.85 (2.39)

Table 9.7. Survival analysis results, among women aged 12-18 years.

	Unadjusted Hazard Ratio (95% CI)		Adjusted Hazard Ratio (95% CI)	
Analyte	**Continuous**	**Binary**	**Continuous**	**Binary**
Coplanar PCBs				
ΣPCB	0.80 (0.56-1.14)	0.87 (0.60-1.26)	0.80 (0.56-1.14)	0.87 (0.60-1.26)
Non-dioxin-like PCBs				
ΣPCB[a]	**0.68 (0.51-0.90)**	0.78 (0.57-1.08)	--	--
Underweight	--	--	--	--
Normal	--	--	**0.49 (0.31-0.79)**	0.66 (0.42-1.05)
Overweight	--	--	0.81 (0.44-1.50)	0.79 (0.42-1.49)
Obese	--	--	--	--
Toxic Equivalents				
Mono-ortho substituted PCBs[b]	0.76 (0.55-1.04)	0.64 (0.50-0.82)	0.85 (0.61-1.18)	--
Non-Hispanic White	--	--	--	**0.47 (0.32-0.71)**
Non-Hispanic Black	--	--	--	0.82 (0.52-1.30)
Mexican American	--	--	--	0.86 (0.46-1.62)
Dibenzo-p-dioxins[c]	0.79 (0.44-1.43)	0.85 (0.54-1.34)	0.82 (0.49-1.37)	0.81 (0.57-1.15)
Chlorinated dibenzofurans[b]	1.00 (0.48-2.06)	1.00 (0.68-1.48)	0.93 (0.52-1.69)	0.96 (0.67-1.38)
PBDEs				
ΣPBDE[b]	1.01 (0.87-1.17)	1.00 (0.77-1.30)	--	--
Non-Hispanic White	--	--	1.04 (0.85-1.26)	1.06 (0.71-1.56)
Non-Hispanic Black	--	--	**1.28 (1.05-1.55)**	1.18 (0.75-1.88)
Mexican American	--	--	**1.30 (1.05-1.62)**	1.17 (0.82-1.67)
PFCs				
ΣPFC[b]	0.96 (0.60-1.54)	0.99 (0.63-1.55)	--	1.13 (0.77-1.67)
Underweight	--	--	--	--
Normal	--	--	1.17 (0.78-1.76)	--
Overweight	--	--	1.27 (0.38-4.29)	--
Obese	--	--	1.17 (0.33-4.10)	--

[a]adjusted for income and BMI
[b]adjusted for race and BMI

[c]adjusted for BMI

Table 9.8. Survival analysis of the association between quartiles of persistent organic pollutant serum concentration and age at menarche.

	Adjusted Hazard Ratio (95% CI)		
Analyte	**Q2 vs. Q1**	**Q3 vs. Q1**	**Q4 vs. Q1**
PCBs (ng/g lipid)[a]	0.96 (0.64-1.44)	0.86 (0.58-1.29)	0.69 (0.46-1.04)
Coplanar PCBs (ng/g lipid)[a]	0.89 (0.69-1.16)	**0.56 (0.38-0.84)**	0.70 (0.44-1.12)
Non-dioxin-like PCBs (ng/g lipid)[b]	1.05 (0.71-1.56)	0.82 (0.58-1.15)	0.72 (0.46-1.14)
Dioxin-like PCBs TEQ[b]	0.93 (0.74-1.16)	**0.61 (0.41-0.90)**	0.71 (0.43-1.16)
Dibenzo-p-dioxins TEQ[c]	1.15 (0.81-1.63)	0.94 (0.62-1.43)	0.99 (0.68-1.45)
Chlorinated dibenzofurans TEQ[b]	0.86 (0.56-1.34)	0.96 (0.73-1.25)	**0.70 (0.50-0.99)**
PBDEs (ng/g lipid)	1.07 (0.75-1.52)	0.93 (0.58-1.50)	0.95 (0.69-1.30)
PBB153 (ng/g lipid)[d]	--	--	--
12-25 years	0.75 (0.49-1.13)	0.86 (0.54-1.37)	0.75 (0.47-1.20)
26-40 years	0.75 (0.37-1.49)	0.91 (0.49-1.67)	1.03 (0.53-2.00)
PFCs[e]	--	--	--
12-25 years	0.80 (0.43-1.47)	1.08 (0.73-1.61)	0.82 (0.59-1.13)
26-40 years	0.65 (0.37-1.15)	1.07 (0.75-1.54)	0.81 (0.51-1.28)

[a]adjusted for age and income
[b]adjusted for age, race and income
[c]adjusted for age
[d]adjusted for income
[e]adjusted for race and income

Figure 9.1. Flowchart of eligibility and exclusions.

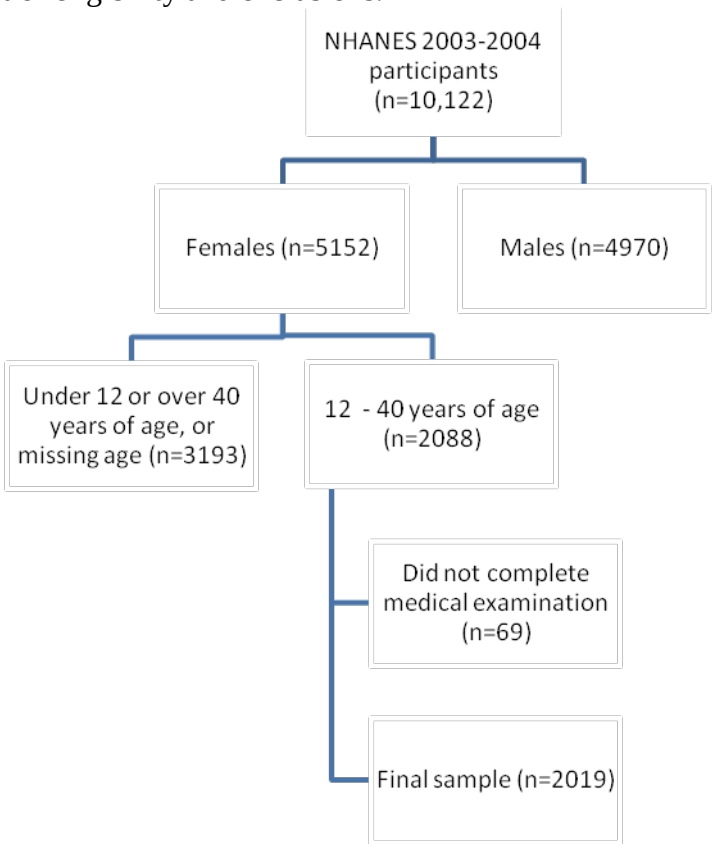


Figure 9.2a. Median and quartiles of lipid-adjusted serum levels of dioxins, furans and coplanar PCBs.

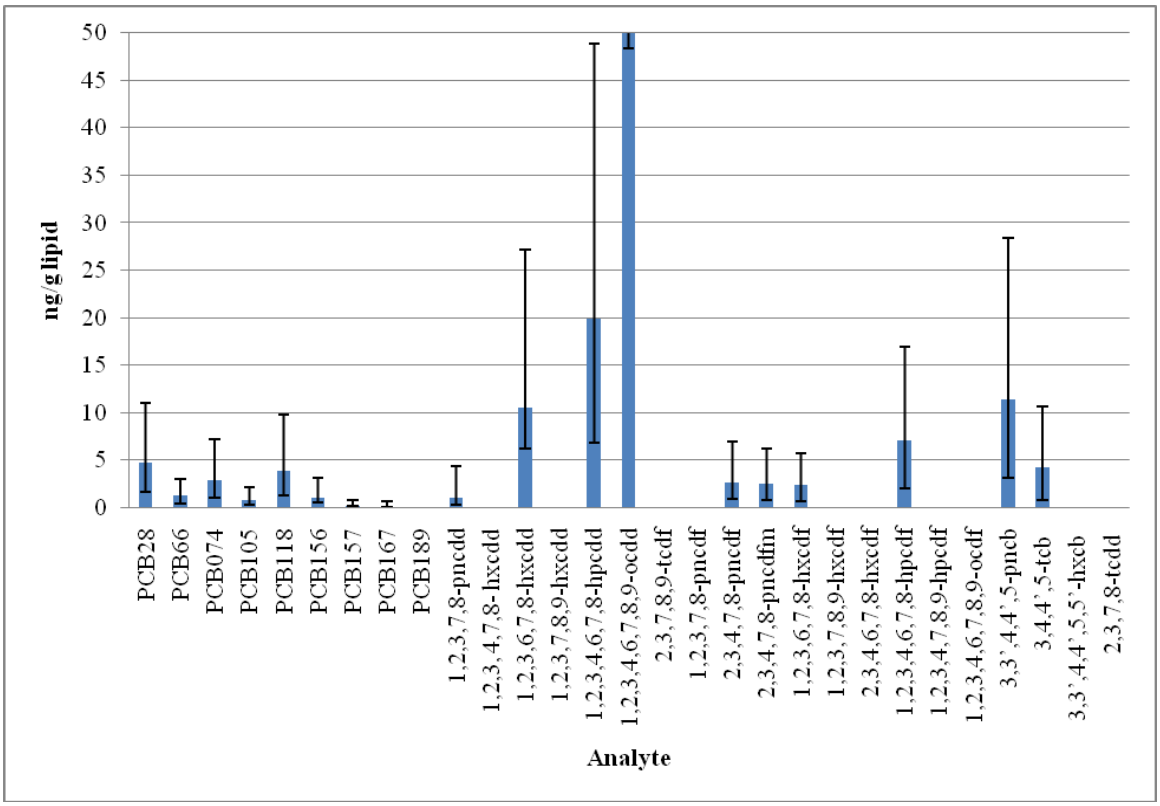


Figure 9.2b. Median and quartiles of lipid-adjusted serum levels of summed coplanar PCBs.

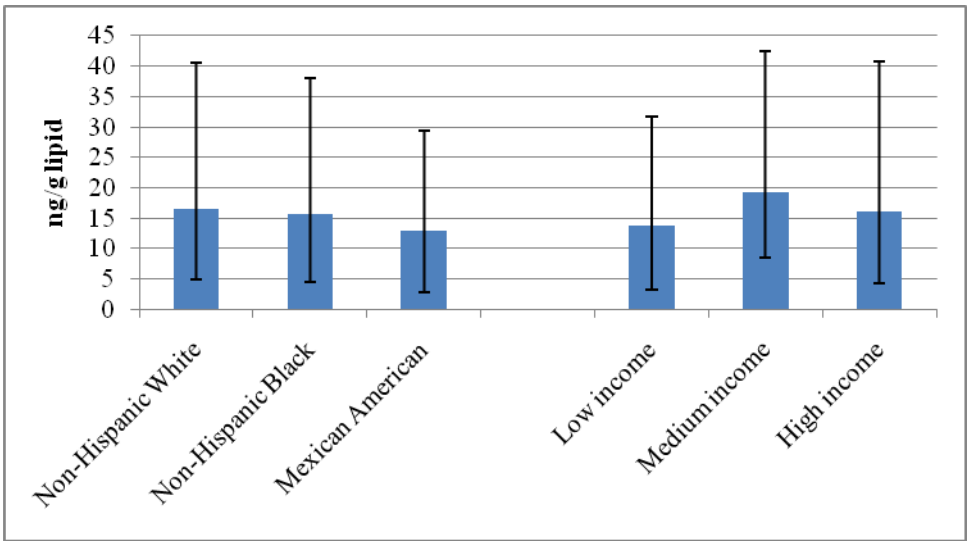


Figure 9.3a. Median and quartiles of lipid-adjusted serum levels of non-dioxin-like PCBs.

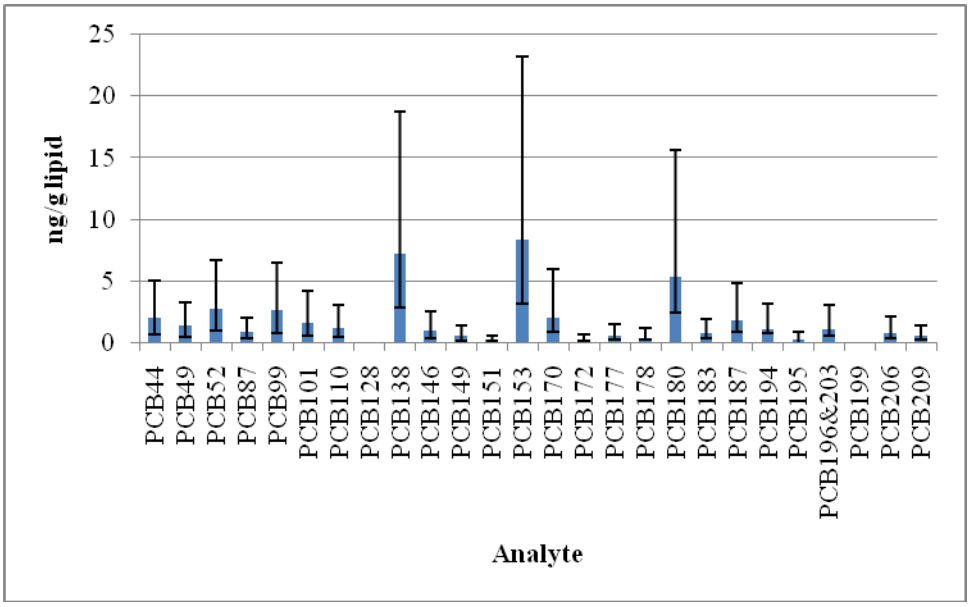


Figure 9.3b. Median and quartiles of lipid-adjusted serum levels of summed non-dioxin-like PCBs.

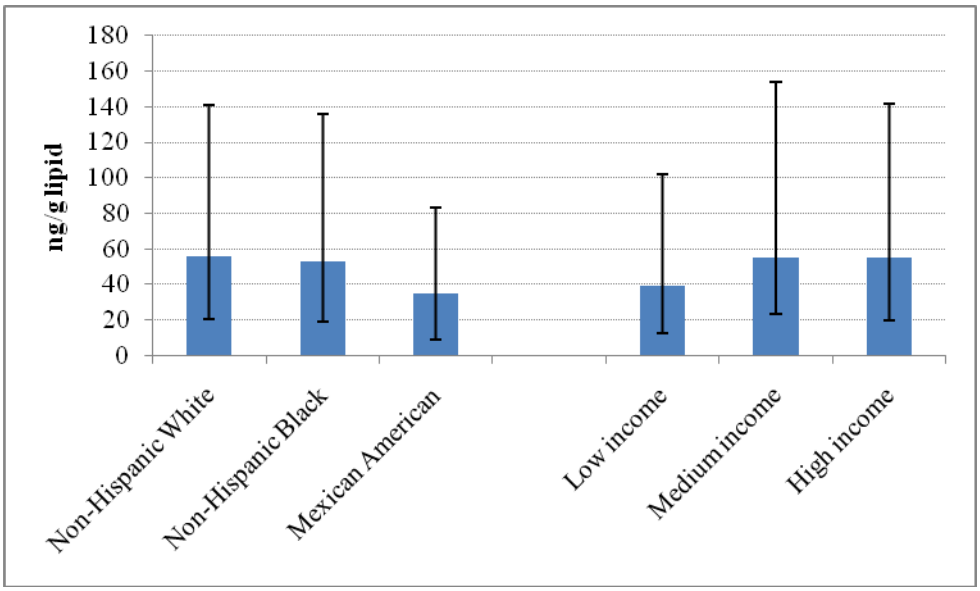


Figure 9.4a. Median and quartiles of lipid-adjusted serum levels of PBDEs.

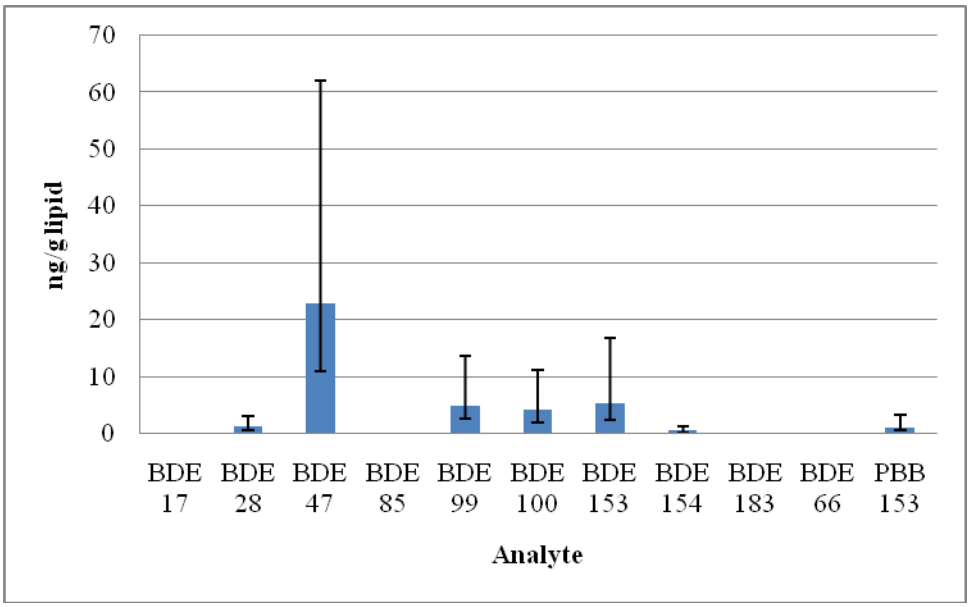


Figure 9.4b. Median and quartiles of lipid-adjusted serum levels of summed PBDEs.

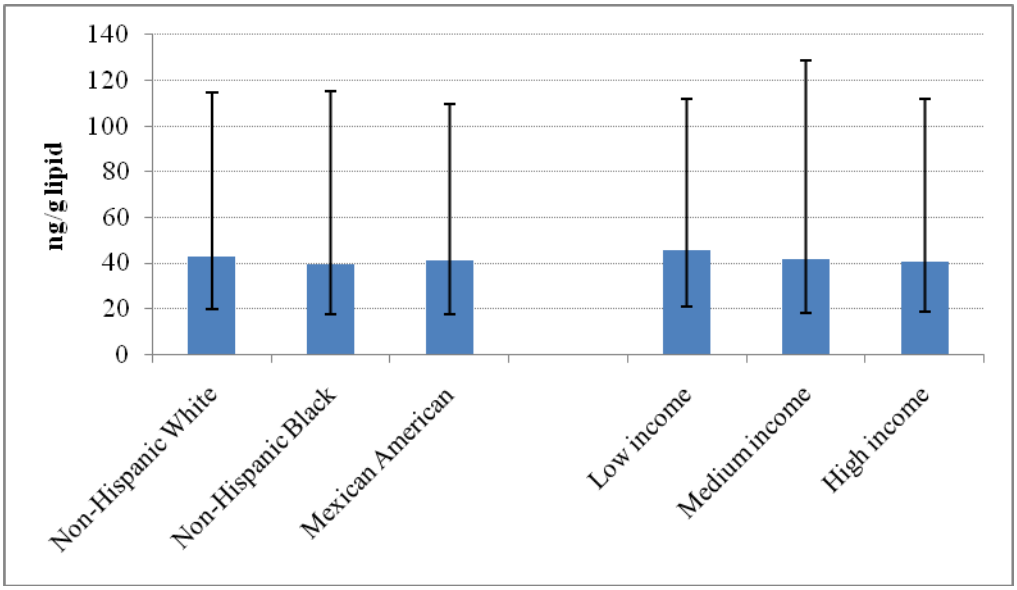


Figure 9.4c. Median and quartiles of lipid-adjusted serum levels of PBB 153.

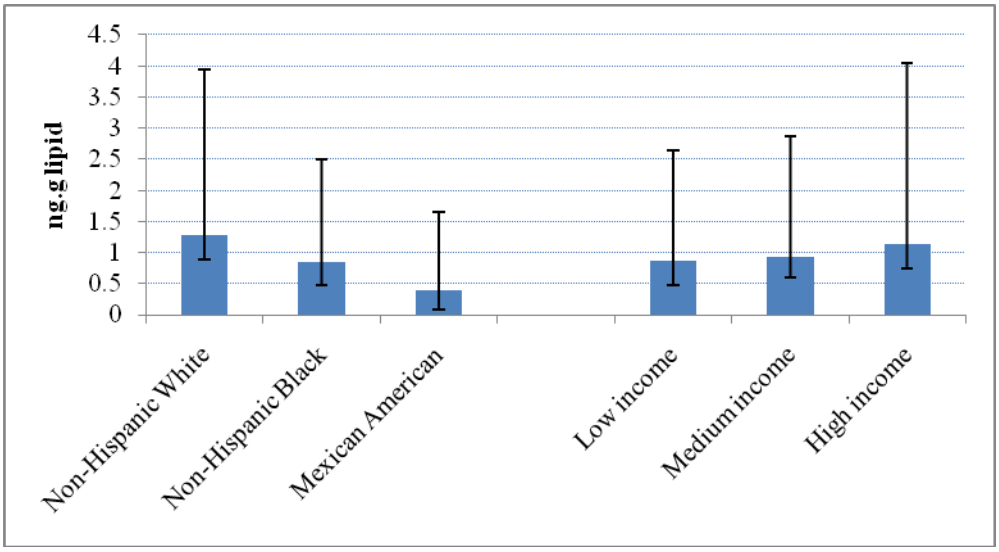


Figure 9.5a. Median and quartiles of serum levels of PFCs.

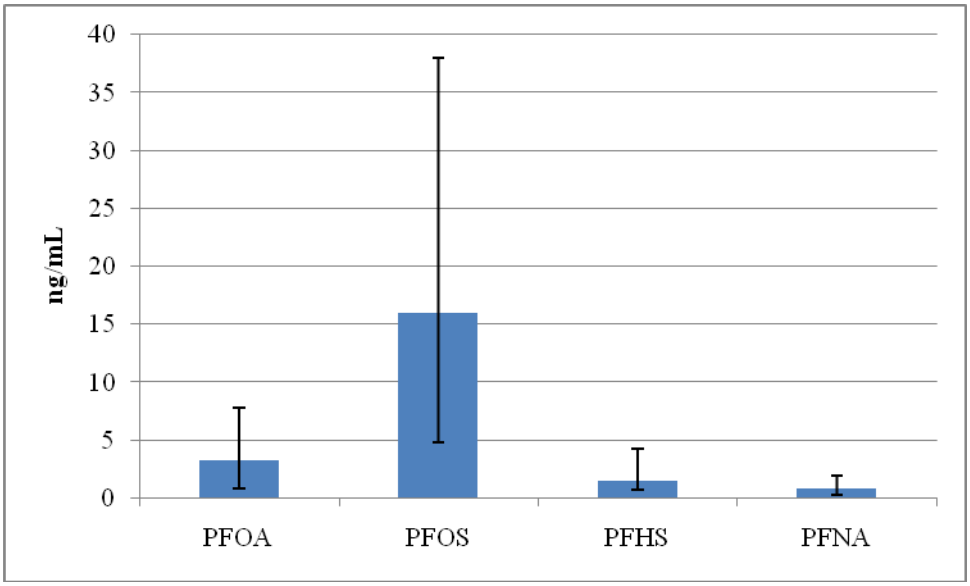
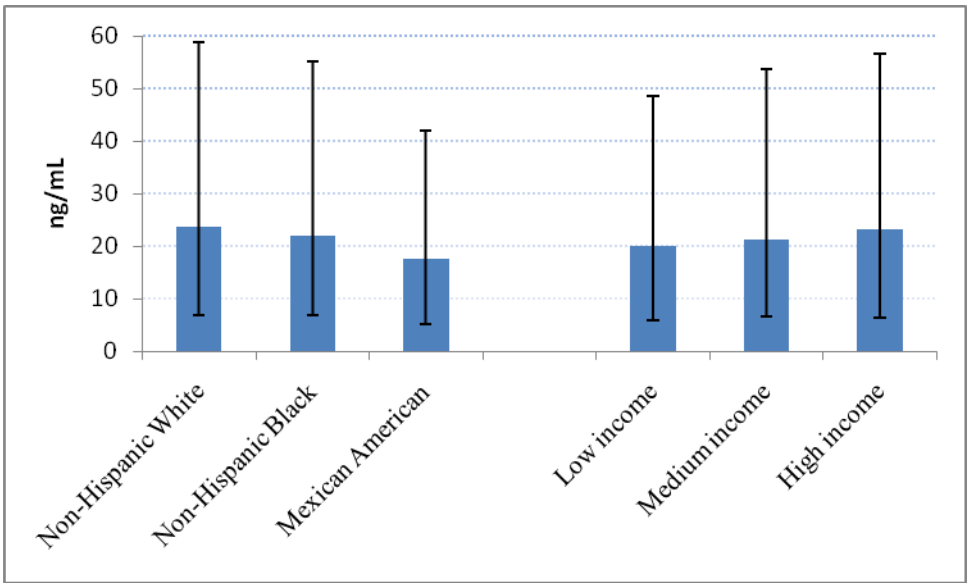


Figure 9.5b. Median and quartiles of serum levels of summed PFCs.



Chapter 10. Conclusions

The adolescent period is a critical time of growth and development. Timing and course of pubertal development affect both psychological and physiological health, and there may be long-term consequences associated with disrupted timing of puberty. Further, due to the complex interaction between multiple body systems during puberty, there is potential for disruption by environmental exposures from the prenatal period onward. We must understand the course of puberty in contemporary adolescents, to generate population norms for development, evaluate secular trends, and identify potential influence of exposure to common environmental chemicals. However, the study of puberty involves certain methodological challenges. This dissertation has identified and utilized innovative techniques to best illustrate the process of puberty among contemporary British girls, and to explore the association between exposure to persistent organic pollutants and age at menarche in Britain and in the United States.

First, the initiation of puberty was described for girls participating in the Avon Longitudinal Study of Parents and Children (ALSPAC). These results confirmed that girls experience different initiation pathways to puberty, and that pathway may be related to various maternal and child characteristics, particularly body mass in both the mother and the child. As a further methodological contribution in studying the initiation of puberty, a bivariate survival analysis approach was used to estimate the correlation between ages at entry into stage 2 of breast and of pubic hair development. Using a novel application of a likelihood maximization technique to the bivariate normal model, we

found that approximately half of the variation in timing of breast development is explained by the variation in timing of pubic hair development.

The progression through subsequent stages of puberty was also described. Girls in the ALSPAC cohort had a similar interval of time between initiation of puberty and menarche as an earlier British cohort, but a slightly earlier age of initiation of puberty and of menarche. Although these results support the theory of a possible secular trend toward earlier maturation, the duration of puberty does not appear to be accelerated. Using an ordinal probit model to accommodate the repeated measures data, several factors were found to be associated with breast and pubic hair development, including maternal at menarche, both maternal and child BMI, race/ethnicity, and birth order.

Variability in exposure to persistent organic pollutants, and the association between such exposures and age at menarche, was assessed using two different populations. First, the association between maternal serum concentrations of polyfluoroalkyl compounds during pregnancy, and earlier age at menarche was examined in the ALSPAC cohort. PFC serum concentrations, both total and for individual compounds, varied by maternal characteristics. However, gestational PFC exposure during pregnancy did not appear to be associated with age at menarche in this cohort. A second study evaluated exposure to brominated flame retardants, dioxins and dioxin-like compounds, polychlorinated biphenyls, and polyfluoroalkyl compounds among women in the United States. Serum concentrations of these pollutants varied by demographic characteristics, and the pattern of exposure was different for each chemical type. Differences in serum concentration by age, race/ethnicity and income were noted, and may be due to differences in living situation, diet, and choice of/access to consumer

goods. Further, PCBs and PBDEs were found to be associated with altered age at menarche.

The findings of this dissertation lead to further research questions and opportunities. In looking at the association between demographic characteristics and timing of breast and pubic hair development, more studies are needed to determine causal relationships for observed associations, as well as the impact of variations in development on future health outcomes. Further, the interplay between maternal body composition and child composition needs to further explored, along with relationship to somatic growth throughout childhood. The maximum likelihood approach used to estimate the correlation between initiation of breast and pubic hair development provides a novel approach for assessing the relationship between timing of milestones in growth and development. Although a bivariate normal survival model was chosen for these research questions, the approach could also be used for other, and for higher order distributions. The variation in exposure described among women in the United States and Britain highlights the need to identify vulnerable populations who may be highly exposed to certain chemicals, and consequently more at risk for adverse health consequences. Finally, associations between certain persistent organic pollutants and age at menarche identified in this work, need to be replicated and further explored.

Understanding patterns of development is necessary to elucidate underlying biological mechanisms and potential pathways for variation in timing and progression of developmental milestones. Exposure to environmental chemicals may disrupt normal endocrine system and gamete development, with subsequent consequences for sexual maturation, growth and function. However, evidence from studies of human female

pubertal development is limited and sometimes conflicting, despite the fact that exposure to persistent organic pollutants is nearly ubiquitous. This dissertation has used data from a contemporary British longitudinal cohort and a representative cross-sectional sample of United States women to describe patterns of pubertal development, variation in exposure to persistent organic pollutants, and associations between such exposures and age at menarche.

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Appendix A: Institutional Review Board withdrawal notice

EMORY UNIVERSITY | Institutional Review Board

FROM: Donna Dent, MISM, MS
Lead, Research Protocol Analyst
Emory University IRB

TO: Michele Marcus, PhD
Principal Investigator

CC:			
	Flanders	William	Epidemiology
	Maisonet	Mildred	Epidemiology
	Yorita	Krista	Epidemiology

DATE: May 8, 2008

RE: **Notification of Submission Determination: No IRB Review Required**
IRB00008919
Evaluating the relation between pre-natal exposures and pubertal development of female offspring using data collected as part of the Avon (U.K.) Longitudinal Study of Parents and Children (ALSPAC)

The above-referenced study has been vetted by the Institutional Review Board (IRB), and it was determined that it does not require IRB review because it does not meet the definition of "Research involving Human Subjects" or the definition of "Clinical Investigation" under applicable federal regulations. Accordingly, IRB review is not required.

45 CFR Section 46.102(f)(2) defines "Research involving Human Subjects" as follows:

Human subject means a living individual about whom an investigator (whether professional or student) conducting research obtains:

(1) data through intervention or interaction with the individual, or
(2) identifiable private information

Intervention includes both physical procedures by which data are gathered (for example, venipuncture) and manipulations of the subject or the subject's environment that are performed for research purposes. Interaction includes communication or interpersonal contact between investigator and subject. Private information includes information about behavior that occurs in a context in which an individual can reasonably expect that no observation or recording is taking place, and information which has been provided for specific purposes by an individual and which the individual can reasonably expect will not

be made public (for example, a medical record). Private information must be individually identifiable (i.e., the identity of the subject is or may be ascertained by the investigator or associated with the information) in order for obtaining the information to constitute research involving human subjects.

In addition, the IRB has determined that the study is not a "Clinical Investigation" under applicable Food & Drug Administration regulations because it does not involve a test article and does not otherwise meet the requirements of the definition of "Clinical Investigation" as set forth in 21 CFR Section 50.3(c).

Please note that any changes to the protocol could conceivably alter the status of this research under the federal regulations cited above. Accordingly, any substantive changes in the protocol should be presented to the IRB for consideration prior to their implementation in the research.

Sincerely,

Donna Dent, MISM, MS
Lead, Research Protocol Analyst
Emory University Institutional Review Board
This letter has been digitally signed

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