Environmental Impact on Reproductive Tissue Development, Puberty, and Endocrine-Based Diseases

HYPOTHESES SUBMITTED TO NCS STUDY DESIGN WORKING GROUP AND FEDERAL ADVISORY COMMITTEE

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Introduction/General Principles

It has become widely recognized over the past decade that genetics, or inherited predisposition, may be only a small contributor to the cause of some adult endocrine-based diseases. In particular, cancers of the breast, prostate, and testes; inflammatory diseases such as endometriosis, benign prostatic hyperplasia and prostatitis; conditions such as osteoporosis, Alzheimers, infertility, amenorrhea, and polycystic ovarian syndrome (PCOS) are diseases in which environmental factors have been implicated as major contributors to the disease state. There is little evidence in the literature to pinpoint the specific environmental factors that associate with these conditions. That may well be due to the fact that ”lifelong”, and particularly early life, exposures of individuals contribute to these various afflictions.

In utero environment, comprising maternal hormone concentrations, integrity of the placenta, availability of nutrients, and exposure to environmental toxicants, may influence risk of many of the described conditions. Furthermore, childhood exposures including from mothers' milk, childrens' diets, and childrens' other interactions with their environments, may affect childhood growth patterns. Changes in growth can affect the onset of puberty and may be related to a variety of disease outcomes. These early exposure periods may also be important in correct “wiring” of the hypothalamic-pituitary-gonadal axis (HPGA). Development of the central nervous system is particularly sensitive to endocrine

fluxes, of both steroidogenic and non-steroidogenic origins. Many environmental toxicants, when tested in animals, have had effects on the developing brain as the primary target (Cooper and Goldman, 1988). Furthermore, many environmental influences examined in animals have demonstrated adverse effects on reproductive outcomes such as organ development, timing of puberty, estrous cyclicity, libido, and fertility (as described in detail within the Fertility and Early Pregnancy working group proposal).

We propose here a ”life course approach to disease development”, in which environmental exposures during early sensitive periods of reproductive development predispose individuals to elevated risk for adult disease. There is strong evidence from animal models and indirect data from epidemiological studies that suggest: 1) early life exposures are correlated with changes in pubertal timing and 2) altered pubertal timing or reproductive organ development relates to several disease states. Increases in rates of precocious puberty in girls and reproductive tract developmental abnormalities in boys make inclusion of these hypotheses in the National Children’s Study imperative.

Specific Hypotheses Related to a Lifecourse Approach to Prevention of Precocious Puberty and Reproductive Tract Disease in Adolescence

Proposed Core Hypotheses –Female Precocious Puberty

1. Identifiable factors in our environment, present early in a child’s development, contribute to the dramatic decrease in the age at which girls undergo the early signs of puberty.
    - Data from 17,000 girls between 3 and 12 years of age (Herman-Giddens et al.,1997) indicates that the early signs of puberty are occurring about 1 year earlier in Caucasian girls and nearly 2 years earlier in African-American girls compared to previous studies.
    - Central nervous system abnormalities such as tumors are responsible for 8-30% of precocious puberty cases (Chalumeau, et al., 2002).

2. Precocious puberty in young women is correlated with an increased risk of endocrine-based conditions such as dysmenorrhea, peak bone mass, endometriosis, and early life breast cancer.
    - Factors affecting risk of endometriosis include dysmenorrhea, early menarche, and shorter menstrual cycle length (Cramer and Missmer, 2001).
    - Precocious puberty may alter timing of peak serum estradiol levels and the maintenance of normal circulating hormone status, which are important determinants of peak bone mass and later osteoporotic fracture risk.
    - Breast cancer ranks first among cancers in the world's women (Sasco, 2001) and research questions remain related to events early in life. Risk factors for breast cancer include early menarche and high exposure to estrogens, either endogenous (high levels of free or not

bound SHBG estrogens) or exogenous (hormone replacement). No human data is available on the increased risk related to exposure to environmental estrogens or to toxicants that can alter aromatase activity, hormone receptor binding or leptin and fatty acid metabolism gene expression.

Male Reproductive Tract Development

3. Pre-conceptional and gestational exposures to environmental contaminants increase the risk for hypospadias, cryptorchidism, and other developmental defects of the male reproductive tract.
    - In more than 80% of newborns with malformations of genitalia, no genetic defect has been demonstrated (Boisen et al., 2001).
    - This hypothesis can be well tested in conjunction with the specific aims outlined by the Fertility and Early Pregnancy work group.

4. Developmental defects of the reproductive system at birth or as a young boy significantly increase the risk of testicular cancer as an adult.
    - Boys with undescended testis are at higher risk of developing testicular cancer. Significant data also suggests that endocrine disruptors in our environment affect individuals with a hereditary predisposition, which is about 20% of the male population (Boisen et al., 2001). Further, estrogenic or antiandrogenic environmental toxicants act *in utero* and during infancy to affect developmental endpoints (see F&EP proposal).

Public Health Significance:

- There is great public interest in cancer prevention and treatment, as the estimated U.S. costs for all cancer diagnoses and treatments are approximately $160 billion.
- Testicular cancer appears in young men beginning at puberty with a peak incidence age 25-30. Incidence of testicular germ cell cancer is increasing (from 1940-1980, 4-fold increase), with an estimated 7,500 new cases this year. The rate of hypospadias and cryptorchidism has doubled over 20 year periods. Sperm concentrations, previously correlated with male fertility, have decreased by half between 1940 and 1980 (Giwercman et al., 1993).
- Given the secular trend toward earlier puberty, the percentage of girls with precocious puberty (signs of puberty prior to age 8) rose from 2.5% in 1969 to 10% in the 1990's (Chalumeau et al., 2002). In fact, the PROS and NHANES III study data estimate that between 12-14% of 8 year-old girls have Tanner stage II or higher breast development (Lee et al., 2001). In addition to increased costs from pediatrician and specialist visits, there is increased psychological stress in children with precocious puberty and abnormally developed reproductive organs.

- Endometriosis affects approximately 1.6 in 1000 women aged 15-40 (without symptoms), and approximately 21-50% of adolescents with pelvic pain or dysmenorrhea (Cramer and Missmer, 2001).
- Osteoporosis is remarkably common. One out of two women and one in eight men will have an osteoporotic fracture, and associated nursing home and hospital costs total $13.8 billion each year. Approximately 25% of hip fracture patients die within 1 year.

Justification for a Large, Prospective, Longitudinal Study

1. **Large.** There are two main reasons for the large sample size of this study. First, in order to specify the associations of interest, subgroups of limited size (due to specifics of the affliction) will need to be compared. For example, it is estimated that approximately 37% of 7 year old, and 52% of 8-year-old African-American girls showed signs of precocious puberty, while corresponding numbers for Caucasian girls were 6% and 16%, respectively (Herman-Giddens et al., 1997). Of those afflicted with precocious puberty, hospital-based studies have demonstrated that 8-33% of the girls present abnormalities of the central nervous system as a cause of the condition. Another significant factor of precocious puberty in Caucasian girls is obesity (Kaplowitz et al., 2001). Using figures from previous smaller studies, less than 6000 African-American girls would be available from the 100,000 children enrolled in the NCS to estimate the effect of environment on precocious puberty, distinct from obesity and central nervous system origins (assuming 40% of girls are African-American, 40% of those have precocious puberty, 10% have CNS abnormalities and 20% are obese). These 6000 girls could then be evaluated for their environmental exposure to xenoestrogens, phthalates, and polybrominated biphenyls (an example of an environmental mixture that could result in precocious breast development and other signs of puberty). The number of Caucasian girls would be about one-third of that, due to the fact that the incidence of precocious puberty is lower and is more often due to obesity. Clearly, environmental exposures may have a causal role in obesity, so cannot be evaluated distinctly from it. Environment may also cause CNS abnormalities that lead to precocious puberty. Therefore, only this large of a study would provide the number of adolescents to evaluate the impact of these various risk factors and their interactions.

   Second, there are no large studies that have evaluated environmental influence from fetal life to adolescence. In addition to the large sample size needed for main effects and effect modification by time-independent covariates, the life course approach necessitates a large sample size. The environmental factors that the children of the U.S. are exposed to may be acute or chronic (making environmental mixture evaluation even

more difficult). Evaluation of sensitive time periods or cumulative dose would only be possible through prospectively collected data in a large cohort with variable exposure patterns. Acute exposure during very early life may cause a reprogramming of the brain that manifests in later life disease or specifically to alter onset of puberty or hypospadias incidence. Fetal exposures may also result in altered development of the reproductive tissues of the infant and increased risk for inflammatory diseases, infertility and adult cancers. The interaction of the successive growth patterns and exposure potentials during fetal life, infancy, and later childhood may give us more information than any one period alone. Only a very large sample, such as the 100,000 proposed for NCS, will allow the investigations of these interactions.

2. **Prospective.** Historical cohorts lack most variables of current scientific interest in the area of endocrine disrupting factors. Prospectively collected data are needed for reliable information on many exposures of interest. For example, maternal and childhood diet, supplement and medicine use, serum hormone profiles, toxicant exposures during pregnancy and childhood, placental and cord blood specimens, infant and childhood anthropometry, and blood specimens for genetic polymorphisms and environmental exposure assessment would serve as important sources of information from which to collect specific data.

3. **Longitudinal.** Evaluation of risks related to a variety of factors over time can only be adequately evaluated with prospective data. Development of all reproductive tissues mentioned is inherently a longitudinal issue, initiated during fetal life and culminating in adverse effect during adolescence (or infancy for some male defects and adulthood for cancers). Additionally, it is suggested that the environmental factors affecting tissue development may have acute effects during pregnancy and lactation, but chronic effects during childhood. Data are required from many time points along the life course, and it is hypothesized that adverse effects early in life will affect the risk of adverse outcomes later in life. To expand upon the precocious puberty example, the long-term use of estrogen and placental protein-containing creams, cosmetics and hair products by young African-American girls; habits that are thought to contribute to their risk for precocious puberty (Tiwary, 1998; Li et al., 2002), could be evaluated in the child and her parents starting at time of enrollment in the NCS. This single route of exposure could then be compared with other environmental factors to estimate risk for precocious puberty. Other evaluated factors may include formula used (soy or milk-based), maternal dietary supplements, diet as a child, and maternal serum estradiol levels during pregnancy; information that is difficult to obtain or recall unless planned for in a study.

Scientific Merit

Our knowledge base about the adverse effects of individual environmental toxicants on reproductive genitalia, accessory organ and breast development, whether or not those compounds are transferred to the fetus or young child via the placenta or breast milk, and their bioaccumulation in laboratory animals is much broader than that in humans. Animal studies have provided some definition of developmental periods for reproductive tissues during which they are most sensitive to xenobiotics. Additionally, few animal studies have addressed well the issue of toxicant mixture effects. However, it is clear from the animal data that many classes of xenobiotics have the potential to affect the reproductive tissue development, puberty, estrous cyclicity, fertility and adult endocrine-based disease risk, especially when exposed as a fetus/infant. Most of the human data on risk of disease in reproductive tissues following toxicant exposure exists due to accidental, acute exposures (i.e., TCDD and PCBs), or unknown side effects of pharmaceutical drugs (DES). Also, it is unclear if it is individual susceptibility to various toxicants or the mixture of xenobiotics they are exposed to that increases disease risk for individuals or subpopulations. Only a hypothesis-driven study of the size and length of that proposed by the NCS will accurately address these issues.

These proposed studies would be the first of their kind at several levels. They can:

1. Establish which environmental toxicant mixtures our children are exposed to during various developmental periods of their life and allow estimation of their impact (concentrations, bioaccumulation, placental/lactational transfer). This could greatly impact risk assessment of compounds of similar structure or mode of action.
2. Provide the power to establish whether or not fetal and infant exposures to environmental factors are influencing the increasing rates of cyptorchidism, hypospadias, precocious puberty, and dysmenorrhea in children/adolescents.
3. Increase the probability of determining biomarkers of toxicant exposure (due to the prospective nature of the study), thereby facilitating preventative measures.
4. Enable correlations of disease outcome later in life with endocrine milieu early in life (prospective and longitudinal needed). A large portion of the risk origin for the afflictions/diseases mentioned is not known, but hormonal changes or reprogramming of the brain caused by the environment is suggested.
5. Provide concrete data to facilitate the understanding of the interrelationships between puberty and disease end-points such as peak bone density, breast cancer, endometriosis, aberrant reproductive organ development and testicular cancer.

6. Determine if a correlation exists between early life environmental exposures and development of serious afflictions with no acceptable animal model available, such as endometriosis and prostate cancer.
7. Facilitate discovery of biomarkers of effect following reported disease outcomes (all facets of the study design required).

Potential for Innovative Research

The fact that so little is known about the impact of environmental toxicants on the early development of children creates an enormous potential for innovative research within these hypotheses. The reason that we do not have answers to the questions proposed here is that a hypothesis-driven study of this size and design has never been undertaken. Questionnaire design and sufficient collection and storage of biological measurements/samples early and throughout the child's first 20-21 years of development will be critical in driving the innovative aspects of this study. It will be this information that provides the opportunity to determine gene-environment interactions, such as gene polymorphisms that alter metabolic clearance of a toxicant, change steroid receptor binding potential or destroy normal protective mechanisms within a tissue. Additionally, identifying endocrine-disrupting compounds in our environment that impact the HPGA of the developing child could significantly decrease endocrine-related disease of adults, such as menstrual disorders, prostatic inflammation, endometriosis, and potentially testicular and breast cancer incidence. Out-year reporting (reporting to age 50 or 60, not further sampling) of cancer occurrence, inflammatory disorders, mental afflictions, sub/infertility and osteoporosis will further improve the innovative aspects of this study.

Evaluation of the impact of environmental exposures to our children's health is a complicated, but critical, undertaking that can be accomplished. Recent studies (Brody et al., 2002; Rudel et al., 2001) have demonstrated new methodologies for reconstructing residential exposures and accurate means to determine levels of hormonally active agents and carcinogens in residential air and dust. Technologies to evaluate toxicant levels in non-invasively obtained biological samples of parents and children are appearing in the literature, as well as the methods to use those tissues for genetic evaluations and biomarkers of effect or exposure. Breast milk and cord blood, samples usually not available when questioning early life contribution to disease, may provide crucial information on the maternal contribution of the child's early toxicant exposure.

The life course approach to evaluating disease risk factors from our environment will require sampling from early life (conception) through adolescence, in addition to the large number of children proposed (100,000). Without a prospective and longitudinal approach, we will limit our understanding of endocrine-disrupting compounds on our children's health. With our increased abilities to detect environmental toxicants in homes and biological samples, comes elevated numbers of compounds on the FDA approval list. Correlated with the increased number of chemicals on the market has come increases in

cancer incidences, inflammatory disease, precocious puberty and other reproductive tissue abnormalities in children around the world. The NCS has the potential to more fully evaluate the impact of environmental agents on children's health than any previous study.

Potential for Synergy with Other Working Groups

There is potential to collaborate with these working groups: Nutrition, Growth, and Development; Gene-Environment Interaction; Fertility and Early Pregnancy; Environmental Exposure; Medicines and Pharmaceuticals.

Feasibility

- Sensitive periods for exposures – fetal, infancy, childhood, adolescence. Evaluation of multiple time points for sensitive periods and impact of cumulative exposure.
- Critical measurement time points – maternal hormonal status (repeated measures in sera), fetal ultrasound, cord blood or placenta, breast milk samples (several), reproductive organ development through puberty (typical exams), sera and non-invasively obtained sampling from infants/children, pubertal endpoints (including new, more specific biomarkers of puberty, hormone profiles, growth rates), bone mass measurements and growth charting, abdominal/pelvic ultrasounds at puberty, peak bone mass measurements, and bone mineral density at age 20; detailed questionnaires.
- Environmental monitoring – residence, day care, school periodic swipes. Measurements of many high use or persistent compounds and their metabolites in sera, breast milk, urine, and water supply.
- Sample size needs – large number of recruited families critical in assessing life course approach to adult disease and mixture effect of environmental toxicants.
- Out year reporting – necessary to get full picture of early determinants of adult disease. Reporting system only. Specifically important for cancers, inflammatory disease of uterus and prostate, osteoporosis, and infertility.
- Short, direct questions can be assessed in sub-sample of the group. Evaluation among risk factors can also be addressed in subgroups (1000-2000). (i.e., the effect of maternal genestein intake during pregnancy on estradiol and SHBG levels in male and female cord blood.)

Ethical considerations:

- Any abnormalities identified in the blood collection or in anthropometric measurements would be noted and appropriate follow-up medical care would be recommended.
- The influence of DNA collection for genetic evaluation and RNA for gene expression profiling presents ethical questions and is hopefully being addressed by the gene-environment working group.

References:

Giwercman, A., E., Carlsen, N. Keiding, and N.E. Skakkebaek. 1993. Evidence for increasing incidence of abnormalities of the human testis: A review. *Environ. Health Perspect. Suppl.* 101(Suppl. 2), 65-71.

Li, S-T. T., P. Lozano, D.C. Grossman, and E. Graham. 2002. Hormone-containing hair product use in prepubertal children. *Arch. Ped. Adol. Med.* 156.

Cramer, D.W. and S.A. Missmer. 2001. The epidemiology of endometriosis. *Ann. NY Acad. Sci.* 11-22.

Lee, P.A., H.E. Kulin, and S.S. Guo. 2001. Age of puberty among girls and the diagnosis of precocious puberty. *Pediatrics.* 107, 1493-1494.

Boisen, K.A., K.M. Main, E. Rajpert-De Meyts, and N.E. Skakkebaek. 2001. Are male reproductive disorders a common entity? The testicular dysgenesis syndrome. *Ann. NY Acad. Sci.* 90-99.

Chalumeau, M., W. Chemaitilly, C. Trivin, L. Adan, G. Breart, and R. Brauner. 2002. Central precocious puberty in girls: An evidence-based diagnosis tree to predict central nervous system abnormalities. *Pediatrics*. 109, 61-67.

Kaplowitz, P.B., E.J. Slora, R.C. Wasserman, S.E. Pedlow, and Marcia E. Herman-Giddens. 2001. Earlier onset of puberty in girls: Relation to increased body mass index and race. *Pedriatrics* 108, 347-353.

Tiwary, C.M. 1998. Premature sexual development in children following the use of estrogen- or placenta-containing hair products. *Clin. Pediatr.* 37, 733-740.

Rudel, R.A., J.G. Brody, J.D. Spengler, J. Vallarino, P.W. Geno, G. Sun, and A. Yau. 2001. Identification of selected hormonally active agents and animals mammary carcinogens in commercial and residential air and dust samples. *J. Air Waste Manage. Assoc.* 51, 499-513.

Brody, J.G., D.J. Vorhees, S.J. Melly S.R. Swedis, P.J. Drivas, and R.A. Rudel. 2002. Using GIS and historical records to reconstruct residential exposure to large-scale pesticide application. *J. Exp. Anal. Environ. Epi.* 12, 64-80.

Sasco, A.J. 2001. Epidemiology of breast cancer: an environmental disease? *APMIS* 109, 321-332.

Cooper, R.L., J.G. Goldman, and L. Tyrey. 1998. The hypothalamus and pituitary as targets for reproductive toxicants. In: Reproductive and

Developmental Toxicology, ed. K. Korach. Marcel Dekker, Inc. (New York). Pp. 195-210.

Herman-Giddens, M.E., E.J. Slora, R.C. Wasserman, et al., 1997. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the PROS network. *Pediatrics*. 99, 505-512.