THE NATIONAL CHILDREN'S STUDY

RESEARCH PLAN

SEPTEMBER 17, 2007 – VERSION 1.3

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PREFACE

Growing up healthy is every child's right and every parent's dream for their children. As a nation, we have made significant advances in improving child health and development over the past century by identifying the causes of many diseases; by developing preventive measures, treatments, and cures; and by improving the overall health status of our children. Still, children today suffer high rates of conditions and chronic diseases that interfere with health and development: Asthma, developmental disorders, obesity, preventable injuries, and other problems. The progressive improvement of our children's health during the last century has reached a plateau and now threatens to move backward. In fact, our children are experiencing major increases in chronic health conditions, especially obesity, asthma, and learning disabilities. Many experts believe that fundamental changes in children's environments appear to be a common pathway for these increases.[1,2]

As many health and safety practices from past generations have been validated or dismissed based on new evidence, we have come to understand that the environments our children live in are profoundly important. From the air they breathe to the food they eat, from where they live to how they live, the environments in which our children grow affect their lifelong health and well-being. As described in the following research plan, multiple studies point to the association of various environmental exposures with problems in our children's health and development, such as air pollution and allergens with asthma, poor diet with obesity, and pesticides with impaired neurodevelopment. Today these problems stand among the most pressing public health concerns in the United States. Yet, with the prevalence of these conditions remaining stubbornly persistent or on the rise, few studies can confirm more definitive links that lead to prevention strategies. In addition, with threats of terrorism, violence, and other stress-inducing experiences becoming a daily exposure for many families, our children face unprecedented challenges to their well-being. Consequently, understanding and protecting our children's health and safety must be a national priority.

The National Children's Study reaffirms the federal government's commitment to the health and well-being of children by drawing together the nation's top experts on child health and the environment in an unprecedented collaboration. Multiple federal agencies, national non-profit groups, community health care providers, and more than 100,000 families stand poised to help child health move forward in the 21st century. The goals of the Study complement government efforts to challenge individuals, communities, and professionals to take action to ensure that good health and long life are enjoyed by all.

I am pleased to present this proposed Research Plan for The National Children's Study to inform scientific reviewers, professional colleagues, contributors, and all who are interested in this ground-breaking initiative that addresses these major challenges to our children's future. The Plan describes the Study's background, design, and measures, and the rationale for their selection, in sufficient detail so that readers can understand the basis of the Study and how it will be carried out. This plan was developed with input from scientists and other professionals across the country and from multiple federal agencies, especially the National Institute of Child Health and Human Development and the National Institute of Environmental Health Sciences at the National Institutes of Health, the Centers for Disease Control and Prevention, and the U.S. Environmental Protection Agency, with full awareness of competing

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[1] Van der Lee, J.H., Mokkink, L.B., Grootenhuis, M.S., Heymans, H.S., & Offringa, M. (2007). Definitions and measurement of chronic health conditions in childhood. *Journal of the American Medical Association, 297*, 2741-2751.

[2] Perrin, J.M., Bloom, S.R., & Gortmaker, S.L. (2007). The increase of childhood chronic conditions in the United States. *Journal of the American Medical Association, 297*, 2755-2759.

priorities, limitations of cost, and acceptable burden on participants. We welcome constructive comments and proposals for how The National Children's Study might address these pressing health concerns of our nation's children even more effectively. Comments and communication about the Research Plan may be submitted by e-mail to: ncsinfo@mail.nih.gov .

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PRÉCIS

Background

Our nation has made great strides in reducing or eliminating classic childhood illnesses such as measles, mumps, and chicken pox. Significant advances have been made to improve child health and development by identifying the causes of many diseases; by developing preventive measures, treatments and cures; and by providing resources to support health care for children. Trends in childhood illnesses have emerged such as increased rates of childhood asthma, alarming numbers of children diagnosed with autism, and an obesity epidemic. This new childhood morbidity threatens to undo the progress made in child health promotion and disease prevention and to add a significant new cost burden to the economy.

In the late 1990s, numerous experts called for new data to better understand child health and development. In 1999, the President's Task Force on Environmental Health and Safety Risks to Children recommended a large longitudinal study of children to fill knowledge gaps about environmental influences on child health and development. The Children's Health Act of 2000 authorized and directed a consortium of federal agencies, led by the National Institute of Child Health and Human Development (NICHD) in partnership with the Centers for Disease Control and Prevention (CDC), the United States Environmental Protection Agency (EPA) and the National Institute of Environmental Health Sciences (NIEHS), to plan and to conduct the study.

The importance and timeliness of this study are based on factors that include the demonstrated and profound effects on child health of environmental exposures, such as lead in early childhood and alcohol during pregnancy; the special vulnerabilities of children to environmental exposures compared to adults; known ongoing exposures, such as prevalent levels of nonpersistent pesticides or hours of media exposure per day in young children; and evidence for environmental contributions to or causes of high-impact conditions, such as autism, developmental disabilities, asthma, and obesity. Science and technology have advanced to a point where it is possible to examine the individual and combined effects of genetic and environmental exposures, genetic variation, and multiple outcomes over life stages in the same individuals. The study design and data collected are determined by requirements necessary to test an integrated set of core hypotheses regarding the relations of environmental and genetic factors with priority outcomes in children, and later in adults.

Goal and aims

The goal of the National Children's Study (NCS) is to provide information that will ultimately lead to improvements in the health, development, and well-being of children. The primary aim of the NCS is to investigate the separate and combined effects of environmental exposures (chemical, biological, physical, and psychosocial) as well as gene-environment interactions on pregnancy outcomes, child health and development, and precursors of adult disease. In addition to this broad purpose, the Study has several specific goals:

(1) Determine the presence or absence of effects, both harmful and helpful, related to the timing, frequency, magnitude, and duration of specific chemical, physical, biological, and psychosocial exposures in children's environments from preconception to adulthood.

(2) Determine possible environmental contributions to, or causes of, specific diseases and conditions of children, including, but not limited to, prematurity and other outcomes

of pregnancy, neurological and developmental disorders, psychiatric and behavioral disorders, altered physical development and sexual maturation, obesity and insulin resistance, asthma, and injuries.

(3) Determine how genotypic variation and mechanisms, and the interaction of genes with environmental factors, influence disease risk and developmental trajectories in children.

(4) Serve as a national resource for future studies of child health and development by providing a rich database and repository of environmental and biological samples and information that can be used to address future questions and hypotheses.

Methods

This longitudinal cohort study will follow a representative sample of approximately 100,000 children born in the United States. Children will be followed from before birth until age 21.

Sampling and recruitment. The Study will employ a national probability sampling approach to select locations for conduct of the Study. The sampling design utilizes a multistage clustered approach. In the first stage, 105 locations (generally corresponding to single counties) were randomly selected from all U.S. counties. Seven of the locations will serve as the Vanguard Locations and will participate in the pilot phase of the Study. Because the focus of the Study includes assessment of the impact of exposures that occur early in pregnancy, both pregnant women and their partners and women of childbearing age comprise the initial target population for enrollment in the Study Locations. At the time of enrollment, participants will be asked to provide written informed consent for participation in the Study. Three distinct groups will be enrolled and followed: pregnant women and their partners; couples planning pregnancy; and women not currently planning pregnancy but with some probability of becoming pregnant during the four-year enrollment timeframe.

Follow-up. The initial follow-up of women enrolled in the study prior to pregnancy (the preconception cohort) will vary with each woman's probability of pregnancy. Women with a high probability of becoming pregnant (generally a subset of women trying to become pregnant) will receive an in-person visit and as many as three telephone contacts during the four months following enrollment. In contrast, women with the lowest probability of becoming pregnant will be contacted annually by phone. It is anticipated that during the enrollment period, a woman's probability of becoming pregnant will not be stagnant. Data collection schedules will be modified based on the most current information on each individual's probability of pregnancy.

Once women are pregnant, follow-up visit schedules will be identical for all women and children enrolled in the Study regardless of the initial probability of pregnancy. A minimum of six in-person visits are planned from the first trimester of pregnancy through age 3. Three of these visits are in the home, and three are in a clinical setting (one of which is the place of delivery of the infant). After age 3, in-person visits should occur every two to three years with an additional home visit after each change of permanent residence. Remote data collections (e.g., telephone, computer, or mail-in questionnaires) will occur between face-to-face contacts. The expected frequency of contact (face-to-face or remote) is approximately every three months through age 1, every six months through age 5, and annually thereafter. For a sample of children enrolled in the Study, visits will also be made to child care and school settings for collection of environmental samples and observational data.

The schedule of in-person contacts and phone interviews from pregnancy through age 21 are outlined in the table below:

Timing and Location of Study Contacts

Age of child	Location of visit
Preconception	As outlined above
1st trimester	Home
2nd trimester	Phone
3rd trimester	Clinic
Birth	Place of delivery
3 months	Phone
6 months	Home
9 months	Phone
12 months	Home
18 months	Phone
24 months	Phone
30 months	Phone
3 years	Clinic
5 years*	To be decided
7 years	To be decided
9 years	To be decided
12 years	To be decided
16 years	To be decided
21 years	To be decided

*Timing and location of visits from 5 years onward is provisional

Anticipated biologic specimens include blood, urine, hair, and nail clippings from mothers and children; blood, urine, and hair from fathers; cord blood, umbilical cord and placental tissues, and meconium collected at or around the time of delivery; vaginal swabs, and breast milk from mothers. Anticipated environmental samples include air, dust, soil, food, and water.

Expected contributions of the Study

The NCS is in a unique position to answer questions regarding the effects of environmental exposures on the long-term health of children. The focus on exposures prior to and early in pregnancy and the breadth of planned exposure and outcome measurements are unique features of the Study. As technology evolves, stored data specimens (biologic and environmental) will provide a valuable resource to answer questions for future generations.

The Study's prospective longitudinal design will permit an in-depth examination of the effects of environmental exposures as they unfold over the course of development. This will include an unprecedented, process-oriented understanding of how exposures at particular points in development lead to both immediate and long-term consequences for children, and what circumstances, characteristics, or genetic predispositions mediate or moderate the relation between exposure and outcome. The size and representative nature of the sample will permit both valid inferences about the U.S. population as a whole and exploration of subgroup-specific patterns of adaptation and maladaptation.

The data collected for the NCS will also provide a platform for future research. Data and biological and environmental samples will be available for future studies as science evolves and new questions arise. The NCS will serve as an exceptional resource both for science and for society.

PART I: BACKGROUND AND SIGNIFICANCE

1. BACKGROUND

Patterns of illness among children in the United States and other industrially developed nations have changed substantially during the past 100 years (Bloom & Dey, 2004). Before and during the first half of the last century, infectious disease comprised the principal threat to children. In contrast, the major illnesses and disorders that impair health, growth, and development today are chronic conditions stemming from the complex interaction of environmental exposures and inherent genetic factors. Some label this the "new pediatric morbidity" (Haggerty, 1975). These conditions include: premature births (Ananth, Joseph, Demisse, & Vintzileos, 2005); asthma (Mannino et al., 2002); injuries (Thornton, Craft, Dahlberg, Lynch, & Baer, 2002); childhood cancer (Linet, Ries, Smith, Tarone, & Devesa, 1999); neurodevelopmental disorders, such as learning disabilities, dyslexia, mental retardation, attention deficit/hyperactivity disorder, and autism (Boyle, Decoufle, & Yeargin-Allsopp, 1994; Newschaffer, Falb, & Gurney, 2005; Scahill & Schwab-Stone, 2000; Shaywitz, 1998); obesity and type 2 diabetes (SEARCH Writing Group, 2004); birth defects such as hypospadias (Paulozzi, 1999); and cardiac defects (Towbin et al., 2006). Addressing the causes and contributors to these and similar chronic conditions is the major challenge to public health practitioners and pediatric researchers today, and constitutes the frontier that must be crossed if the health and well-being of children in developed countries is to move forward. The National Children's Study is designed to address these issues with robust science and the ability to generalize the data to the U.S. population.

The National Children's Study's design rests on the principle that both health and susceptibility to disease are determined by dynamic processes that occur throughout life. Perturbations ("insults") that impact health states may occur any time from preconception through adult life. These insults can affect viability, differentiation of major organ systems, somatic growth, and the development of functional processes including maturation of metabolic systems. A range of determinants acting either in concert or synergistically may impact growth and development. These include the built and natural environments with their chemical and physical factors, the social environment, individual behaviors, and biological factors including genetics. Of particular importance are the earliest stages of human development, pregnancy and early childhood, when cell division, differentiation, and maturation are most rapid.

These health determinants may influence development in many ways. For those with high potency when acting at critical periods of development, such as thalidomide or Accutane, severe birth defects will result in most exposed offspring. Most environmental factors, however, are not so potent. More often, factors operating at critical or sensitive periods of development will interact with other factors over the life course to raise or lower the risk of adverse health outcomes. These factors may be genetic or non-genetic. For example, accelerated weight gain during childhood is associated with increased risks of diabetes and cardiovascular outcomes later in life; this phenomenon is accentuated among children born with restricted fetal growth (Barker, 2005; Bhargava et al., 2004). The risk of orofacial clefts due to maternal cigarette smoking is markedly increased in children with certain genetic traits and/or reduced folic acid intake (Lammer, Shaw, Iovannisci, & Finnell, 2005; Shaw et al., 2005; Shaw, Wasserman, Murray, & Lammer, 1998). Only with this appreciation of the complexity of interactions among genetic and environmental factors will we be able to inform the next generation of caregivers about effective prevention and treatment to lower the burden of common chronic conditions of childhood and later-onset diseases that arise from early developmental insults.

1.1 The Children's Health Act of 2000

Faced with the challenge of how to address the potential risks of many environmental factors that may be affecting the health and development of children, the President's Task Force on Health Risks and Safety Risks to Children concluded in 1999 that a large study to define the actual risks associated with broad environmental exposures is an essential first step. Following the recommendation of the task force, the U.S. Congress passed the Children's Health Act of 2000, which directed the National Institute of Child Health and Human Development (NICHD) to conduct a national longitudinal study of environmental influences on children's health and development. The National Institute of Environmental Health Sciences (NIEHS), the Centers for Disease Control and Prevention (CDC), and the U.S. Environmental Protection Agency (EPA) joined the NICHD in planning this study.

The Children's Health Act of 2000 (Public Law 106-310 Sec. 1004) specified that the study should extend from the prenatal period to adulthood, following a sample of children across development. It should investigate the short-term and long-term influences of physical, chemical, biological, and psychosocial environmental exposures on children's health and development, including not only physical health, but behavioral, emotional, and educational outcomes as well. The study should elucidate both those factors that protect children from detrimental outcomes and those that put them at risk, including sufficient diversity to address the existence and consequences of health disparities among children in the United States. The scientific rationale for this program of research, now named the National Children's Study, is described below.

1.2 Rationale for the National Children's Study

1.2.1 The Public Health Burden of Childhood Chronic Conditions

While there are many important conditions of childhood that have grave effects on certain individuals and families, there are some that also place a great burden on the population because of their prevalence, severity, and/or cost. For example, there are increasing concerns about the large (and perhaps growing) number of American children who have one or more major chronic health or developmental conditions. As many as 17 percent of children have some type of developmental disorder (Boyle et al., 1994), about 21 percent have a diagnosable mental or addictive disorder with at least minimum impairment (U.S. Department of Health and Human Services, 2000), and about 7 percent have asthma (Mannino et al., 1998). The NCS is particularly poised to examine these conditions because it is a large study of the general population. Through the extensive planning process of the NCS, the following areas emerged as primary outcomes around which the Study's core hypotheses have been generated: pregnancy outcomes; neurodevelopment and behavior; asthma; obesity and growth; injury; and reproductive development. Additionally, the NCS is committed to assessing predictors of healthy development. The data collection process will also allow the examination of a range of health outcomes that extend beyond those identified in this Study.

The priority outcome areas were chosen not only because of their importance to public health, but also because a research study of the scope and magnitude of the NCS is required to understand their origins and course. Since many of the outcomes may arise as a consequence of in utero exposures, study of these outcomes must begin before birth. Additionally, a variety of exposures likely contribute directly, indirectly, and interactively to these outcomes. A full understanding of their etiology requires a study covering a range of exposures. Genetics could also play a role both in the origin and expression of disorders, thus a complete study must include an exploration of direct genetic contributions and of gene-environment interactions. Furthermore, each outcome has a meaningful range of manifestations over the course of development, including sensitive periods for exposures, different ages of onset, and changes in

nature or severity over development. Only a longitudinal study can track these outcomes as they unfold during childhood and adolescence. Finally, to examine these exposure-outcome relations in a definitive manner, the Study must have sufficient power, and thus sufficient sample size, to explore both normative and low-prevalence outcomes. The NCS will follow a representative sample of 100,000 children from before birth to age 21 and will include assessments, collected through a variety of modalities, of chemical, physical, psychosocial, and biological exposures, as well as genetics. Incorporating both breadth and depth of investigation, the NCS will be particularly well suited to provide scientists and practitioners with the tools to address these new childhood morbidities, and to promote health and well-being in our children.

1.2.1.1 Pregnancy Outcomes

Low birth weight and preterm delivery are highly correlated and continue to be among the major refractory causes of infant mortality and childhood morbidity (Gutbrod, Wolke, Soehne, Ohrt, & Rigel, 2000). Identified environmental factors for increased risk of preterm birth, which include maternal smoking (Kyrklund-Blomberg, Granath, & Cnattingius, 2005), chemical agents (Gonzales-Cossio et al., 1997; Hinckley, Bachand, & Reif, 2005), infection (Andrews, Hauth, & Goldenberg, 2000; Pararas, Skevaki, & Kafetzis, 2006; Romero, Espinoza, Chaiworapongsa, & Kalache, 2002), stress (Hobel, 2004; Park, Park, Lockwood, & Norwitz, 2005), and even air pollution (Rogers & Dunlop, 2006), all point to environmental exposure etiologies. More recent reports point to more complex interactions between environmental and genetic factors. Several possible genetic variations have been described that place some women at particular risk of premature births with certain exposures, such as infection (Varner & Esplin, 2005) and cigarette smoke (Wang et al, 2002).

Birth defects are the leading cause of infant mortality and are responsible for more than 8,000 (approximately 20 percent) of the 40,000 infant deaths that occur annually (CDC, 1998). Following on the morphologic birth defects of fetal exposure to alcohol (Jones, Smith, Ulleland, & Streissguth, 1973), there are concerns about other birth defects such as hypospadias that have increased in recent years along with exposures to phthalates and other endocrine active compounds (Paulozzi, 1999; Rogan, Gladen, Guo, & Hsu, 1999; Weiss, 2002). There are also concerns about central nervous system defects, such as anencephaly, spina bifida, and hydrocephaly, and their association with diabetes, with lesser alterations possibly associated with altered glucose metabolism (Anderson et al., 2005).

1.2.1.2 Neurodevelopment and Behavior

In contrast to birth defects which are structural in nature, developmental disabilities are recognized because of abnormalities in functioning that emerge as a child ages. Almost 20 percent of all children in the United States are reported to have some type of developmental disability (Boyle et al., 1994), including approximately 2 percent of school-age children with a serious developmental disability (Crain, 2000). Conditions that are representative of developmental disabilities include mental retardation, cerebral palsy, attention deficit/hyperactivity disorder (ADHD) and autism. Numerous exposures in utero and during infancy, most notably lead (Lidsky & Schneider, 2003), alcohol (Mattson & Riley, 1998), and nurturing (Bradley et al., 1989), have been identified as affecting neurological and cognitive development. The causes of most cases of mental retardation, however, are unknown (Yeargin-Allsopp, Murphy, Cordero, Decoufle, & Hollowell, 1997). Recent evidence reveals the potential contribution of known neurodevelopmental toxicants to developmental disabilities at levels of exposure well below currently recognized levels of toxicity (Lanphear, Vorhees, & Bellinger, 2005; Schober et al., 2003). Previously unidentified environmental agents, including persistent and nonpersistent pesticides as neurotoxicants, may also play a role in developmental disabilities (Kofman, Berger, Massarawa,

Friedman, & Jaffar, 2006; Rice & Barone, 2000; Weiss, 2000). The cost of diminished child functioning due to environmental toxicants is substantial (Grosse, Matte, Schwartz, & Jackson, 2002; Salkever, 1995; Weiss, 2000).

The etiology of neurodevelopmental disorders can be complex and difficult to specify. For example autism is a neurodevelopmental disorder that was once believed to be rare (i.e., 4-5 per 10,000 children); however, the number of individuals receiving services for autism has increased dramatically in the past 10 years. The current prevalence of autism and the broader group of autism spectrum disorders stands at about 3-6 per 1,000 children (Gillberg & Wing 1999; Hirtz, 2000; Rutter, 2005; Yeargin-Allsop et al., 2003). Although autism has a strong genetic component (The Challenges of Autism, 2000), environmental and social factors are also thought to play a significant role in its expression, and a number of environmental agents have been suspected of interacting with genetic factors to cause the apparent increase of autism.

1.2.1.3 Child Health and Development

In addition to investigation of specific disorders of childhood, an understanding of child health and development involves examination of individual differences and children's trajectories through time on measures of health, well-being, social and emotional development, and cognitive development and achievement.

Early developmental deficits can compromise subsequent social and academic success. While most children enter kindergarten having mastered basic skills, a significant percentage lags behind in key domains. Between 18 to 42 percent of preschoolers are estimated to lag behind their classmates significantly in their preparedness for learning (West, Denton, & Germino-Hausken, 2000). In the realm of behavior and conduct, approximately 12 percent of infants and toddlers have significant behavioral or emotional problems (Briggs-Gowan, Carter, Skuban, & Horwitz, 2001). Such problems unfold in complex ways over time, however, as research indicates that less than 50 percent of children with conduct problems during the toddler or preschool period continue to have significant problems one to two years later (Baillargeon et al., 2007; Lavigne et al., 1998). Additionally, children who show early signs of social competence tend to become even more prosocial with development (Baillargeon et al., 2007). Nonetheless, many children with deficits in emotional, social, and cognitive skills at school entry are likely to have both ongoing conduct problems, and difficulties with academic achievement (Wentzel & Asher, 1995).

Many different experiences and exposures have the potential both to affect child health and development at sensitive periods and to change children's developmental trajectories. For example, sensitive parenting and secure infant-parent attachment during infancy predict children's subsequent competence and healthy social functioning (Thompson, 1999). In contrast, parental mental health problems can lead to disturbances in parent-child interactions (Jameson, Gelfand, & Kulcsar, 1997), and the strategies that a young child uses to relate to a mentally distressed parent can become persistent, resistant to change, and can develop into a long-term behavioral pattern of response (Field, 1995; Lyons-Ruth, Wolfe, Lyubchik, & Steingard, 2002). Contexts outside the family can also have great impact on children. Early experience in high-quality center-based child care predicts better vocabulary skills, but also slightly elevated aggressive behavior in middle childhood (Belsky et al., 2007). The sensitive periods for exposure and trajectories of functioning are multifaceted and are also likely moderated by genetic and physiological factors (Curtis & Cicchetti, 2003). Longitudinal research with a sufficiently large and representative sample is needed to untangle these intricate pathways.

1.2.1.4 Asthma

Among children, asthma is the most common chronic illness (National Academy of Sciences, Institute of Medicine, 2000). Asthma prevalence in the United States, estimated from the National Health Interview Survey (NHIS) by the American Lung Association (American Lung Association Epidemiology and Statistics Unit, 2006), shows the prevalence of asthma increased 85 percent from 1982 through 1996 to an estimated 14.6 million persons (55.2 per 1,000). This increase was 76 percent in children younger than 18, or 4.43 million persons in 1996 (62.0 per 1,000). This trend paralleled increasing asthma hospitalization and death rates in children (Akinbami, 2006; American Lung Association Epidemiology and Statistics Unit, 2006). In 2004, the prevalence of doctor-diagnosed asthma reached 30.2 million Americans (104.7 per 1,000), including 6.5 million children younger than 18. Almost 4 million children younger than 18 were estimated to have experienced an asthma attack in 2004. Prevalence data in the United States, both from the 12-month prevalence (before 1997) and 12-month attack prevalence of asthma (since 1997), were highest among children ages 5-14, higher among Blacks compared with whites, and higher among females than males (Akinbami, 2006; American Lung Association Epidemiology and Statistics Unit, 2006). Approximately 38 percent of the hospital discharges related to asthma in 2004 were in children younger than 15, although only 21 percent of the U.S. population was younger than 15.

Asthma is associated with substantial physical and behavioral disability among children. Thirty percent of children with asthma reported activity limitation compared to 5 percent of children without asthma, and asthma was estimated to account for 10 million missed school days and 13 million physician contacts among children in 1988 (Taylor & Newacheck, 1992). This is an underestimate of the current burden because of increasing trends in asthma prevalence and associated morbidity (Mannino et al., 2002). The annual estimated cost of pediatric asthma in the United States in 1997 was $6.6 billion (Landrigan, Schechter, Lipton, Fahs, & Schwartz, 2002).

In 2004, the total cost of asthma was estimated at $16.1 billion, including $11.5 billion in direct health care costs and $4.6 billion in indirect costs (lost productivity) (National Institutes of Health, 2004). The severe forms of asthma account for a disproportionate amount of the direct costs. Malone, Lawson and Smith (2000) estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs. Asthma also poses a substantial and increasing public health burden because of school absences and restriction of children's usual physical and social activities (Newacheck & Halfon, 2000).

Asthma is a complex disease characterized by pulmonary obstruction due to inflammatory response within central and peripheral airways. Asthma has a variety of clinical phenotypes, which carry implications for disease etiology, evolution, and severity (Martinez, 2000; Martinez & Helms, 1998). Current understanding of the etiology and severity of asthma focuses on individual response to a range of interacting immunogenic and immuno-protective factors (Busse & Lemanske, 2001): air pollution and bioaerosols (including allergens, endotoxin, and mold); respiratory tract infections; maternal stress; dietary antioxidants; and early exposure to bacterial and microbial products. This focus opens a range of potential research areas that address interactions between host response (e.g., individual inflammatory response, genetic makeup), potential inflammatory triggers (e.g., ozone, particulate matter, and other airborne pollutants; viral infection; animal or fungal antigens), and potential protective factors (e.g., early exposure to bacterial endotoxin, dietary antioxidants).

1.2.1.5 Obesity and Growth

The prevalence of overweight among children is greater than 15 percent among children age 6 or older, and this prevalence has increased during the past 40 years (Ogden, Flegal, Carroll, & Johnson, 2002). Being overweight as a child is a risk factor for being overweight as an adult (Serdula et al., 1993) and is associated with increased risk of type 2 diabetes, hypertension, and coronary artery disease (Freedman et al., 2001). Being overweight as a child also increases the risk of developing type 2 diabetes before age 21 (Sinha et al., 2002).

The best estimate of the prevalence of type 2 diabetes among those younger than 21 in the United States is about 0.1 percent based on National Health and Nutrition Examination Survey (NHANES) data from 1988-1994 (Fagot-Campagna, Saaddine, Flegal, & Beckles, 2001). Given the increase in overweight among children, it seems reasonable to assume that the prevalence now is higher than 0.1 percent—but by how much is unclear. Although type 2 diabetes may not be common enough for the NCS to examine with sufficient power, insulin resistance or closely related conditions, such as metabolic syndrome, are outcomes that would occur with sufficient frequency among subjects younger than 21 and could serve as both outcomes and markers for adult disease. Insulin resistance is considered the underlying abnormality in metabolic syndrome. Metabolic syndrome, according to the World Health Organization and as modified by Laaksonen et al. (2002), is defined by fasting hyperinsulinemia, impaired fasting glycemia or diabetes, and the presence of at least two of the following: abdominal obesity, dislipidemia (hypertriglyceridemia or low HDL cholesterol), or hypertension. Such a definition is feasible for detection in large-scale epidemiologic studies and identifies those who are at high risk of developing type 2 diabetes. The prevalence of metabolic syndrome among adults, as compared with the prevalence of type 2 diabetes, is about four-fold greater (Laaksonen et al., 2002). Investigations based on NHANES III data indicated that approximately 4 to 10 percent of adolescents ages 12-19 have metabolic syndrome (Cook, Weitzman, Auinger, Nguyen & Dietz, 2003). Thus, it is reasonable to assume that an outcome definition of metabolic syndrome like the definition presented above would have a prevalence rate above 0.2 percent. This means the NCS would have sufficient power to examine metabolic syndrome in relation to a wide range of exposure levels.

Mounting evidence suggests prenatal factors and early childhood experiences may influence the development of disease later in life (Barker, 1992). Altered fetal growth has been related to increased risk of cardiovascular disease, hypertension, and diabetes in adulthood (Barker, 1995; Barker & Osmond, 1986; Barker, Winter, Osmond, Margetts, & Simmonds, 1989; Poulter, Chang, MacGregor, Snieder, & Spector, 1999). Accelerated childhood growth is related to the risk of breast cancer in women (Ahlgren, Melbye, Wohlfahrt, & Sorensen, 2004) and to impaired glucose tolerance in adulthood (Hales et al., 1991).

1.2.1.6 Injury

Both unintentional injuries (e.g., motor vehicle crashes, suffocations) and intentional ones (interpersonal violence, child maltreatment, self-inflicted injuries) exert a tremendous toll in childhood. Beyond the first year of life, unintentional injuries are the leading cause of mortality in every age group until age 44 years (Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, 2007). In the teen years, homicide and suicide are the second and third leading causes of death, respectively. Fatal injuries represent only a small portion of the problem; it is estimated that in 2001 more than 230,000 children younger than 21 were hospitalized for an injury and approximately 9.7 million were treated in an emergency room and released (Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, 2007). The economic burden of injuries for persons of all ages was estimated at $406 billion in 2000, including $80.2 billion in medical care costs and $326 billion in

lost productivity (Finkelstein, Corso, & Miller, 2006). For children and adolescents younger than 14, the total economic burden in 2000 was estimated at more than $50 billion.

Similar to other outcomes, injuries result from exposures in multiple domains and represent the convergence of individual behaviors (e.g., risk taking, aggression), the physical environment (e.g., road embankment, access to weapons), and societal factors (e.g., access to emergency care). Many serious injuries result in significant impairment with lifelong consequences for health and development. From this perspective, injuries are not only an important outcome to investigate in the NCS, but also an exposure that alters trajectories of development in multiple outcome domains of interest. This could occur through direct effects (e.g. a head injury causing direct brain damage) or through more subtle pathways (e.g., the emotional effects of the event leading to post-traumatic distress; changes in level of physical activity due to physical limitations imposed by injury).

Haddon and other injury prevention pioneers conceptualized injuries as the consequence of human exposure to energy in ways that resulted in an injury (Haddon, 1964; Stapp, 1957). This idea expanded the field to analysis and study of physical forces and how to modify their impact on humans as a conceptual framework for the control and prevention of injuries (Haddon, 1970). Identification of the combination of individual, environmental, and societal factors that result in injury is critical for the development of effective interventions. Childhood injury prevention experts recommended conducting longitudinal cohort studies to identify environmental risk and contextual factors and understand how they can be modified to reduce injuries (Committee on Injury and Poison Prevention, 1996; Scheidt, 1988). Careful analyses of multiple conceptual frameworks for injury prevention emphasize that a temporal perspective and acknowledgement of the complex interplay of societal and environmental factors are critical (Andersson & Menckel, 1995). Thus, moving beyond the surveillance and cross-sectional methodologies (Scheidt et al., 1995) to longitudinal studies of sufficient size is essential to separate confounders and isolate causal relations that can provide the basis of effective preventive strategies (Rivara, 1999).

1.2.1.7 Reproductive Development

Hypospadias is one of the most common congenital anomalies, affecting 27-55 of every 10,000 births in the United States (Paulozzi, 1999; Paulozzi et al., 1997) or 0.8 percent of male live births (Pohl et al., 2007). Cryptorchidism affects 3 percent of full-term male newborns (up to 7.7 percent of low birth weight infants) decreasing to about 1 percent by age 1 (Pohl et al., 2007). Reports of increasing trends for hypospadias (Paulozzi, 1999; Paulozzi et al., 1997) and cryptorchidism (Paulozzi, 1999) in the United States and other countries and secular trends toward decreasing age at menarche and other measures of puberty onset in boys and girls (Herman-Giddens et al, 1997; Herman-Giddens, 2006; Kaplowitz et al., 2001; Lee et al., 2001), have created concerns about the etiological factors behind these trends. These factors include better nutrition or perhaps over-nutrition; earlier and greater growth; increasing incidence of obesity; and socioeconomic or environmental factors.

Documented exposures of children and pregnant women to compounds that have potential reproductive toxicity support the importance of studying environmental determinants of age at puberty. Exposure of children and pregnant women to hormonally-active agents (HAAs, also called endocrine disruptors) is widespread in America (CDC, 2003), and animal studies suggest the potential for toxicity at current levels of exposure (Vom Saal & Hughes, 2005). For example, cross-sectional data from NHANES III (Selevan et al., 2003; Wu et al., 2003) suggest that higher blood lead levels may be associated with a delay in the onset of puberty in girls, paralleling similar findings in animals. Precocious puberty was reported in girls who were both exposed in utero to the fire retardant FireMaster, which contained polybrominated biphenyls (PBBs), and breast-fed by mothers who were exposed to the fire retardant

(Blanck et al., 2000). Bisphenol A, a weak estrogen (Pottenger et al., 2000), is a high production volume chemical used in a variety of applications, including manufacturing flame retardants, resins, and plastics. Human exposure may arise in a number of circumstances, for instance, when foods are contaminated by heated plastics. Blood levels of bisphenol A in pregnant women (Schonfelder et al., 2002) are similar to those found in pregnant rats that give birth to offspring with bisphenol A-induced reproductive toxicity (Howdeschell, Hotchkiss, Thayer, Vandenbergh, & vom Saal, 1999; Pottenger et al., 2000; Rubin et al., 2001). Atrazine is a widely used herbicide. In a population-based probability sample of children ages 3-13, about 3 percent of children had detectable levels of an atrazine metabolite in their urine, and urban-rural differences in levels were not statistically significant. Recent experiments in peripubertal rats show that atrazine in doses of 30 milligram per kilogram orally per day for as long as 25 days delayed the onset of puberty (Ashby et al., 2002). It is not clear if the doses effective in animal experiments result in urinary metabolite levels like those seen among children with detectable levels.

Lack of accurate information on the level and timing of past exposures to HAAs has limited most previous studies of the potential human impacts of known and suspected HAAs. This limitation will be directly addressed by the prospective design of the NCS because exposures to chemicals will be measured during pregnancy, in breast milk, and in the perinatal period before the appearance of health effects. The measurement of multiple outcomes related to single, multiple, and continuous or repeated exposures is only possible with a large longitudinal study. The potential for cumulative effects on the reproductive system can only be discerned through the use of a large longitudinal sample that allows repeated measures of exposure and evaluation of reproductive outcomes through time. Measures or biomarkers of exposure are available for most HAAs of interest and will allow linking of exposures at specific life stages with early or late reproductive outcomes. Measures of gene prevalence and gene expression will permit examination of genetic polymorphisms that may influence gene-environment interactions and will allow assessments of genetically determined inter-individual differences in susceptibility to HAAs.

Since the effects of HAAs are gender specific, it will be necessary to study exposure-outcome links separately in males and females, which will reduce the sample size for each case to approximately 50,000. Susceptible subgroups related to genetic polymorphisms may require additional subgroup studies.

1.2.2 Environmental Factors That May Influence Childhood Chronic Conditions

This section provides the rationale for emphasis of the NCS on an array of environmental factors and their impacts across domains and time. These include the natural and built environments with their attendant chemical, physical, and biological factors; the social environment; individual behaviors; biological factors; and genetics. It should be emphasized that health states are determined by interactions among genetic and non-genetic factors, and that these interactions may change over time.

1.2.2.1 Chemical Exposures

There is increasing and ample evidence that children experience a significantly greater vulnerability to the effects of chemical exposures than do adults in similar environments (Anderson, Diwan, Fear, & Roman, 2000; International Programme on Chemical Safety [IPCS], in press). A National Academy of Sciences Committee on Pesticides in the Diets of Infants and Children identified four fundamental differences that contribute to children's heightened susceptibility to toxic chemicals (National Research Council, 1993): (1) Children have disproportionately heavy exposures to environmental toxicants as a consequence of their greater intake kilogram-for-kilogram of food, water,

and air coupled with their unique behaviors, in particular their oral exploratory behavior in infancy; (2) Children's metabolic pathways, especially in the first months after birth, are immature. In many instances, children are less able than adults to excrete and/or detoxify toxic compounds; (3) Children are undergoing rapid growth and development, which makes them more vulnerable to environmental toxicants; (4) Children have more years ahead of them to develop chronic diseases that may be initiated by their exposures than do adults. Although broad windows of sensitivity during development can be identified for many systems, information on exact timing of sensitivity, and on any preventable factors, is limited. This lack of information reinforces the importance of detailed exposure assessment.

The chemical environment in which children live has also changed with regard to known risks of several decades ago (Lioy, 1999; National Research Council, 1991). Today there are more than 80,000 synthetic chemicals, most developed since the 1950s (Environmental Protection Agency [EPA], 1998a). These include plastics, pesticides, fuels, building materials, antibiotics, chemotherapeutic agents, flame retardants, and synthetic hormones. Children are at especially high risk of exposure to the 2,800 synthetic chemicals produced in quantities of one million tons or more per year (Environmental Protection Agency, 1998b). These high-production-volume (HPV) chemicals are the synthetic materials dispersed most widely in air, food, water, and consumer products in homes, schools, and communities (EPA, 2001). Recent national surveys show quantifiable levels of HPV chemicals have been detected in the bodies of most Americans as well as in the milk of nursing mothers (EPA, 2003).

Although much remains to be learned about associations between the environment and disease in children, accumulating evidence suggests chemical, physical, and biological factors contribute to disease causation and severity. Numerous pollutants in the indoor environment—second-hand tobacco smoke, mold and mites, cockroach droppings, animal dander, and certain pesticides (CDC, 2005; Gergen et al., 1999)—have been identified as triggers for childhood asthma. Reduction in children's exposures to these indoor pollutants has been shown to reduce frequency of asthma (Lioy, Freeman, & Millette, 2002). Evidence indicates that ambient air pollutants—fine particulates, ozone, oxides of nitrogen, and diesel exhaust—also increase the incidence of asthma and trigger asthmatic attacks (Kattan et al., 2005; Salam, Li, Langholz, & Gilliland, 2004). Reduction in ambient air pollution has been associated with reduction in the number of hospitalizations due to asthma and other respiratory diseases (Friedman, Powell, Hutwagner, Graham, & Teague, 2001; Gauderman et al., 2004; Suh, Bahadori, Ballarino, & Spengler, 2000). Drinking water may have low-level concentrations of a number of chemical contaminants, such as pesticides, phthalate plasticizers, and byproducts of water disinfection. Animal studies indicate that some of the phthalate plasticizers have anti-androgenic properties and may cause birth defects (Blount et al., 2000; Barlow et al., 2003). Childhood cancer has long been linked to ionizing radiation. More recently, benzene, 1, 3-butadiene, and pesticides have been etiologically associated with childhood malignancies (Andrade et al., 2006; Daniels et al. 2001). A recent National Academy of Sciences study suggests that at least 28 percent of developmental disabilities in children may be caused by environmental contaminants acting alone or in combination with genetic factors (Bigbee et al., 1999; Lee, Cantor, Berzofsky, Zahm, & Blair, 2004; National Academy of Sciences, Committee on Developmental Toxicology, 2000; Slotkin, 1999). Although the concentrations of such contaminants may not be sufficiently high to cause overt acute toxicity among exposed individuals, the safety of low-level exposures to such chemicals in utero or during early childhood is unclear and is a serious concern.

The various routes and patterns of exposure in the environment can impact internal absorption and biological effects (EPA, 1998). Very little research is available about differences in patterns of exposure (e.g., short-term, peak, cumulative, chronic, or intermittent). The importance of considering exposures to a single or mixture of chemicals through all relevant pathways and routes as an aggregate exposure is exemplified by studies of chorpyrifos (IPCS, in press), lead (Albalak et al., 2003; Garcia Vargas et al., 2001; Morgan et al., 2005), arsenic (Pineda-Zavaleta et al. 2004), and DDT for malaria control (Carrizales et al., 2006; Diaz-Sanchez, Rumold, & Gong, 2006). These studies report high

levels of exposure from multiple routes with the largest contributor sometimes resulting from unexpected sources. How different patterns of exposure, such as peak exposure or cumulative exposure from all sources and pathways through time, determine the overall risk to individuals is not well understood (Herrera, Ochoa, Franco, Yanex, & Diaz-Barriga, 2006). Some studies of organophosphate pesticides (OP) point to greater susceptibility to cumulative exposure to OPs in children compared to adults (IPCS, in press). Thus far, however, the measurement methodology, capability, and adequate study size have not been available in any study to begin to understand the impact of routes and patterns of exposures (Wessels, Barr, & Mendola, 2003).

1.2.2.2 Physical Exposure: The Built Environment

The physical environment in which children live has prompted concerns about potential health effects. A higher proportion of children in America live in cities and suburbs than ever before, and the built environment has been shown to be capable of influencing children's physical and mental health and their risk of disease (EPA, 2003; Department of Agriculture, 2003; Frumkin, 2002; Galvez, Frieden, & Landrigan, 2003; Horowitz, Colson, Hebert, & Lancaster, 2004; Jackson, 2003). The adverse effects of the modern built environment are magnified in low-income, predominantly minority, urban communities where crowded streets, lack of outdoor play-spaces, limited access to fresh and healthy food, and substandard housing contribute to substantial and well-documented disparities in health (Morland, Wing, & Diez-Roux, 2002; Morland, Wing, Diez-Roux, & Poole, 2002; Sallis, Bauman, & Pratt, 1998; Sallis, Kraft, & Linton, 2002; Sallis et al., 1990). Recognition is increasing that characteristics of the built environment may influence diet and activity patterns and, as a result, increase the risk of obesity (Ewing, Schmid, Killingsworth, Zlot, & Raudenbusch, 2003; Frank, Andressen, & Schmid, 2004). Humpel et al. (2002) observed that physical environmental factors show consistent associations between the built environment and physical activity behavior. They also noted that availability of and access to bicycle paths, footpaths, health clubs, and swimming pools, as well as favorable aesthetics (e.g., indicating that it is pleasant near the home) are associated positively with physical activity. Thus, the physical environment is an important predictor of physical activity change and related health outcomes (Berrigan & Troiano, 2002; Berrigan, Troiano, McNeel, Disogra, & Ballard-Barbash, 2006). Physical activity of youth appears to be determined by many factors, including the physical environment, but the long-term influence of the built environment on children's physical activity is largely unexplored with about 75 percent of the extant literature being cross-sectional in nature (Sallis, Prochaska, & Taylor, 2000). A more recent review shows that physical activity in childhood exerts its strongest influence in diseases that have in common altered stress, inflammation, and leukocyte function, such as asthma and arthritis (Schwarzenberg & Sinaiko, 2006; Van Gaal, Mertens, & De Block, 2006). The impact of physical activity on critical periods of development in children need not be limited to the walking child, since assisted exercise in preterm infants has been shown to increase body weight and improve bone strength.

1.2.2.3 The Psychosocial Environment

The psychosocial environment plays a critical role in healthy development. Substantial evidence points to the complex and dynamic role that psychological and social environmental influences play in development, and in the creation and amelioration of health disparities. Concentrated poverty, racial segregation, and high levels of crime contribute to poor health, developmental deficits, and high levels of risk behaviors among individual residents (Aneshensel & Sucoff, 1996). Yet to be explored are the mechanisms and the interactions with genetic and other exposure factors needed to guide interventions. National and local public policies influence the resources available to individuals and families and their ability to manage health-related aspects of their lives. The functioning of families, the

most crucial element of the psychosocial environment for young children, is affected by economic, policy, social, and cultural dimensions of the environment.

Evidence and practical experience attest that parenting practices, as just one critically important component of a child's psychosocial environment, can have a profound impact on a child's development and outcome (Borkowski, Ramey, & Bristol-Power, 2002). There is an increasing body of evidence based on animal research which elucidates pathways that explain how early social environment can cause lasting changes in gene expression which remain into adulthood (Barr et al., 2004; Newman et al., 2005). It is also known that abuse and unstable parent-child relationships can lead to behavioral disorders and increased morbidity and mortality (Shonk & Cicchetti, 2001; U.S. Department of Health and Human Services, 2004). Suomi's (2004) research demonstrates that in non-human primates marked differences in maternal nurturing interact with genetic variations in certain neurotransmitters resulting in dramatically different outcomes for the offspring. This suggests mechanisms for human behavioral development, and potential avenues for targeted interventions in humans (Champoux et al., 2002; Suomi, 2004). The observation that certain parenting styles are associated with a young child's risk of being overweight creates important questions about identifying the mechanisms for this association and its interactions with genetic and other factors. Understanding these gene-social environment interactions is both a pressing need and an emergent opportunity (Tholin, Rasmussen, Tynelius, & Karlsson, 2005) that can best be addressed by the National Children's Study.

Psychosocial environmental influences appear to interact with physical and chemical exposures in complex ways. Environmental justice literature (Brulle & Pellow, 2006; Bullard, 1983; Bullard, 1990; Bullard & Wright, 1993) suggests that the impact of exposure to toxic substances may be greater in communities that have low levels of education and have poor access to health services. Social factors may also confound relations between physical exposures and health. For example, an association between exposure to an environmental toxicant and violent behavior may be misinterpreted as causal when, in fact, poverty causes the physical exposure and violence. Social factors and physical exposures may also modify or mediate the effects of one another on health outcomes. Sorting out these influences is essential if researchers are to understand why some children are healthy and thrive while others do not.

1.2.2.4 Biological Factors

A child's biologic environment ranges from in utero interaction with maternal physiology to nutritional, infectious, and allergenic exposures throughout childhood and adolescence. Accurate serial assessment of a child's multifaceted biologic exposures is important to understand the etiology and severity of NCS outcomes from preterm birth and congenital anomalies to insulin resistance and schizophrenia in adolescence.

Infection, inflammation, and stress

Maternal or early childhood exposure to many different organisms has been implicated in the subsequent development of outcomes to be studied in the NCS, including preterm birth (Andrews, Hauth, & Goldenberg, 2000; Goepfert et al., 2004; Pararas, Skevaki, & Kafetzis, 2006), neurodevelopment and psychiatric disorders (Hagberg & Mallard, 2005; Rapoport, Addington, Frangou, & Psych, 2005), and asthma (Garcia-Garcia et al., 2007; Sigurs et al., 2005). In contrast to the direct suppurative effects of infection, such as the cognitive and hearing losses associated with bacterial meningitis, the nature and timing of some putative associations suggests the distal influence of host inflammatory mediators produced in response to infection. However, the nature of the relation between in utero exposure to infection or inflammation and subsequent outcomes has been difficult to study in humans. For example,

the epidemiological literature suggests a strong association between maternal viral infection and subsequent schizophrenia in offspring (Bagalkote, Pang, & Jones, 2001; Yolken & Torrey, 1995). Animal studies demonstrate that in utero or early life exposure to circulating cytokines result in neuronal lesions compatible with schizophrenia (Gilmore, Jarskog, Vadlamudi, & Lauder, 2004; Meyer et al., 2006). Establishing a direct relation between prenatal inflammatory exposure and subsequent schizophrenia has been impossible because of time lags between exposure and outcome, which limit potential preventive and therapeutic strategies. The potential relation between in utero inflammation and autism has similar characteristics (Chauhan & Chauhan, 2006; Meyer et al., 2006), and the ability of the NCS to capture these early exposures offers similar opportunities for advancing etiologic understanding and prevention and treatment possibilities.

The relation between early infection or inflammation and asthma poses additional questions concerning the influence of immune response and subsequent disease. Numerous studies suggest that viral infection during infancy is associated with increased asthma risk (Garcia-Garcia et al., 2007; Sigurs et al., 2004). A related body of literature that is often presented under the rubric "hygiene hypothesis" suggests early exposure to infectious products, perhaps bacterial products in particular (Braun-Fahrlander et al., 2002), protects against subsequent development of asthma. Attempts to untangle this relation have focused on the impact of type and timing of infection on development of a strong Th-1 lymphocyte response as opposed to the persistence of Th-2 immunologic response associated with asthma and atopy (Effros & Nagaraj, 2007). Recent studies have suggested this is complicated even further by the timing of exposure to non-infectious allergens such as dust mite or animal dander (Holt & Sly, 2002).

An additional contributor to immune system development is exposure to maternal stress unrelated to infection and inflammation (Elenkov, 2004; von Hertzen, 2002). Genetic variation in the structure or activity of specific molecular mechanisms, particularly Toll-like receptors, also seems to influence the already complex relations (Vercelli, 2006). Serial measures of maternal and child infection and inflammatory response, emotional and physiologic stress, timing of exposure to a variety of potential antigens, and genomic analysis within the NCS will be integrated to enable an increased understanding of asthma etiology and the potential to develop new preventive and ameliorative strategies.

Elevated maternal glucose or diabetes

Compared to infants born to women without diabetes, infants born to women with a diagnosis of diabetes or other evidence of elevated blood glucose have an increased risk of congenital anomalies (Farrell, Neale, & Cundy, 2002; Guerin, Nisenbaum, & Ray, 2007; Nielsen et al., 2005; Schaefer et al., 1997; Sharpe, Chan, Haan, & Hiller, 2005). The amount of additional risk varies depending on the nature of the diabetes and the degree of maternal hyperglycemia. This may suggest a simple dose-response mechanism. However, a range of disparate major and minor defects with different embryologic origins is influenced by maternal hyperglycemia (Schaefer et al., 1997; Nielsen et al., 2005). Animal models suggest that one potential pathway through which maternal hyperglycemia disrupts normal embryologic development is via oxidative stress damage following increased fetal glucose metabolism (Loeken, 2006). The fetal oxidative stress response can influence selective dysregulation of individual gene expression and have differential effects on organogenesis depending on the timing and degree of maternal hyperglycemia. This mechanism may explain the similar effects of maternal hyperglycemia on the development of multiple and diverse organ systems. The potential role of oxidative stress in the etiology of at least some birth defects also dovetails with possible mechanisms of other exposures to be investigated in the NCS including infectious and inflammatory sequelae, diet, and respiratory pollutants.

Diet and nutrition

Aspects of maternal and child diets that are important to multiple outcomes within the NCS include overall caloric and macronutrient intake and potential exposure to pesticides or other chemical contaminants. Collection of additional dietary information will enable elucidation of potentially more subtle influences of diet on health and disease.

For example, in both human and animal diets with a high glycemic index and glycemic load, measures of a food's post-consumption impact on blood glucose (Frost & Dornhorst, 2005) have been associated with increased risk of obesity and type 2 diabetes which is independent of the diet's caloric content (Ludwig, 2002; Pawlak, Kushner, & Ludwig, 2004; Schulze et al., 2004). Further understanding of these presumptive relations is necessary if optimal interventions to curb the increase of obesity and related morbidity are to be developed.

A nutritional factor that may play an important modifying or protective role in relation to multiple NCS outcomes is dietary anti-oxidants. Oxidative stress has been hypothesized to play an etiologic role in numerous outcomes, including neurodevelopment and psychiatric conditions (Chauhan & Chauhan, 2006; Meyer et al, 2006); asthma (Effros & Nagaraj, 2007); birth defects (Loeken, 2006); and diabetes (Duncan & Ines Schmidt, 2006; Esposito et al., 2002). Evidence from human and animal studies regarding the ability of diets high in antioxidants to protect against disease is mixed (Abela, Howe, Oakes, & Webster, 2005; Devereux et al., 2006; Litonjua et al, 2006; Murray, Simpson, Kerry, Woodcock, & Custovic, 2006). The potential benefits of such a diet, however, retain biologic plausibility. For example, culture studies suggest the ability of antioxidants to prevent neuronal damage, although the developmental timing is crucial (Perry, Norman, Litzburg, & Gelbard, 2004). The longitudinal collection of systemic nutritional measures and dietary characteristics starting in utero and continuing through adolescence may help elucidate the role of oxidative stress in diseases and offer potential interventions.

1.2.2.5 Genetic Factors

The past decade has witnessed a virtual explosion in the development and application of genomic methodology and research that have direct application to the NCS. The majority of common disorders in children and adults are now recognized as having a "complex" multifactorial etiology, wherein multiple genetic and environmental factors play a role in disease causation (Kelada, Eaton, Wang, Rothman, & Khoury, 2003; Moore, 2003; Zondervan & Cardon, 2004). It is the interaction or multiplicative effects, rather than the sum of these factors, that likely underlies disease risk. These complex relations require that studies of disease causation assess each of these multiple factors in a common cohort of individuals as opposed to assessing different factors in different cohorts. In addition, changes in epigenetic factors and the association with environmental factors necessitate a longitudinal approach. This requires the kind of comprehensive assessment of genetic and environmental risk factors for disease in the same individuals and the large number of study participants to provide adequate statistical power (Garcia-Closas & Lubin, 1999) proposed in the National Children's Study.

The sequencing of the human genome provides powerful research tools to identify genetic variation that contributes to health outcomes (International Human Genome Sequencing Consortium, 2001; Venter et al., 2001). In the past, association studies using candidate genes have been the mainstay of epidemiologic investigations of the role of genetic and environmental factors in children's health. More recently, rapidly changing and increasingly affordable technology and information from the International HapMap Project have made whole genome association studies using haplotype tagging single nucleotide polymorphisms (SNPs) a reality (The International HapMap Consortium, 2005). The HapMap project has lessened the task of measuring millions of SNPs by using linkage disequilibrium to identify a reduced set

of “tag” SNPs for capturing variation throughout the genome (Johnson et al., 2001; Wall & Pritchard, 2005). As affordable technology becomes available, complete sequencing of the genome of NCS participants will be possible. Emerging systems biology approaches to genomic analyses, which seek to understand how different biologic systems are interconnected (Bogyo & Cravatt, 2007; Li & Burmeister, 2005) and how both the components and their relations can change over time, will benefit from repeated phenotypic and genomic measures in the NCS.

The Study will also have the power to examine gene-environment interactions from a developmental perspective in a new way. It will provide the opportunity to evaluate specific genetic factors in subgroups of mothers, fathers, and children in the Study. It will be a rich source of data that can be used to investigate the mechanisms behind complex diseases such as autism and asthma, the quantitative contribution of genetic variation to common conditions such as obesity, and the impact of gene and environment interactions on behavior and health outcomes. Multiple gene-environment and gene-gene interactions play a key role, creating the need for complex, computer-intensive forms of analysis. The analysis of genomic data is a field of much active research (Chatterjee, Kalaylioglu, Moslehi, Peters, & Wacholder, 2006; Heidema et al., 2006; Thornton-Wells, Moore, & Haines, 2004). Analysis of genotype effects, multi-locus genotype-genotype interactions (e.g., epistasis), and gene-environment interactions can be conceptualized in a regression analysis framework for different types of outcomes where the predictor variables include SNP genotypes, environmental exposures, epistasis (e.g., interactions) among SNPs, and SNP-environment interactions. Methods developed for analyzing high-dimensional data such as microarray gene expression, massively parallel signature sequencing (MPSS), and evolutionary trees of haplotypes may also be utilized. New analytic methods can be expected to emerge in the future and researchers analyzing the NCS genomic data will apply the best methods available in every phase of the process.

1.2.3 Conclusion

There is a well established vulnerability to the effects of environmental exposures for the embryo, fetus, infant, young child, and even the developing adolescent. There is a broad array of environmental exposures that have been identified as possible threats to children’s health and development, only a few examples of which are noted above. Only for a small number of these exposures has empirical and theoretical evidence of their specific effects on children been established. Likewise, conditions and diseases in children that represent the major health threats of the new morbidity continue to challenge the researchers who seek to understand their genetic and environmental causes. The convergence of these experiences and scientific observations was a compelling rationale for the President’s Task Force to recommend, and for Congress to direct, that NICHD conduct a longitudinal study of environmental influences (including physical, chemical, biological, and psychosocial) on children’s health and development with a national scope, a large sample size, and a breadth of measures that are capable of identifying the environmental and genetic factors contributing to the major diseases and conditions that affect our children.

The representative, probability-based sampling approach ensures the Study sample will reflect the broad racial and ethnic diversity of the United States. The full NCS sample will consist of approximately 78,000 white, 19,000 Hispanic, 15,000 Black, 5,000 Asian, and 2,000 American Indian participants. Also, approximately 20 percent of the cohort will be from rural areas. This makes the NCS the most comprehensive, long-term study of this size for Hispanic children, Black children, and children from rural settings.

The size and diversity of the cohort will allow the NCS to generate large amounts of data and better characterize the disparities among subpopulations, including uncovering more subtle disparities than previously recognized. Disparities of interest to the NCS cut across a variety of the Study exposures and outcomes, from differences in the prevalence of preterm birth to variations in exposure to pesticides across communities to differences in the types of injuries children suffer.

Future researchers may find even more topics that prove timely and important. The wide range of study hypotheses that can be addressed using the NCS data also translate into a wide number of disparities that can be examined using the large data set. For many possible study questions, adequate power exists to perform analyses at the subgroup level.

2.2.3 Case-Control Studies

Many of the Study hypotheses can be addressed effectively and most efficiently through nested case-control studies. The prospective collection and careful storage of all Study data (biospecimens, environmental samples, stored images, etc.) will allow investigators to limit many of the expensive analyses to smaller subsets of identified cases and their matched controls.

Perhaps most importantly, this approach will allow the investigation of hypotheses formulated in the future. Some of the greatest values of this Study are the establishment of a databank of longitudinal measures and a repository of both environmental and biologic specimens that will allow future investigators to address important questions of clinical and public health relevance.

3. PRELIMINARY STUDIES

As part of the National Children's Study's conceptualization, the President's Task Force on Environmental Health Risks and Safety Risks sought advice concerning exposure measurement and study design from a panel of experts involved in recent or current major longitudinal studies. These included the Collaborative Perinatal Project, The Danish National Cohort Study, the Bogalusa Heart Study, The Avon Longitudinal Study, The Women's Health Initiative, The Framingham Heart Study, The Nurses Health Study, and HMO-based studies. In addition to strong endorsement and encouragement for a national longitudinal study of children's environmental health, the panel recommended the development of specific hypotheses that would frame the study and assure the most critical contemporaneous health concerns of children were not neglected. Additionally, the work group exhorted the planners to be bold and ambitious to ensure the study would be worth the considerable expenditure of time and resources (Iowa Department of Human Services, 2003).

3.1 Review of Existing Longitudinal Studies and Databases

Before the planning and initiation of a new large and expensive study proceeded, an inventory and review of longitudinal studies was commissioned by the National Center for Health Statistics and undertaken by the Lewin Group (2000). The review examined existing resources for assessing the possibility of addressing the Study goals without embarking on an entirely new study and identified needs for longitudinal research by the Centers for Disease Control and Prevention. This search sought to identify possible duplication of efforts by the proposed longitudinal cohort study. To identify virtually all of the significant longitudinal studies, two databases served as primary sources of identification: Medline and the listing of National Institutes of Health (NIH)-funded studies at the Community of Science, a network of scientists and research organizations on the Internet. Searchers used the terms "longitudinal studies," "cohort study," and "risk assessment." From more than 37,000 citations, the search identified 154 studies that met the criteria of longitudinal (studies must collect data at two points in time), longer than one year, prospective, observational (as opposed to interventional), general population (as opposed to disease specific), and meaningful sample size (generally 1,000 and greater) conducted in the United States. The Lewin inventory did not include studies that could be identified only through the behavioral, psychological, or social science literature or studies of occupational health. The studies from the initial search cover an array of health conditions in youth and adults, including, but not limited to, asthma, behavioral health, cancer, and child development.

A systematic review of all available longitudinal cohort studies found no study capable of answering the questions and concerns that led to proposed National Children's Study regarding potential long-term effects in children from environmental exposures. Although the Lewin inventory identified 62 longitudinal studies of youth and their health outcomes, only five met the criteria of a predominantly U.S. sample, sample recruitment during pregnancy or early infancy, and sufficiently large sample size (greater than 10,000). Of these five, only one, the Early Childhood Longitudinal Study (ECLS-B) Birth Cohort (National Center for Education Statistics, 2000) met the above criteria and could possibly be adapted or used as a framework for a large longitudinal cohort study of environmental factors and children's health. The goal of the ECLS-B is to assess the health, growth, and developmental factors critical for school readiness and achievement. It identified a nationally representative sample of approximately 15,000 children at birth and is performing examination batteries at 9, 18, 30, and 48 months of age. Because the ECLS-B recruited participants at birth, the issues involved in recruiting during the prepregnancy or early pregnancy period still needed to be identified. Thus, the ECLS-B excluded the possibility of observing effects of prenatal and infancy exposure and did not collect data for any chemical or biological exposures.

Ongoing population-based studies of the National Center of Health Statistics were also considered as resources to address concerns about environmental effects in children. These included the National Health and Nutrition Examination Survey (NHANES), the National Survey of Family Growth (NSFG), the National Maternal and Infant Health Survey (NMIHS), the National Health Interview Survey (NHIS), and vital statistics. Of those surveys, only NHANES met key criteria of activity that is done on a continuous or relatively frequent interval, and of the ability to collect physical measurements of the child or environment, or biomarkers, in the context of the effort. While NHANES serves extremely important surveillance and monitoring functions, it is not a cohort study and its cross-sectional design does not permit it to identify the kinds of exposure-outcome relations critical to the goals of the NCS. NHANES collects data on approximately 5,000 people per year selected to be a nationally representative sample of the U.S. population of all ages (Centers for Disease Control and Prevention, National Center for Health Statistics, 2007). In the course of this effort, mobile examination centers (MEC) and technical personnel travel around the country collecting the data. Since NHANES is representative of all ages, the numbers of children are relatively few overall, and it would take many years to gather information on the number of children required for the work proposed on the NCS. The importance and uniqueness of the proposed Study is its ability to examine exposures very early in development, including intrauterine exposures. Given its household sampling frame, NHANES would contain too few pregnant women to enable detailed analysis. Most importantly, NHANES is not designed to do multiple assessments in specific individuals over time.

3.2 NCS Planning and Methods-Development Studies

More than 2,500 scientists and other professionals had input on the NCS. Guided by the Interagency Coordinating Committee (ICC) of scientists and staff of the federal funding agencies (HHS, NICHD, NIEHS, CDC, EPA) a federally chartered advisory committee (NCSAC) was established under the Federal Advisory Committee Act. The NCSAC established 22 Working Groups, comprised of federal and non-federal scientists, that focused on specific scientific areas or aspects of the study (see Appendix J for a list of working groups). Most of the Working Groups focused primarily on defining the domain-related hypotheses (see Chapters 4 and 7 and Appendix A for details of hypotheses) and study methods that were subsequently reviewed by the NCSAC and incorporated by the ICC as the Study core hypotheses.

The Study planners used a range of approaches to address the numerous issues and questions they faced, including large conferences, workshops, scientific reviews or "white papers," and actual methods-development studies that were labeled "pilot studies." Five large assemblies were held to exchange information and science related to the Study and to provide venues for the Advisory Committee and Working Groups to conduct activities. Thirty-one extremely useful workshops with subject-matter experts have been conducted thus far to define and to clarify scientific issues and methods that could be applied to the various constructs of interest to the Study. For example, workshops on dietary assessment and on the collection and use of genetic information helped identify measurements and assays applicable to the Study and eliminate inappropriate or unfeasible measures. Along with reports from the respective Working Groups, the workshop proceedings were used for input and as a starting place for specific protocol planning. Reports from the workshops are posted on the Study's Web site: www.nationalchildrensstudy.gov.

For a number of aspects of the Study, more detailed reviews and analyses of the scientific literature were needed to inform decision making, and literature reviews or white papers were also commissioned to provide essential guidance for a number of critical issues. For example, to decide the Study's sampling strategy, a series of papers was commissioned to review topics including alternative sampling strategies; the impact of anticipated recruitment and retention rates on sampling options; the

impact of sampling options on core hypotheses; and cost estimates for sampling options. In addition, a series of methods development or pilot studies was conducted to answer specific questions or to develop specific methods. Such studies varied in methods and objectives. For example, focus groups were conducted, using a variety of sources (i.e., young mothers, adolescents, health providers), regarding attitudes and perceptions related to the NCS. One study evaluated the feasibility of three-dimensional versus two-dimensional ultrasound for measurement of fetal growth, which led to the more economical decision to use two-dimensional ultrasound. Another study, testing the feasibility of employing clinical practice sites for data collection venues in the NCS, identified a number of important issues to be addressed where this strategy is used. Thirty-five white papers and 29 pilot or methods development studies have been conducted thus far, and reports are available on the NCS' Web site. Many of these projects have applicability beyond the NCS and have been published in the scientific literature. Lists of the workshops, white papers, methods-development studies, and publications that have come out of these projects appear in Appendix J.

4. AIMS AND HYPOTHESES

4.1 Specific Study Aims

The National Children's Study has several broad aims. These aims will be served through a program of current, carefully designed research questions and the creation of a resource for future research questions. The specific aims of the Study are:

(1) Determine the presence or absence of effects, both harmful and helpful, related to the timing, frequency, magnitude, and duration of specific chemical, physical, biological, and psychosocial exposures in children's environments from preconception to adulthood.

(2) Determine possible environmental contributions to, or causes of, specific diseases and conditions of children, including, but not limited to, prematurity and other outcomes of pregnancy, neurological and developmental disorders, psychiatric and behavioral disorders, altered physical development and sexual maturation, obesity and insulin resistance, asthma, and injuries.

(3) Determine how genotypic variation and mechanisms, and the interaction of genes with environmental factors, influence disease risk and developmental trajectories in children.

(4) Serve as a national resource for future studies of child health and development by providing a rich database and repository of environmental and biological samples and information that can be used to address future questions and hypotheses.

4.2 Core Hypotheses

The rationale for a large longitudinal study with multiple classes of exposure, outcome, and genetic measures to address the Study aims has been described. These aims are the sum of specific hypotheses examining how environmental and genetic factors may affect children's health and development. Thus, the NCS can be can best be understood as a broad program of research comprising multiple separate and overlapping hypothesis-driven studies, each requiring this proposed design and size.

The NCS planners recognize that framing hypotheses is essential to guide study planning and to assure that important questions can be addressed. Nonetheless, not all important or answerable questions are necessary or even possible to state. However, in planning the Study a standard was established that a supporting hypothesis must be required for inclusion of measures or design elements in the Study. Within broad priority exposure and outcome areas, the NCS has framed 28 well-defined core hypotheses to fulfill its aims to ascertain whether exposures to environmental factors either adversely or positively affect the health and development of children and whether certain health conditions of children result from environmental exposures.

To derive the core study hypotheses, the NCS relied on the expertise and input of a Federal advisory committee (National Children's Study Advisory Committee [NCSAC]), its working groups, and the general public. Within priority areas, many hypotheses were proposed by working groups and other entities and then considered by the NCSAC, which made recommendations concerning their relevance

and prioritization. Ultimately, the NCS Interagency Coordinating Committee[1] established the core Study hypotheses (see Table 4-1). A more detailed listing by priority outcome area appears in Appendix A-1. Fully documented and referenced hypotheses across different priority areas are found in Appendix A-2. These hypotheses identify relevant environmental exposures including physical, chemical, biologic, and psychosocial factors that affect the identified priority outcomes, including pregnancy outcomes, neurodevelopment and behavior, injury, asthma, obesity and growth, child health and development, and reproductive development. Many hypotheses also take into consideration the vital impact of gene-environment interactions or the effect of access to health care services on health and well-being. The potential avenues of investigation are too numerous to cite, but a number of specific study questions have been developed to assure that key measures are obtained, and that the sample of participants and the study design are adequate to address the questions. Acknowledging that science evolves, this list of hypotheses is expected to change as additional existing hypotheses are refined, omissions of important questions are identified, and other hypotheses become outdated.

[1] The Interagency Coordinating Committee is comprised of senior Federal staff scientists assigned since 2000 to lead the development of the NCS on behalf of the lead Agencies supporting the NCS: the Department of Health and Human Services, National Institute of Child Health and Human Development, National Institute of Environmental Health Sciences, Centers for Disease Control and Prevention, and the U.S. Environmental Protection Agency.

Table 4-1. Hypothesis Topics of the National Children's Study

- Birth defects from impaired glucose metabolism
- Increased risk of preterm birth from intrauterine exposure to mediators of inflammation
- Increased risk of fetal growth restriction, preterm birth, birth defects and developmental disabilities in children born through assisted reproductive technologies
- Maternal subclinical hypothyroidism and neurodevelopmental disabilities/adverse pregnancy outcomes
- Non-persistent pesticides and poor neurobehavioral and cognitive skills
- Prenatal infection and neurodevelopmental disabilities
- Gene-environment interactions and behavior
- Prenatal and perinatal infection and schizophrenia
- Family influences on child health and development
- Impact of neighborhood and communities on child health
- Impact of media exposure on child health and development
- Social institutions and child health and development
- Influences on healthy development
- The role of prenatal maternal stress and genetics in childhood asthma
- Exposure to indoor and outdoor air pollution, aeroallergens, and asthma risk
- Dietary antioxidants and asthma risk
- Social environmental influences on asthma disparities
- Early exposure to structural components and products of microorganisms decreases the risk of asthma
- Environmental exposures interact with genes to increase the risk of asthma and wheezing in children
- Obesity and insulin resistance from impaired maternal glucose metabolism
- Obesity and insulin resistance from intrauterine growth restriction
- Breastfeeding associated with lower rates of obesity and lower risk of insulin resistance
- Fiber, whole grains, high glycemic index and obesity and insulin resistance
- Genetics, environmental exposures, and type 1 diabetes
- Repeated mild traumatic brain injury and neurocognitive development
- Behavioral exposures, genetics, and childhood or adolescence onset aggression
- Antecedents and resiliency to traumatic life events in childhood
- Hormonally active environmental agents and reproductive development

PART II: STUDY DESIGN AND METHODS

5. CHALLENGES AND APPROACHES

5.1 Design Challenges and Approaches

A major challenge in designing the National Children's Study is to balance the power of the large sample size, enrollment of women early in (or prior to) pregnancy, longitudinal follow-up, and breadth of exposure and outcome measurements with the real life considerations of participant burden and cost. Because the Study is observational, respondent burden must be commensurate with the motivations and limited benefits of participation. Using other longitudinal observational studies (Golding, 2004) as a guideline, the NCS will try to limit any single face-to-face data collection to no more than half a day (four hours). Similarly, procedures must pose no more than minimal risk, and biospecimens will be limited in quantity. Thus, only some of the many possible clinical assessments are appropriate, and only a finite number of tests can be run on each specimen. Funds are also limited, thus the planned tests or procedures implemented in the entire cohort must be carefully selected. A core set of contacts and data collections with Study participants has been proposed, and forms the framework for the Study. It is expected that sub-studies and adjunct studies will supplement the full Study, utilizing additional, more in-depth data collections on targeted areas of interest and involving subsets of the full Study population (see Chapter 16).

5.2 Measurement Challenges and Approaches

A number of approaches will address the measurement challenges of this large, longitudinal, epidemiological study. The best measures possible will be employed in the full Study, but they may not always mirror more in-depth assessments used in more circumscribed research studies because of the cost, time, and burden involved. Again, it is expected that these issues can be addressed in sub-studies or adjunct studies. For some measures, for example certain of the unstable and expensive environmental chemical assays that are important to assess in the entire population, a validation subsampling methodology will be considered to reduce costs while still including critical exposure measures (Strauss, Lehman, Morara, & Ryan, 2003). It is likely that more extensive measures focused on specific areas will be utilized in anticipated sub-studies or adjunct studies.

To limit respondent burden, questionnaires developed for use in the core protocol incorporate short versions of scales or selected targeted subscales, when possible and appropriate. Moreover, a number of the questions are viewed as "screens" and will be followed by a lengthier set of questions for those with a positive screen. Face-to-face data collections are supplemented with mail-in or computer-based self-administered data questionnaires. These additional remote data collections not only provide valuable data for the Study, but also serve as a means to maintain meaningful, frequent contacts with Study participants.

5.3 Processes for Continuing Protocol Development

As a longitudinal study of more than 20 years, the NCS will face demands of continually changing and advancing science, technology, and methods. Wherever possible, the Study must anticipate and accommodate evolving science and technologies. For example, a large National Institutes of Health (NIH) program to develop efficient, inexpensive, and accurate exposure assessments is underway. It is

likely that some of the methods from this initiative would increase the power of environmental assessments for the NCS (http://www.gei.nih.gov/exposurebiology/index.asp). Development of technologies and methodologies for genomic measurement and analyses will continue to evolve rapidly during the Study. It is expected that as this field advances and expands, so too will the opportunities for understanding gene-environment relations and mechanisms of disease. Information management systems (IMS) can be expected to be outdated at least every 5 years, and the IMS for the NCS is being built to allow incorporation of changes as they occur.

Accordingly, the Study's protocol will require continual planning for each successive phase of the cohort as the participants advance through life. The initial research plan and protocol focus on the first phase of pregnancy and on infancy. Subsequent plans and phases will address preschool, elementary school, early adolescence, and late adolescence. Planning the specific methods for these respective protocols will be undertaken as the cohort approaches each phase with enough time for careful planning and implementation but close enough to the needed protocol so methods can be as current as possible.

As in the planning of the Study thus far, future protocol planning will seek to include the best scientific input possible. To seek input and guidance on specific issues, The Study will continue to utilize the Federal Advisory Committee, ad hoc workshops, and literature reviews and white papers. When needed, methods development and pilot studies will be conducted to resolve issues and to refine measures for each respective phase. In contrast to the first several years of Study planning, the Study will not have numerous expert working groups under the Advisory Committee. Instead, investigators from the 30-40 Study Centers and Coordinating Center will provide ample depth and breadth of expertise in the form of subject matter expert teams for input into planning the respective protocols. These working teams will propose methods and measures for the respective protocols to the staff of the Program Office and Coordinating Center. The Steering Committee of Principal Investigators will consider and propose decisions regarding Study priorities and similar issues, and the Interagency Coordination Committee will continue to provide review and oversight with regard to the Study's ability to address the goals and missions of the respective federal agencies. Though committed to input from broad scientific expertise, ultimate decision-making authority rests with the Director of the National Institute of Child Health and Human Development and, on his behalf, the Director of the NCS.

6. STUDY DESIGN

6.1 Overview of Study Population

The National Children's Study is a longitudinal study that will enroll and follow over time a nationally representative sample of approximately 100,000 children born in the United States. Participation in the Study is voluntary, so potential Study participants can choose not to participate at any time. The Study calls for collecting information on children from birth through age 21. However, to enable assessments of exposures and risk factors during critical periods of embryonic and fetal development, some information must be collected from women before they become pregnant, and additional information must be collected while the woman is pregnant. Thus the Study plan is to select a sample of women of child-bearing age, to request that each eligible woman participate in the Study, and to follow these women over a fixed period of time. If an eligible woman informs the Study that she is planning on becoming pregnant or she is "at risk" of becoming pregnant, certain information will be collected on preconception exposures and risk factors. If any eligible woman in the selected sample becomes pregnant, information will be collected during her pregnancy. The collection of mother's information will begin as early in the pregnancy as possible and, at the time of birth, her child will be enrolled in the sample of children. It is recognized that not all pregnancies will result in a live birth; the Study protocol addresses human subject concerns and issues related to adverse pregnancy outcomes.

Thus, for the NCS, children/births are sampled through the mothers. Because not all pregnancies are planned, not all mothers will have preconception information collected. The Study target is to enroll at least 25 percent of pregnancies prior to conception and to identify and enroll a cumulative total of 90 percent of pregnancies before the end of the first trimester of the pregnancy. For most Study locations, births will be enrolled during a 4-year period with a target of 250 births per Study location per year. As described below, there are 105 Study locations in the national design. Most locations correspond to a single county, but some are comprised of multiple counties.

6.2 Inclusion and Exclusion Criteria

The sample design described below calls for recruiting women into the Study primarily through household sampling. All women who are in the first trimester of pregnancy at the time of initial contact with the Study are eligible for inclusion. Additionally, women between ages 18 and 44 at the time of initial contact who are not pregnant are eligible for enrollment and follow-up for pregnancy. If at any time in the enrollment period it is determined that a particular woman cannot become pregnant, she will not be followed. The frequency and intensity of follow-up of women who are not pregnant depends on the woman's probability of becoming pregnant as described in Section 6.4.2.

If a woman enrolled in the Study gives birth during the 4-year enrollment period, the newborn is included in the Study provided the mother resides in a household that is part of the Study sample at the time of the delivery. All births to mothers who meet the initial eligibility criteria are eligible for Study enrollment, including children born to surrogate mothers, those expected to be adopted or assigned to foster homes, and births to women who are on active duty in the military.

Women who are cognitively impaired or mentally ill are not eligible if they are not able to understand fully the Study requirements and grant informed consent.

6.3 Sampling Strategy

A number of study and sampling design options were considered for the NCS (see Sample Design Options and other related documents available at http://www.nationalchildrensstudy.gov/events/advisory_committee/other_work_062004.cfm). There are advantages and disadvantages to each of the candidate approaches, however, after careful consideration and upon the advice of the NCSAC, a national probability sample of all U.S. births was chosen as the design that best fulfills the following goals:

- Collection of high quality, objective data to minimize measurement biases
- Avoidance of selection biases and other biases that could lead to invalid inferences concerning exposure/outcome relations
- Ability to capture the diversity of the U.S. population such that both the range and diversity of exposures and outcomes are represented
- Ability to generalize results of the NCS to the U.S. population

The sample design for the NCS is a multistage probability sample of births in the United States where the births are identified from a sample of households. The design includes two or three stages of sampling.

The first stage of sampling was the selection of primary sampling units (PSUs), which correspond to single counties or groups of contiguous counties. The second stage is the selection of smaller geographic areas (segments) from within the primary sampling unit. In general, these segments comprise city or suburban blocks or combinations of blocks and roughly correspond to neighborhoods. The third stage, which applies only to very densely populated segments, involves the selection of groups of households from within the segments. Each stage is detailed below.

6.3.1 Selecting Study Locations

The process for selection of Study locations was based on the need to achieve representative coverage of the United States with respect to geographic areas, metropolitan/nonmetropolitan areas, and demography. All decisions on sample design options considered costs, coverage, statistical reliability, and practical concerns of the protocol. Cost models and logistical aspects of the NCS data collection led to the design decision to use 105 study locations.

The probability of a county being selected as a PSU is based on the number of births to residents of that county. Because the number of births in a county at a future date cannot be known, data on resident births (births based on the mother's residency at the time of birth) from four recent years (1999-2002, the most recent four-year period available at the time) were used as an estimated measure of size for sampling the PSUs.

The 3,141 U.S. counties were categorized into 18 large strata defined by metropolitan status (metro, nonmetro) and geography (nine census divisions). Within each of the 18 large strata, the total number of births determined the initial number of smaller strata. Based on their number of births, 13 counties were large enough to be designated as self-representing units (also referred to as certainty units). For three of these counties, the number of births was so large that each county was assigned multiple PSUs. Los Angeles County was assigned four PSUs, Cook County, IL, (containing Chicago) was

assigned two, and Harris County, TX, (containing Houston) was assigned two. These are units that were "certain" to be selected into the probability sample based on their large number of births. Thus, the design contains 13 locations but 18 PSUs that are considered self-representing.

The remaining 3,128 counties were placed into smaller strata. Within each of the 18 large strata, these smaller strata were formed to be of roughly equal size. The smaller strata were defined in terms of the size of county or the percent of births with specific characteristics. The characteristics used to define the smaller strata were percent of births to Native American women, percent births to Asian women, percent births to Hispanic women, percent births to Black women, and percent low birth weight. After all strata had been formed, one PSU per strata was selected with a probability proportional to size (i.e., number of births).

A minimum measure of size for a PSU was established as 2,000 births during a 4-year period (or an average of 500 births per year). If a county was selected that had fewer than 500 births per year, geographically adjacent counties in the same stratum were added until the PSU met the minimum measure of size. In a few cases, that criterion could not be achieved. For such cases, an additional PSU was selected.

The final first stage sample comprised 110 PSUs in 105 locations: 26 locations are non-self-representing PSUs from nonmetropolitan strata; 66 locations are non-self-representing PSUs from metropolitan strata; and 13 locations with 18 PSUs are from self-representing metropolitan strata. While this design is generally consistent with an equal probability sample design, differences in the sizes of the strata relative to the PSU probability of selection results in some variation. See Appendix B for a map of study locations.

6.3.2 Sampling within Locations (PSUs)

To meet the analytic needs of the Study, a total sample size of 1,000 enrolled live births is the target for each sampled PSU. With an enrollment period of 4 years, a sample size of 250 enrolled live births per year in each PSU is needed. (The Vanguard Centers have an additional year of enrollment and thus have 1,250 targeted births.) Because each selected PSU has greater than 250 births expected per year, a sample of births within each PSU must be designed and selected. This leads to the second stage of selection for the NCS. It is not feasible to take a simple random sample of births within each PSU. The second stage of the NCS design consists of forming small geographic units within a PSU called segments (or secondary sampling units) and then selecting a sample of those segments for inclusion into the Study.

6.3.3 Segment Sampling

To increase the operational efficiency, reduce costs, and provide for more useful representation of neighborhood-level characteristics, the segments within the PSUs are "clusters" of households. A geographic classification used by the U.S. Census Bureau (blocks nested with block groups, block groups nested within census tracts) is used to form segments. An advantage of using census geography is that data from other sources for these units can be linked to the sampled segments.

Prior to the formation of segments in a PSU, a target number of sampled segments is established. This number is primarily based on operational considerations and varies between PSUs. For most PSUs, it is expected that the number of sampled segments will be between 10 and 15. In general, a smaller number of segments are targeted in more rural, less densely populated PSUs that cover large areas; in more densely populated PSUs with larger numbers of births, the number of sampled segments

may be larger. The segments are constructed to be as uniform in size as possible within a PSU, but slight departures from the target segment size are expected.

As was done for the selection of PSUs, segments will be stratified to improve the precision of estimates and to ensure the sample is representative with respect to the stratum definitions. The NCS segments will be formed by combining a number of census blocks or block groups. Stratification can be done either before or after segments are formed. When stratification is done beforehand, the characteristics of the block groups can be used to form strata and only block groups in the same strata are then combined to form segments. These segments are homogenous with respect to the stratification variables but may not be geographically contiguous, thus increasing data collection costs. When stratification is done afterward, contiguous block groups can first be clustered to form segments and then "similar" segments are grouped to form strata.

It is expected that the segment stratification scheme will vary from PSU to PSU, with a goal of achieving locally defined neighborhoods as segments. (It is hoped that using locally defined neighborhoods will increase study participation rates and facility data collections at the community level.) Within most PSUs, geographic stratification will be used either as the sole stratifying variable or in combination with other variables. Geographic stratification is useful because many of the characteristics that differentiate subpopulations (such as income, race/ethnicity, educational attainment, and environmental measures) tend to be geographically clustered.

The strata are formed as equal in size as possible so that with approximately equal-sized segments, an approximately equal probability sample of segments is obtained. In some cases, it is desirable to allow for some variations in stratum sizes within a PSU to construct more homogenous strata than an equal-sized-strata scheme would permit. If the strata vary in size within a given PSU, the segments also vary in size across strata to equalize the sampling fraction within each stratum. For example, if one stratum is twice as large as another stratum within a given PSU, the segments within the first stratum are constructed to be twice as large as the segments within the second stratum.

In some cases, the strata are not geographically contiguous. This is typically the case when variables other than geography are used for segment stratification. In these cases it is necessary that each disjointed part of a stratum be large enough to form complete segments with minimal variation in segment size.

One challenge in having PSUs that have different sizes (number of births) is the large variation in the number of possible segments across PSUs. For example, among the Vanguard Centers, the smallest PSU has only 11 segments whereas the largest has approximately 1,800. A large number of segments causes difficulties in both forming and reviewing segments. In order to use resources more efficiently, a three-stage sampling protocol is used for large PSUs (typically those with more than 500 segments).

In large PSUs, geographic units are formed within strata and these geographic units, which vary in the total number of estimated births, are sampled with the probability of selection proportionate to the size of the geographic unit. Within each stratum, exactly one geographic unit is selected. Segments are then formed within the sampled geographic unit to be equal in size. Across strata, the segments are made equal in size if the strata are equal sized, or vary in size proportionate to the variation in stratum sizes if the strata are not equal sized. Within each sampled geographic unit, exactly one segment is randomly selected.

6.3.4 Listing and Enrollment

In selected segments, household screening is attempted in all households (dwelling units [DUs]) in the segment. The exception is a very large segment, which cannot be subdivided during segment formation. In such segments, DUs are subsampled. If one of these large segments is selected, the segment is divided into "chunks" and then a chunk is randomly sampled for listing and enrollment. For example, suppose a given segment is twice as large as the target segment size and consists of two very large apartment buildings that contain approximately equal numbers of DUs. In that case, each apartment building is a chunk, and one of the two is randomly selected to be retained in the sample. Other approaches for chunking (depending on the situation) include using floors of apartment buildings or block faces as chunks.

Household screening is attempted in each sampled DU, and all eligible women are enrolled. The scheduled monitoring of eligible women is dependent on each woman's likelihood of becoming pregnant. Women more likely to become pregnant are contacted more frequently (see Section 6.4.2). In some instances, the composition of the household will change or the DU will have new occupants. To enroll births from mothers in these situations, all DUs will be contacted at least once a year. This contact will be used to update the status of enrolled women's likelihood of pregnancy and thus her schedule for follow-up visits.

6.3.5 Rollout of PSUs

A sample of seven PSUs was selected to serve as the Vanguard Centers. These seven Vanguard Centers will serve as a platform to develop methodologies and procedures that will be refined and implemented throughout the Study. The remaining 98 PSUs will be introduced in three waves. The specific plan for the subsampling of the PSUs into the waves is currently under consideration. Pilot data collection is planned to begin in the Vanguard Centers in mid-2008, data collection in the first wave of additional PSUs is planned to begin in mid-2009 with the second wave two years later and the final wave two years after that.

The 98 PSUs not covered by the Vanguard Centers will be covered in the subsequent waves by the addition of Study Centers. Each Study Center will oversee participant recruitment and data collection at one to three geographically proximal study locations. The Vanguard Centers and Study Centers will work with the NCS Coordinating Center and the NCS Program Office to ensure effective development and implementation of study procedures.

6.3.6 Subsamples

In addition to the core set of measurements collected from all study participants, a number of data collections are being considered that involve collection of survey information, samples, or biological specimens from a subset of the total population or only at the community level. One example would be to reduce the proportion of samples obtained with nonmeasurable concentrations of an environmental substance. Questionnaire information on recent pesticide applications could be used to determine what homes will have air samples collected for nonpersistent pesticides since the air concentrations of these chemicals tend to decrease over time. Pesticide measurements in drinking water are currently being planned only in rural areas for homes using private wells since municipal water system information would be available for other locations and pesticide concentrations in drinking water in urban areas are often below detection limits. In some cases, environmental samples will be collected but not analyzed (e.g., metals in dust) unless biomarker concentrations (e.g., blood levels) indicate higher exposures have

occurred, and there is a need to determine the media or sources contributing to this exposure. Additionally, the large sample size of the National Children's Study affords the opportunity for more in-depth studies of subsamples within the framework of the longitudinal cohort study. A mechanism for adjunct study proposals is described in Chapter 16. Finally, to optimize the study's ability to incorporate state-of-the-art measurements, including some too costly or too burdensome for implementation in a sample of 100,000, the use of a validation sampling approach might be considered for certain measures. In this approach, a simple or less costly assessment is paired with the more costly or burdensome approach in a planned subsample of the population. For example, personal monitoring may be the best way to measure direct exposure to air pollutants or pesticides, but the cost and intrusiveness of this monitoring make this impractical to use on the entire cohort. The relation between the two assessments of the same domain is used to characterize and adjust for "measurement error" in the analysis of exposure-outcome relations for the entire cohort, although the majority of the study participants receive only the simpler, less expensive assessment. Similarly, a matrix approach for other applications (e.g. varying times of assessment) is also being considered.

6.4 Participant Recruitment

6.4.1 Recruitment Goals

The goal of recruitment is to obtain the highest response rate possible to reduce the potential for nonresponse bias. The minimum goal for combined response and coverage in each location will be between 65-75 percent. Study locations with traditionally lower survey participation rates will have lower targets. For example, in highly urban areas response rates for surveys are often considerably lower than in other settings.

To assess the impact of nonresponse bias, studies will be undertaken to assess the differences between responders and nonresponders. Lower response rates are acceptable only if it can be demonstrated that the nonrespondents are missing at random, or if a nonresponse assessment provides an adequate statistical procedure to adjust NCS estimates for nonrandom missingness. This combination of rigorously conducting the Study to obtain response rates as high as feasible along with studying the characteristics of nonrespondents is consistent with new standards and guidelines developed and distributed by the Office of Management and Budget.

6.4.2 Enumeration of Households

Within selected segments, all households will be enumerated to identify women of child-bearing age living in the household. This enumeration will be conducted in person by trained interviewers using computer-assisted personal interviewing techniques. An adult household reporter (age 18 or older) will be asked to answer questions about the number of household members, the number of males and females, and for females, their ages and their relationships to the household reporter. To ensure coverage of all dwelling units within each structure, questions will also be asked about other dwelling units that may not be easily visible or obvious, and therefore may have been missed during the listing process.

Two groups of age-eligible women (18-44) are targeted for enrollment: women who are in their first trimester or pregnancy, and women who are at some probability of becoming pregnant during the four-year enrollment period. After the age-eligible women are identified from the household enumeration, a separate pregnancy screener will be completed with each woman to determine her status. This will be done using a standardized set of questions related to her age, history of prior births, contraceptive use, and sexual activity. To ensure privacy these questions the pregnancy screener will be

administered in-person using computer-assisted self-interviewing techniques, which allow the woman to enter her responses directly into the computer. An audio feature of this will be included to read the questions to the woman to further ensure privacy and to circumvent possible literacy issues.

Women who are not currently pregnant, and who are not actively trying to become pregnant, or who are trying to become pregnant but based on the pregnancy screening have a relatively low probability of becoming pregnant, will be categorized as either "low probability" or "moderate probability." These groups will receive periodic phone contacts to determine if they have either become pregnant or, based on a limited set of screening questions, have moved to the group at higher probability of pregnancy. Women who are at high probability of becoming pregnant will be enrolled in the preconception cohort and actively followed for four menstrual cycles following enrollment. It is estimated that 55.2 percent of women in this group will become pregnant during this timeframe.

There will be periodic rescreening of households in selected segments to monitor for "move-ins" and other changes in the composition of the household living at each address. This periodic rescreening will take place only for those households where no eligible women are identified (estimated to be approximately 70 percent of all households). For those households with women being followed as part of the Study, scheduled contacts will be used to update information about household membership. This will be an important mechanism for monitoring changes in household composition as well as for identifying young women who "age in" (i.e., turn 18) during the four-year enrollment period.

6.4.3 Recruitment through Prenatal Care and Other Mechanisms

The primary mechanism for recruiting women for the Study is by contacting them in their households and encouraging them to participate in all phases of the Study. Some women, however, will move into sampled segments after the segments have been screened (and prior to the recontacts discussed above). Since children born to women living in the sampled segments are eligible, other mechanisms are needed to identify and recruit these women.

A supplemental mechanism to recruit eligible women (those living in the sampled segments) is through providers of prenatal care, birthing centers, and hospitals. All of the requirements of those sampled in households must be satisfied by these women, so this is simply another technique for identifying and recruiting eligible women from sampled households. In addition to increasing the Study's ability to cover the mobile population that otherwise would be missed, this supplemental recruitment also provides another opportunity to encourage participation from women who previously chose not to participate in the Study when contacted in the household screening. While this method is useful in reducing nonresponse and undercoverage, it does not provide full data from the pre-pregnancy and early pregnancy data collections and is thus viewed as a supplemental approach.

6.5 Community Outreach and Engagement

The NCS values community engagement, but it will not follow a strict community-based participatory research model. Community-based participatory research is defined as a collaborative research approach designed to ensure and organize participation in all aspects of the research process and action, emphasizing participation by the communities affected by the issue being studied, by representatives of organizations, and by researchers. Because the protocol includes data collection from multiple study sites to answer specific study questions that require a national sample, it was not possible to define the core study questions and initial protocol development through input of local communities or to account for their varied needs. However, principles of community-based research will be applied when

feasible and appropriate. A partnership with each community will be formed to ensure mutual respect and the establishment of an enduring relationship. Genuine community engagement offers the hope of enhancing recruitment, retention, and participant satisfaction.

Since the beginning of planning, the NCS has undertaken a range of community engagement activities to lay the groundwork for Study Center activities. Between 2000 and 2005, the NCS conducted many focus groups to obtain community perspectives on informing communities about the NCS, gaining the support of communities, recruiting and retaining participants, and NCS sampling and visits. Additionally, the establishment of working groups, the Study Assembly, and the Federal Advisory Committee allowed ongoing community input into the Study plans. The Vanguard Centers are working within local communities to prepare for recruitment. Study Centers will continually share experiences with and learn from each other in implementing community engagement plans.

Ideally, Study Centers will be able to build upon prior local community networks and relationships. However, the unique sampling strategy, data collection intensity, and length of the NCS necessitate different approaches to working with communities than previous studies or projects. To build trust, enhance the credibility of the Study, and ensure community engagement on the local level, during the first year of the Study the investigators from the Centers will conduct community needs assessments to identify children's environmental health issues in the target community. These assessments will focus on community concerns regarding the core NCS protocol and additional concerns (e.g., health issues) that may be considered for inclusion in the core protocol at all sites or as a specific sub-study focus in the particular site. Community activities will include identification of community representatives and resources and recruitment of community partners to facilitate engagement. Examples include advance contact with community leaders to gather information about the community, town meetings, and listening sessions. Key community members will be recruited and engaged in support of the Study in activities such as acting as a spokesperson for the Study, providing insight into local issues to enhance the relevance of the NCS for their community's health, and serving on community advisory boards. Reliance on secondary data sources like environmental and geographic data can actually enhance these activities. Previous studies have shown the importance of involving community members, either in the actual data collection for the study or as liaisons to special populations such as the medically underserved. These approaches will be utilized at the Study Centers to the extent possible.

Prior to the enrollment period, each Study Center will increase the awareness of the Study among community residents. Building on the community engagement efforts and involvement of community members described above, a variety of strategies will be used to announce the NCS enrollment period. Examples include press releases, appearances on local television and radio shows, and other methods to increase community excitement and interest. Wherever possible, these activities will involve joint participation of study staff and community members. These press and public relations activities will have the technical support of the Coordinating Center and the NCS Program Office, with the approval of the NCS Project Officer.

Throughout the Study, the Study Centers will involve and solicit input from the community. Examples of ongoing activities include establishing a community advisory board, partnering with other organizations to host events or forums, incorporating community leaders into the Study Center structure, and building referral networks between the Study and organizations. Steps for community engagement will vary depending on the characteristics and experiences of the communities and the Centers, and it is expected that the most effective approaches will vary. Once data collection begins, communities will be interested in learning about Study findings. Aggregate findings will be shared with individual participants and communities through newsletters, publications, and other means. The community perspective can inform NCS researchers on ways to be sensitive to unique cultural and political issues and to concerns

within each community when communicating results. Because the NCS is a long-term research effort, attention to sustaining community relationships will be very important.

6.6 Data Collection Schedule

The following section provides an overview of the data collection schedule at each contact until the child reaches age 1. For details about the measurements (e.g. content of questionnaires, targeted analytes in biological or environmental samples, or specifics of physical assessments) and their relation to hypotheses, see Appendices E through I.

The data collection schedule for the NCS includes a variety of data collection modalities at each participant contact. A comprehensive schedule of in-person visits in the home or a clinical setting, contacts by telephone, contacts through self-administered forms, and other contact methods has been carefully constructed to minimize respondent burden while enabling measurement of key exposures and outcomes at critical points from before pregnancy through the postnatal period and beyond. Although a framework of anticipated contacts with the study participants is provided through age 21, details of the data collections are specified only for the visits occurring before pregnancy, during pregnancy, at and around birth, and during the first 24 months of the child's life, with the most detailed information through 12 months. Less detail is presented for the preschool period, and provisional details only are presented for subsequent data collections. As technology advances new tools should become available to measure key constructs, and specifying measurement strategies too far in advance might serve to limit the use of cutting-edge advances. Thus, in developing the Study's protocol, maximum flexibility has, and will continue to be, retained with respect to specifying the timing and location of participant contacts for the later years of the Study.

Table 6-1. Current Schedule and Site of In-Person Contacts with Study Participants

Prior to pregnancy*: home	3 years: clinic
First trimester: home	5 years***: to be decided
Second trimester: clinic**, ultrasound only	7 years: to be decided
Second trimester: clinic, ultrasound only	9 years: to be decided
Third trimester: clinic, full visit and ultrasound	12 years: to be decided
Birth: delivery location	16 years: to be decided
6 months: home	20 years: to be decided
12 months: home	

* For women enrolled in the pre-pregnancy cohort (see Section 6.4.2)

** Only if the woman has not had early clinical ultrasound for gestational age dating

***Timing and location of visits from 5 years onward is provisional

6.6.1 Prior to Pregnancy

As described in Section 6.4.2, women who are determined to be at "high probability of pregnancy" will be invited to enroll in the Study's pre-pregnancy cohort. The first data collection for this group will be in the home prior to pregnancy and will include an interview with the enrolled woman, collection of biological specimens and environmental samples, and a brief physical examination. At this visit, women will also be given dietary questionnaires to complete and return to the Study Center. Multiple pregnancy test kits also will be provided and the women will be instructed to use them around the time of their expected menses to enable identification of pregnancy as early as possible. As soon as a woman learns she is pregnant, she will be asked to obtain a self-collected urine sample for assessment of

transient environmental exposures and to contact her local Study Center. For a woman who is not reporting a positive pregnancy test, a series of telephone contacts will occur beginning one month after the initial home visit to ascertain if she has become pregnant and to update contact and environmental exposure information. If after four months there is no pregnancy, the woman will be moved to a lower probability of pregnancy group (either low probability or moderate probability, as defined in Section 6.4.2).

After the initial screening visit, women who are determined to have a low or moderate probability of pregnancy will be asked to contact the Study if their intent to become pregnant changes or if they become pregnant. Both groups will be contacted by telephone by Study Staff every six months for the group with a moderate probability of pregnancy and yearly for the low probability group. The phone contacts will be used to confirm that there has been no change in residence, that the female is still eligible for the Study, and that there has been no change in their probability of pregnancy. If a woman in either the low or moderate probability group became pregnant during the four-year enrollment period, she would be invited to participate in the Study beginning with the appropriate pregnancy visit. Women at low or moderate risk of pregnancy at the initial screening who later move to the higher probability group (e.g., women using reliable birth control who, on rescreening, are no longer using birth control and are actively trying to become pregnant) will be invited to participate in the preconception cohort.

Summary of preconception visits for women with a high probability of pregnancy

- Initial preconception visit (home)
- One month following initial visit (phone)
- Two months following initial visit (phone)
- Four months following initial visit (phone)

6.6.2 Pregnancy

6.6.2.1 Pregnant Women

Two face-to-face visits, one visit for a fetal ultrasound, one more comprehensive clinical visit (including an ultrasound and other assessments), and several phone contacts are planned during pregnancy. The first visit is an in-person contact that will occur as early as possible during pregnancy and will be conducted in the home to allow collection of exposure data during this critical period of early development. In addition to environmental samples taken from the home, the visit will include collection of interview data, biospecimens, and a brief physical examination. Women will be given instructions for completing several self-administered questionnaires, which they will be asked to complete and to mail back after the visit. They will also be provided with a diary to record targeted exposures (e.g., fever) that might be subject to recall bias if ascertained only at planned contacts. Finally, the women will be provided with a health visit log to document visits to clinical providers as well as targeted data items (e.g., blood pressure) (Tang, Ash, Bates, Overhage, & Sands, 2006). Women will be contacted by telephone at approximately 16-17 weeks of gestation to update pregnancy information and environmental exposures. In the mid to late second trimester (approximately 22-24 weeks), women will be invited to receive an NCS-sponsored fetal ultrasound to assess fetal growth. The second core face-to-face data collection will occur in a clinical setting in the early third trimester (approximately 28-30 weeks). The clinical setting was chosen because it can help facilitate the collection of a second standardized

assessment of fetal growth by ultrasound and the collection of other biological specimens and physical assessments. There will also be a brief interview, and women will be given instructions for obtaining easily collected environmental samples from the home that will be mailed back to the Study Center. A telephone contact will again be made at about 36 weeks gestation to update delivery information (i.e., due date, hospital).

6.6.2.2 Early Dating Ultrasound

The Study recognizes the importance of obtaining an early ultrasound to date the pregnancy, to pinpoint the timing of exposures with respect to gestational age, and to assess targeted outcomes accurately, such as preterm birth or fetal and infant growth. Preliminary data suggest that between 40-70 percent of pregnant women from the initial NCS Vanguard Sites will receive a first trimester ultrasound. Thus, at the first Study visit during pregnancy, women will be asked if they already had an ultrasound or if they are scheduled for an early ultrasound. If the answer to either is yes, they will be asked to provide the name of the provider and the needed permissions (e.g., consent and Health Insurance Portability and Accountability Act [HIPAA]) for the Study to obtain results of that ultrasound from the provider. For the women who did not receive an early ultrasound as part of routine care, an ultrasound will be scheduled through the Study. This process was chosen both to decrease the mother's burden and the Study's cost.

6.6.2.3 Biological Fathers

Biological fathers will be invited to participate in the Study. During pregnancy, the primary data collection from fathers is at the time of the first trimester home visit. Targeted data collections include biological specimens, interview data, and a brief physical examination. If an enrolled woman does not want to reveal the identity of the biological father or does not want the Study to contact the biological father, the Study will not contact him. In these instances, the pregnant woman (and her child) would still be eligible for participation in the Study. The father does not necessarily need to live in the same home as the mother for initial inclusion in the Study, however, biological fathers or biological mothers who have no contact with the child following birth will not be followed.

Summary of pregnancy visits

- Early first trimester: (home: mother and biological father)
- First trimester (clinic: ultrasound for women without an early clinical ultrasound)
- 16-17 weeks (phone contact: mother)
- 22-24 weeks (clinical visit, ultrasound only: mother)
- 28-30 weeks (clinical visit with ultrasound: mother)
- 36 weeks (phone: mother)

6.6.3 Following Pregnancy

A number of data collections central to the Study occur at the birth location around the time of delivery. These include a brief maternal interview; the collection of biological specimens (e.g., cord blood, placental tissue, and meconium); information about the delivery and the hospital course of the infant as ascertained through abstraction of obstetric and neonatal hospital records; and a baseline neonatal physical and neurodevelopmental assessment. These collections should require at least two visits by Study staff to the place of delivery - one around the time of delivery and a second prior to the infant's discharge. Although the goal is to complete a physical and neurodevelopmental assessment of the child before discharge from the hospital, it is recognized that this will not always be feasible. Thus, if time does not permit assessment in the hospital, a home visit will be made at approximately 1 month after birth.

Following birth, alternating phone and in-person contacts are scheduled every 3 months through age 1 (3-month phone, 6-month in-home visit, 9-month phone, and 1 year in-home visit). After age 1, contacts are every 6 months through age 3 (18, 24 and 30 month phone contacts, and 3-year clinic visit). All contacts include interviews with the primary caregiver to assess both exposures and outcomes of interest. At the 6- and 12-month visits, the primary caregiver and the alternate caregiver (as identified by the primary caregiver) will be interviewed. During each in-person visit, the child will be assessed directly for growth and development and child-parent interactions will be observed. At the home visits, both environmental samples and observational data will be collected. Biological samples will be collected from the child primarily at the 12-month and 3-year visits, although urine will be collected more frequently for measurements of transient environmental exposures. The 3-year clinic visit provides the first opportunity for the measurement of physiologic and physical outcomes (e.g., lung function and body composition) that require larger equipment more easily operated and standardized in a clinical setting. The only biological samples obtained from parents following the birth of the child are salivary samples to measure cortisol as a biomarker of stress, because parental stress is anticipated to have a direct effect on parenting behaviors and child outcomes.

A number of self-administered data collection tools, primarily mail-in questionnaires, will be utilized for more in-depth assessment of some topics than is feasible during the home or clinic visits. Finally, comparable to the data collections during pregnancy, a health visit log for the child will be provided for collection of basic information about clinical visits, including date of visit, type of visit (well child vs. acute), diagnosis, immunizations, etc. Strategies and formats to make the health visit log most valuable to (and thus most utilized by) the participant will be explored during the pilot phase of the Study.

Summary of birth/postnatal visits through 24 months

- Visits around the time of delivery at the place of delivery
- Three months (phone)
- Six months (home)
- Nine months (phone)
- Twelve months (home)
- Eighteen months (phone)
- Twenty-four months (phone)

6.7 Overview of Data Collection for Participant Contacts through 24 Months

This section consists of three tables outlining the contacts between the NCS and Study participants from before pregnancy through the 24-month phone contact. The relations between each participant contact, the relevant data collection modalities for that contact, and the broad domains assessed during that contact are illustrated. Table 6-2 shows the pre-pregnancy and pregnancy contacts for the mother, Table 6-3 outlines the maternal and child contacts from birth through 24 months, and Table 6-4 shows partner contacts.

The appendices include more detailed text and tabular descriptions of the NCS data collection activities. Appendix D describes each of the data collection modalities, and Appendices E through I describe specific exposure and outcome measures as well as potential confounders that are being assessed.

Table 6-2. Prepregnancy and Pregnancy: Maternal Contacts

Visit	Questionnaire and Diary	Biologic Samples	Clinical/Developmental Examination	Environmental Samples
Prepregnancy home visit	Maternal interview Demographics Household composition Medication use Health behaviors Housing characteristics Chemical exposures Product use Occupational exposures Diet	Blood Urine Saliva Vaginal swabs Hair	Anthropometrics Blood pressure	Indoor air House dust
Prepregnancy phone follow-up	Maternal phone interview Diet Chemical exposures	------	-------	-----
First trimester home visit	Maternal interview Demographics* Household composition* Medication use* Health behaviors* Housing characteristics* Chemical exposures* Product use* Occupational exposures* Diet* Medical history Stress and social support Depression	Blood Urine Saliva Vaginal swabs Hair	Anthropometrics Blood pressure Fetal ultrasound (from medical report or clinic visit)	Indoor air House dust Drinking water Soil
Second trimester phone follow-up	Maternal phone interview Major life events Mental health update Medical update Chemical exposures update Housing update	-----	-----	-----
Third trimester clinic visit	Maternal interview Updates on: Demographics Household composition Medication use Health behaviors Housing characteristics Chemical exposures Product use Occupational exposures Diet Medical history Stress and social support Prenatal life events Depression	Blood Urine Saliva Vaginal swabs Hair	Anthropometrics Blood pressure Fetal ultrasound	Indoor air House dust (self-collected and mailed in)

* Updates if in prepregnancy cohort

Table 6-3. Birth through 24 months: Maternal (M) and Child (C) Contacts

Visit	Questionnaire and Diary	Biologic Samples	Clinical/Developmental Examination	Environmental Samples
Birth: At delivery, hospital	Maternal interview Health behaviors (M) Diet (M) Chemical exposures (M) Plans for infant feeding, sleeping, etc.	Blood (M) Urine (M) Cord blood Placenta and cord samples Heel stick (C)	Anthropometrics (C) Dysmorphology and neurologic exam (C) Digital photographs of face and anomalies (C) Chart abstraction (M, C)	-----
3-month phone call	Maternal phone interview Child care Medical update (C)	Breast milk (mailed in at 4-6 weeks)	-------	-----
6-month home visit	Maternal interview Stress and social support Family process and parenting practices Health behaviors (M) Depression and cognition (M) Diet (C) Medical update (C) Medication use (C) Media exposure (C) Child care Chemical exposures Temperament (C)	Urine (C) Hair (C) Saliva (M) Breast milk	Anthropometrics (C) Dysmorphology exam and photos (C) Dermatologic exam (C) Social development observation (M, C)	Indoor air House dust Drinking water Soil Visual assessment of house and neighborhood
9-month phone call	Maternal phone interview Child care Medical update (C) Housing update Chemical and occupational exposures (M, C)	-----	-----	-----

Table 6-3. Birth through 24 months: Maternal (M) and Child (C) Contacts (continued)

Visit	Questionnaire and Diary	Biologic Samples	Clinical/Developmental Examination	Environmental Samples
12-month home visit	Maternal interview Household composition update Family process and parenting practices Health behaviors (M) Diet (C) Medical update (C) Medication use (C) Media exposure (C) Child care Housing update Chemical and occupational exposures (M, C) Language acquisition and social interaction (C)	Blood (C) Urine (C) Hair (C) Saliva (C) Breast milk	Anthropometrics Blood pressure Dermatologic exam Cognitive exam Motor and language assessments Social development observation (child and father, if available)	Indoor air House dust Drinking water Soil Visual assessment of house and neighborhood Noise survey
18-month phone call	Maternal interview Child care Medical update (C) Diet (C) Housing update Chemical and occupational exposures (M, C)	-----	-----	-----
24-month phone call	Maternal interview Child care Medical update (C) Housing update Chemical and occupational exposures (M, C) Life events (M)	-----	-----	Indoor air House dust (self-collected and mailed in)

Table 6-4. Paternal or Partner Contacts

Visit	Questionnaire	Biologic Samples	Clinical/Developmental Examination
First trimester home visit	Partner interview Demographics Household composition Tobacco use Medical history Cognition	Blood Urine Hair	Anthropometrics Blood pressure
6-month home visit	Partner interview Family process and parenting practice Tobacco use Mental health	Saliva	-----
12-month home visit	Partner interview Family process and parenting practice Tobacco use Cognition (if not assessed at first trimester visit)	-----	Social development observation with child

7. SELECTION OF OUTCOME AND EXPOSURE MEASURES

To guide the selection and prioritization of exposure and outcome measures, 28 hypotheses were developed based on the input from multiple federal agencies and scientific experts. The criteria for these core hypotheses were that they be scientifically compelling, have important public health implications, be feasible to test, and clearly justify the need for a prospective birth cohort study of 100,000. These hypotheses can be found in Appendix A. Although it is expected that many other scientific questions will be investigated, the core hypotheses have served as guidelines for prioritization of measures.

Due to the breadth of the National Children's Study, each contact between the participant and NCS personnel must capture information pertinent to multiple exposure and outcome domains. Thus, the length of time a measure takes to administer is an important issue with respect to overall participant burden and retention. Also for these reasons, measures that are generally perceived as invasive or uncomfortable are less likely to be included in the full protocol. Each of the procedures, measurements, and assessments associated with the NCS must meet the criteria for "minimal risk" as defined in the Code of Federal Regulations [§45 CFR 46.102(i)]. In addition, the NCS is committed to minimizing even minimal risks.

7.1 Exposures, Outcomes, Mediators, and Confounders

To some degree, the categorization of certain measurements as an outcome, an exposure, a potential mediator, or a confounder is arbitrary because a factor that is an exposure in one hypothesis may be a mediator, confounder, or outcome for another. For example, childhood obesity can be considered an outcome related to fetal growth and maternal glucose tolerance and also a risk factor for subsequent development of diabetes or cardiovascular disease. For consistency, this document categorizes outcomes and exposures as they are presented in the NCS hypotheses while acknowledging the fluidity inherent in many of the areas. For a more in-depth discussion of mediators and confounders see the Statistical Analysis Plan (Chapter 10).

7.2 Overview of Measures

As described previously, the NCS will engage in a continual process of planning the protocol measures to ensure they reflect the best science and technology available. Protocol development will continue as the children age and new scientific data become available. Specific measurements for each new wave of data collection will begin approximately two years before the measures are needed in the field.

Assessment of exposures and outcomes will utilize tools suitable for a large-scale, longitudinal, multi-site, geographically dispersed epidemiologic study. In general, measurements used successfully in other large studies of child health are most likely to be included in the NCS because they have a record of past performance and will facilitate the comparison of NCS results to those from other studies. However, some novel or less frequently used tools may provide important high-quality data and are included where appropriate for the entire cohort, while others may be more suitable for focused adjunct studies.

7.2.1 Organization of Rationale for Measures Chapters and Appendices

The rationale for outcome and exposure measurement strategies is presented in Chapters 8 and 9. The domains of exposures and outcomes described in these chapters extend from birth to age 21. There is more specificity, however, relating to assessments through infancy since protocol development is still ongoing. These chapters are supported by Appendices E through I, which contain detailed information about the domains of measurement at each participant contact through child age 24 months.

Chapter 8 describes the rationale for measurement strategies with regard to each of the seven priority outcome areas: pregnancy; neurodevelopment and behavior; child health and development; asthma; obesity and growth; injury; and reproductive development.

Chapter 9 describes the rationale for measurement strategies with regard to chemical exposures, physical exposures and environment, psychosocial environment, biological exposures, and genetics.

7.2.2 Overview of Measures Related to Specific Hypotheses

As mentioned previously, the NCS core hypotheses served as guidelines for the selection of outcome and exposure measurement domains. Consequently, in conceptualizing the relation between the outcome and the exposure measurement domains, it is helpful to think about their connections within these core hypotheses. Table 7-1 presents the 28 core hypotheses across the top of the table organized by priority outcome area. Domains of exposures and covariates are listed down the side. For each hypothesis, the exposures and covariates central to that hypothesis are indicated in the table.

Table 7-1. Measures by Hypotheses

	Pregnancy Outcomes				Neurodevelopment and Behavior				Child Health and Development					Asthma						Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Influences on Healthy Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microorganisms Decreases the Risk of Asthma	Environmental Exposure and Gene Interaction and Asthma and Wheezing in Children	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Questionnaire - Mother																												
HH Composition and Demographics		C	C		C		C	C	N	C	C	C		C	C		C	C	N	C	C	C	C		C	N	C
Parental Stress		C				C	N		N	N			N	N												N	N	
Maternal Exhaustion *Prop							C							C												C		
Social Support							N		C				N	C			C									C		
Family Process							N		N	C			N				N					C			N	N	C	
Health Behaviors	C	C					C				C		N	C	C					C	C	C	C			C		C
Diet and Toxicants through food					N											N		N		C	C	N	N	N				C
Media Use									C		N	C																
Maternal Mental Health & Cognition					C		N		N		C	C	N													N	N	
Parenting Style							N		N		C		N													N	N	
Maternal / Paternal Attachment					N		N		N			N	N															
Child Care									N	N	N	N	N															
Neighborhood									C	N		C															N	
Public Policy													N														N	
Housing Characteristics				C	C	C	C	C		C					N	C	C	C	C			C	C		C			C
Occupation / Hobbies				N	N	C	C	C						N	N	C	N	N	N			C	C	N	C			N
Appliance and Product Use				N	N	C	C	C							N	C	N	N	N			C	C		C			N
Use of medicines	C					N	C	N								C		C				C	C			C		
Time and Activity (mother / resident father)				N	N	C	C	C			C				N	C	N		N	C	C	C	C		C			N
Family Environment (observation only)							N		N				N													N	C	
Brief Medical History	N	N	N		C	N		N	N	N	N	C		N	N	N	N	N	N	N	N	N	N	N	N	N	N	C
Child Language Development					N		N		N			N	N															
Child Temperament / Emotional Regulation							N		N				N													N	C	
Socio-Emotional Functioning					N				N	N	N	N	N															
Social Competence / Behavior Problems													N														C	
Child Autism Screening					N		N		N																	N		
Adaptive Behavior							N		C																	N		
Questionnaire - Child																												
Neonatal Neuro-behavior	N		N		N	N				N			N															
General Cognitive Ability					N				N	N	N	N	N															
General Motor Development					N				N	N	N	N	N														N	
Cognitive Processes					N	N			N	N	N	N													N	N	N	
Language Development					N				N	N	N	N	N														N	
Infant Emotion					N		N		N																	N	N	
Parent-Child Interaction							N		N				N													N	N	
Attachment Status					N				N	N	N	N	N														N	
Sensory Function					N				N	N	N	N																
Questionnaire - Father																												
Demographics									N	C		C															N	
Paternal Mental Health and Cognition					C		N		N		C	C	N													N	N	
Social Support							N		C				N	C			C									C		
Experiences with Target Child																												

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes					Neurodevelopment and Behavior				Child Health and Development					Asthma						Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Influences on Healthy Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microoganisms Decreases the Risk of Asthma	Environmental Exposure and Gene Interaction and Asthma and Wheezing in Children	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Questionnaire - Father continued																													
Parenting Style							N		N		C		N														N	N	
Paternal Attachment					N		N		N			N	N																
Child Care									N	N	N	N																	
Tobacco Product Use, Alcohol Use, Illicit Drug Use and Prescription Drug Abuse (5.10)																													
Use of Medicines and Alternative Medicines																													
Brief Medical History																												N	
Occupation / Hobbies				N	N										N		N	N	N					N				N
Family Process							N		N	C		C	N				N					C				N	N		
**Subdomains are shown if they are a confounder/covariate only.																													
Environmental																													
Indoor air																													
PM2.5 - ETS, Pb, Cd, Mn	C	C	C		C	C									N	C	N		N										N
PM10 - PAHs, pesticides	C	C	C	N	N	C	N,C	C							N		N		N							C			
VOCs					C	C		C							N				N							C			
Aldehydes & Ketones							C								N	C	C		N										
NO2															N	C	N		N										
Hg				N	C	C	C	C																	C	C			
O3															N	C	N		N										
CO					C										N	C	N		N										
House dust																													
Allergens, endotoxin															N	C	N		N										
Mold															N	C	N		N										
Metals - Pb, Cd, Mn, As					C	C	C																						N
Pesticides: OPs, Carbamates, Pyrethroids				N	N	C	C	C																		C			
Pesticides: OCs				N		C	C	C																		C			
Drinking water																													
Disinfection Byproducts (DBPs) - HAA9	C	C	C	C				C																					
VOCs	C	C	C		N,C	N,C	N,C	C							N											C			
Metals - Pb, Cd, As			C	C	C	C	C	C																		C			N
Nitrate	N,C	N,C	N,C	N,C																									
Perchlorate				N																									N
Pesticides: OPs, Carbamates, Pyrethroids				N	N	C	C	C																		C			
Pesticides: Atrazine																													N
Pesticides: OCs				N																									
Soil																													
Metals - Pb, Cd, Mn, As						C	C																						N
Pesticides - OPs, Carbamates, Pyrethroids				N	N	C	C	C																		C			
Near CCA treated wood - Cr+6 (as total), As					N	C	C	C																		C			
Visual assessment		C		N	N,C	C		C							N,C	C	N,C			C	C					C			N
Noise survey														C															
Indoor/Outdoor measurements																													

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes					Neurodevelopment and Behavior				Child Health and Development					Asthma						Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Influences on Healthy Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microoganisms Decreases the Risk of Asthma	Environmental Exposure and Gene Interaction and Asthma and Wheezing in Children	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance fom Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Aggression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Environmental - Continued																													
Community air																													
PM2.5								C							N	C	N			N									N
PM10 - PAHs				N	N	C	C	C							C											C			
O_3															N	C	N			N									
NO_x, SO_2															N	C	N			N									
Pollen (non-NCS data collection)										C				C	N		N			N									
Outdoor water samples at homes	C	C	C	N	N	C		C							C				N						C	C			N
Community water systems (non-NCS data collection)																													
Hg				N	C	C	C	C																	C	C			
Perchlorate				N																									N
Pesticides				N	N	C	C	C																		C			N
Nitrate	C	C	C																										
Physical Exam - Mother																													
Anthropometric									C	C	C										C	C	C	C					C
Ultrasound			N																		N	N							
Physical Exam - Father																													
Anthropometric									C	C	C										C	C	C	C					C
Blood Pressure									C	C																			
Physical Exam - Child																													
Anthropometric									N	N	N										N	N	N						
Blood Pressure									N	N																			
Dysmorphology/Physical Exam	N		N							N																			
Physical Activity									C		C										C	C		C					
Biospecimen Collection - Mother																													
Blood																													
Endocrine Panel																													
Cortisol	C						C	N						N												C			
Cortisone	C						C	N						N												C			
Corticotropin releasing hormone (check volume)	C						C	N						N												C			
Cortisol binding globulin	C						C	N						N												C			
CRH binding protein	C						C	N						N												C			
Reproductive																													
Estriol	C						C	N						N												C			
Estradiol	C						C	N						N												C			
Progesterone	C						C	N						N												C			
Infection/Inflammation/Biological																													
CBC (WBC, RBC, Hgb, Hct, MCV,MCH, MCHc, RDW, Plt, MPV, (NE, LY, MO, EO, BA % and #))		N			N	N		N							N			N	N										
cytokines/interleukins		N				N		N										N	N										
Ig types and subtypes and org specific		N				N		N											N										
Rubella (IgM antibody)		N			C	N		N										N											

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes				Neurodevelopment and Behavior				Child Health and Development					Asthma						Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Influences on Healthy Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microorganisms Decreases the Risk of Asthma	Environmental Exposure and Gene Interaction and Asthma and Wheezing in Children	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Biospecimen Collection - Mother (continued)																												
Syphilis (Ig)		N			C	N		N										N										
Varicella (Ig)		N			C	N		N										N										
Herpes Simplex 1&2 (Ig)		N			C	N		N										N										
Hepatitis Profile (a and b) *Medical Records* (Ig)		N			C	N		N										N										
Toxoplasmosis (toxoplasma gondii) (Ig)		N			C	N		N										N										
IgE (cat, dog, cockroach, dust mite, fungi, mouse/rat urine)		N				N		N							N			N	N									
CRP		N			C	N		N										N										
Heat Shock proteins		N			C	N		N										N										
Homocysteine and folate (fasting?) serum or red cells (prenatal vitamin influence)		N			C	N		N								N		N										
Cells T-cell subsets for Th-type *Prop		N			C	N		N										N										
thyroid (TSH and free t4)				N																								
Fasting N3-N6 Fatty Acids *Prop																N												
Antioxidant (vit A/E/Carotenoids)																N												
Vitamin C																N												
%carb deficient transferrin (alcohol)	C	C																								C		C
Glucose Metabolism																												
Fasting C-peptide *Prop	N																			N								
Fasting Glucose	N																			N								
HgbA1C	N																			N								
Insulin like Growth Factor *Prop	N																			N								
Fasting Insulin	N																			N								
Fasting lipids (included in chemical volume)	N																			N								
Genetic Tests																												
DNA, DNA & protein adducts for Exposure Assessment (Chemical Changes)					N														N									
Gene Expression (RNA)					N														N								N	
Epigenetic changes (genomic DNA)					N														N									
Genetic Variation: Paraoxonase Gene, glucokinase, vntr insulin, etc. (DNA)	N				N	C		C											N	N	N	C	C	C		N		
Cryopreserved PBMCs	N				N	C		C											N	N	N	C	C	C		N		
Cell lines *Prop	N				N	C		C												N	N	C	C	C		N		
mitochondrial DNA *Prop	N				N	C		C												N	N	C	C	C		N		
Chemical Exposures																												
lipids, PCBs, organochlorine pesiticides, PBDE, Perfluorinated cmpds(PFOA,PFOS) (4 mL Serum)				N	C																							N
Lead, Mercury, Cadmium (3 mL bld)				N	C																					C		N
Combination of dioxins/furans and all other chemicals (excluding metals)				N	C																							N
Stored Samples																												

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Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes				Neurodevelopment and Behavior				Child Health and Development					Asthma						Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Influences on Healthy Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microorganisms Decreases the Risk of Asthma	Environmental Exposure and Gene Interaction and Asthma and Wheezing in Children	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Aggression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Biospecimen Collection - Mother (continued)																													
Serum	N	N		N	N	N		N						N	N	N		N	N	N								N
Plasma	N	N		N	N	N		N						N	N	N		N	N	N								N
RBCs (folate and fatty acids)	N	N		N	N	N		N						N	N	N		N	N	N								N
Lavender Top	N	N		N	N	N		N						N	N	N		N	N	N								N
Buccal Cells *Prop																													
Nails																													
Organic Hg (ethyl, methyl)				N	C																								N
Hg inorganic				N	C																								N
Hair																													
Cd				N	C																								N
cotinine	C	C		N	N		C							C	N	C											C		C
Hg inorganic				N	C																								N
Organic Hg (ethyl, methyl)				N	C																								N
nicotine	C	C		N	N		C							C	N	C											C	C	C
Saliva																													
Cortisol	C						C	N						N													C		
storage	C						C	N						N													C		
Breast Milk																													
Antioxidants : Vit C/E/ Beta Carotene																N						N							
Component: lipid, proteins, carbohydrates, enzymes, immunoglobulins, minerals, vitamins, cytokines, hormones																N						N							
Chemical Exposures																													
Dioxins/furans; Organochlorine Pesticides; PCBs					C																				N				N
Pesticides					N																				N				N
PBDE (frozen) flame retardant					C																				N				N
Perchlorate					C																				N				N
Metals: Manganese and others					C																				N				N
phytoestrogens					N																				N				N
MTBE methyl tertiary butyl ether (fuel additive)					N																				N				N
Bisphenol A					N																				N				N
Urine																													
Illicit Drug Panel	C	C																									C		C
alcohol marker *Prop	C	C																									C		C
Antioxidants																N													
Catecholamine	C						C							N													C		
Cortisol	C						C	N						N													C		
Asymptomatic bacteriuria		N				N		N										N											
Fertility Monitor																													
Pregnancy Test Kit																													
PCR for identification of specific organisms *Prop		N				N		N										N											
Chemical Exposures																													

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Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes					Neurodevelopment and Behavior				Child Health and Development					Asthma						Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Influences on Healthy Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microorganisms Decreases the Risk of Asthma	Environmental Exposure and Gene Interaction and Asthma and Wheezing in Children	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Biospecimen Collection - Mother continued																													
PFBS, Creatinine, alkyl phenols (Bisphenol A, nonylphenol), Hg(inorganic), As(speciated), perchlorate, halogenated phenols(PCP), phthalates, atrazine, OPs, carbamates, pyrethroids, EBDC/ETU, Cadmium				N	N																								N
PAH (may be storage issues); requires separate analysis (3-4 ml)				N	N										N														N
ICP/MS urine screen *Prop				N	C																								N
cotinine	C	C		N	N		C							C	N	C											C		C
Phytoestrogens				N	N											N													N
Storage	C	N		N	N	N	C	N						N	N	N		N									C		N
Vaginal Swabs																													
Chlamydia		N				N		N										N											
Bacterial Vaginosis		N				N		N										N											
Cultures antibodies		N				N		N										N											
Cultures cytokines		N				N		N										N											
Cultures metalloproteinase		N				N		N										N											
Group B Strep		N				N		N										N											
Gonorrhea		N				N		N										N											
Placenta																													
Cultures antibodies and cytokines		N				N		N										N											
Pathology		N				N		N																					
Chemical Contaminants				N	N										N														N
Umbilical Cord																													
Cultures antibodies and cytokines		N				N		N										N											
Pathology		N				N		N																					
Chemical Contaminants				N	N										N														N
Biospecimen Collection - Child																													
Blood (NOTE: Because of the limited blood draw volume for the child, samples will be stored for future analysis. Final protocol for analytes will be decided in the future.)																													
Infection/Inflammation/Biological																													
CBC (WBC, RBC, Hgb, Hct, MCV,MCH, MCHc, RDW, Plt, MPV, (NE, LY, MO, EO, BA % and #))		N			N	N		N		N				N	N		N	N	N					N				N
Chemical Exposures																													
lipids, PCBs, organochlorine pesiticides, PBDE, Perfluorinated cmpds(PFOA,PFOS) (4 mL Serum)				N	C																				N				N
Stored Samples																													
Serum	N	N		N	N	N	N	N	N	N				N	N	N	N	N	N	N	N	N	N	N		N		N
Lavender Top	N	N		N	N	N	N	N	N	N				N	N	N	N	N	N	N	N	N	N	N		N		N
Guthrie Card at birth	N	N		N	N	N	N	N	N	N				N	N	N	N	N	N	N	N	N	N	N		N		N
Buccal Cells *Prop																													
Hair																													
Cd				N	C																				N				N
cotinine	C	C		N	N		C							C	N	C									N		C		C

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes					Neurodevelopment and Behavior				Child Health and Development					Asthma						Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Influences on Healthy Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microorganisms Decreases the Risk of Asthma	Environmental Exposure and Gene Interaction and Asthma and Wheezing in Children	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Biospecimen Collection - Child continued																													
Hg inorganic				N	C																				N				N
Organic Hg (ethyl, methyl)				N	C																				N				N
nicotine	C	C		N	N		C							C	N	C			N						N		C	C	C
Saliva																													
Cortisol	C						N							N						C	C	C	C	N		N		C
storage	C						N							N						C	C	C	C	N		N		C
Urine																													
Chemical Exposures																													
PFBS, Creatinine, alkyl phenols (Bisphenol A, nonylphenol), Hg(inorganic), As(speciated), perchlorate, halogenated phenols(PCP), phthalates, atrazine, OPs, carbamates, pyrethroids, EBDC/ETU, Cadmium				N	N								C		N				N						N				N
PAH (may be storage issues); requires separate analysis (3-4 ml)				N	N										N				N						N				N
ICP/MS urine screen *Prop				N	C										N										N				N
cotinine	C	C		N	N		C							C	N	C									N		C		C
Phytoestrogens				N	N											N						C	C		N				N
Storage	C	C		N	N	N	C							C	N	N	N	N		N	N	C	C		N		C		N
Meconium																													
Cotinine	C	C		N	N		C							C	N	C									N		C		C
Organophosphate Metabolites				N	N																				N				N
Cord Blood																													
Cortisol	C						N							N						C	C	C	C	N		N		C
Cortisone	C						N							N						C	C	C	C	N		N		C
Corticotropin releasing hormone	C						N							N						C	C	C	C	N		N		C
Cortisol binding globulin	C						N							N						C	C	C	C	N		N		C
CRH binding protein	C						N							N						C	C	C	C	N		N		C
Reproductive																													
Estriol	C						N							N						C	C	C	C	N		N		C
Estradiol	C						N							N						C	C	C	C	N		N		C
Progesterone	C						N							N						C	C	C	C	N		N		C
Infection/Inflammation/Biological																													
CBC (WBC, RBC, Hgb, Hct, MCV,MCH, MCHc, RDW, Plt, MPV, (NE, LY, MO, EO, BA % and #))		N		N	N	N		N		N				N	N		N	N						N				N
cytokines/interleukins		N				N		N		N				N	N		N	N						N				
IgE (cat, dog, cockroach, dust mite, fungi, mouse/rat urine)		N				N		N	N	N				N	N	N	N	N										
Ig types and subtypes		N				N		N	N	N				N	N	N	N	N										
N3-N6 Fatty Acids								C								N				C	C	C	C						
Antioxidant (vit A/E/Carotenoids)								C								N				C	C	C	C						
Vitamin C								C								N				C	C	C	C						
Lymphocyte Subsets Th status (processing difficult)																													
Glucose Metabolism																													
Glucose	N								N	N										N	N	N	N	N					
HgbA1C	N								N	N										N	N	N	N	N					
Insulin like Growth Factor	N								N	N										N	N	N	N	N					

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Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes				Neurodevelopment and Behavior				Child Health and Development					Asthma						Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Influences on Healthy Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microoganisms Decreases the Risk of Asthma	Environmental Exposure and Gene Interaction and Asthma and Wheezing in Children	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Biospecimen Collection - Child continued																												
Insulin	N								N	N										N	N	N	N	N				
lipids (included in chemical volume)	N								N	N										N	N	N	N	N				
Genetic Tests																												
DNA, DNA & protein adducts for Exposure Assessment (Chemical Changes)					N								N											N				
Gene Expression (RNA)					N		N						N									C	C	N		N	N	
Epigenetic changes (genomic DNA)					N		N															C	C	N		N		
Genetic Variation: Paraoxonase Gene, glucokinase, vntr insulin, etc. (DNA)	N				N	C	N	C												N	N	C	C	N		N		
mitochondrial DNA *Prop	N				N	C	N	C												N	N	C	C	N		N		
Chemical Exposures																												
lipids, PCBs, organochlorine pesiticides, PBDE, Perfluorinated cmpds (4 mL Serum)				N	C								C											N				N
Lead, Mercury, Cadmium (3 mL bld)				N	C								C											N		C		N
Stored Samples																												
Serum	N	N		N	N	N	N	N	N	N				N	N	N	N	N	N	N	N	N	N	N		N		N
Plasma	N	N		N	N	N	N	N	N	N				N	N	N	N	N	N	N	N	N	N	N		N		N
RBCs	N	N		N	N	N	N	N	N	N				N	N	N	N	N	N	N	N	N	N	N		N		N
Guthrie Card	N	N		N	N	N	N	N	N	N				N	N	N	N	N	N	N	N	N	N	N		N		N
Lavender Top	N	N		N	N	N	N	N	N	N				N	N	N	N	N	N	N	N	N	N	N		N		N
Whole blood storage PCR	N	N		N	N	N	N	N	N	N				N	N	N	N	N	N	N	N	N	N	N		N		N
Biospecimen Collection - Father																												
Blood																												
Genetic Tests																			N									
DNA, DNA & protein adducts for Exposure Assessment (Chemical Changes)													N/C						N									
Gene Expression (RNA)													N/C						N								N	
Epigenetic changes (genomic DNA)																			N									
Genetic Variation: Paraoxonase Gene, glucokinase, vntr insulin, etc. (DNA)	N				N	C		C											N	N	N	C	C	C		N		
Cryopreserved PBMCs	N				N	C		C											N	N	N	C	C	C		N		
Cell lines *Prop	N				N	C		C												N	N	C	C	C		N		
mitochondrial DNA *Prop	N				N	C		C												N	N	C	C	C		N		
Chemical Exposures																												
lipids, PCBs, organochlorine pesiticides, PBDE, Perfluorinated cmpds(PFOA,PFOS) (4 mL Serum)					C																							
Lead, Mercury, Cadmium (3 mL bld)					C																							
Combination of dioxins/furans and all other chemicals (excluding metals)					C																							
Stored Samples																												
Serum					N	N		N																		N		
Plasma					N	N		N																		N		

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes				Neurodevelopment and Behavior				Child Health and Development					Asthma						Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Influences on Healthy Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microorganisms Decreases the Risk of Asthma	Environmental Exposure and Gene Interaction and Asthma and Wheezing in Children	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Biospecimen Collection - Father continued																												
RBCs (folate and fatty acids)					N	N		N																		N		
Lavender Top					N	N		N																		N		
Buccal Cells *Prop																												
Nails																												
Organic Hg (ethyl, methyl)					C																							
Hg inorganic					C																							
Hair																												
Cd					C																							
cotinine					N																							
Hg inorganic					C																							
Organic Hg (ethyl, methyl)					C																							
nicotine					N																						C	
Saliva																												
Cortisol	C						C							N												C		
storage	C						C							N												C		
Urine																												
Chemical Exposures																												
PFBS, Creatinine, alkyl phenols (Bisphenol A, nonylphenol), Hg(inorganic), As(speciated), perchlorate, halogenated phenols(PCP), phthalates, atrazine, OPs, carbamates, pyrethroids, EBDC/ETU, Cadmium					N																							
PAH (may be storage issues); requires separate analysis (3-4 ml)					N																							
ICP/MS urine screen *Prop					C																							
cotinine					N		C																					
Phytoestrogens					N																							
Storage					N		N																					
Semen																												
Quality (home collection)																												

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

8. RATIONALE FOR OUTCOME MEASURES

The rationale for outcome measures is divided into subsections, one for each of the seven National Children's Study priority outcome areas. The measures outlined in these subsections will allow the NCS to address important hypotheses relevant to these outcome areas. The broad array of data to be collected by the NCS, however, will also enable the examination of countless additional health and developmental outcomes. Some of the outcomes comprise more or less definitive diagnoses, such as preterm birth or congenital anomalies. Others entail the initial assessments of quantitative trajectories that will be measured throughout the Study, such as cognitive development, behavior, or body composition.

8.1 Pregnancy Outcomes

8.1.1 Definition

Proximal outcomes of pregnancy will be the first measures of child health captured by the NCS. Preterm birth and structural congenital anomalies are of specific interest because of the immediate morbidity and mortality associated with those conditions and the potential persistence of associated morbidity and disability throughout life. In addition, growing evidence suggests that deviation from normal fetal growth trajectories, even if not associated with perinatal complications, may be related to cardiovascular or other chronic conditions later in life (Barker, 1994). Preterm birth is generally defined as birth prior to 37 completed weeks of gestation (calculated as the time from the start of the last menstrual period to the time of birth). A structural birth defect is generally defined as malformation of an organ or structure that is present at birth and adversely affects health and development. In addition to documenting major structural anomalies (e.g., neural tube defects, facial clefts, cardiac defects), the NCS also will attempt to measure subtle variations in morphogenesis that may be related to periconceptional chemical or metabolic exposures and later neurodevelopment.

8.1.2 Assessment of Gestational Age and Fetal Growth

While the majority of preterm births occur close to term and are at relatively low risk for severe morbidity, the relatively small proportion of births at the lower end of the viable gestational age range (down to approximately 24 weeks) are at greatly elevated risk for mortality and long-term morbidity. In addition, the quality of fetal growth assessment, either by growth parameters obtained in utero via ultrasound or by using birth weight and anthropometric measures obtained at birth, is dependent on accurate knowledge of gestational age. Thus accurate ascertainment of gestational age is important not only to identify degree of preterm birth, but also to collect accurate fetal growth measures that may be independent variables of interest for many child outcomes throughout the course of the NCS.

In a clinical setting, a fetus's gestational age is based on the date of the last menstrual period. An increasing proportion of women receive a first trimester ultrasound (generally between 8-12 weeks gestation) that also is used to estimate gestational age. These estimates are commonly based on crown-rump length (Hadlock, Shah, Kanon, & Lindsay, 1992), though algorithms using other measures, such as biparietal diameter, are also used.

Measures of gestational age

In the NCS, data for estimation of gestational age will come from a variety of sources. Among all women, questionnaire data will ascertain date of last menstrual period and whether a first trimester ultrasound was or will be obtained. If so, a report of that scan will be collected and used as a basis for gestational age. If a woman does not receive an early clinical scan, then she will be scheduled for one under the auspices of the NCS. Among women enrolled prior to pregnancy, the use of frequent pregnancy tests may provide an additional, potentially accurate, estimate of date of conception. For all women, date of birth should be directly available since collection of maternal and infant samples at birth is a focus of the NCS. In situations where the birth was missed by the NCS, retrospective interview data and review of the labor and delivery and neonatal charts will be used to ascertain birth date and other perinatal information.

Reconciliation of multiple, sometimes discordant, measures of duration of pregnancy is often neither simple nor straightforward. The various measures are based on three conceptually different constructs (Alexander & Allen, 1996): time (days from menstruation or ovulation to birth), size (sonography), or maturity (newborn examinations such as those of Ballard et al., 1991). Menstrual dating is the traditional gold standard, and all other measures were originally validated among women with well-characterized menstrual dates. Especially on the aggregate level, however, uncritical acceptance of menstrual dates leads to gestational age estimates that may be implausible, manifested particularly as a bimodal distribution of birth weight at early gestational ages and a biologically implausible number of pregnancies continuing considerably beyond the expected 280 days (David, 1980). When sonography was compared directly to menstrual dating, it was found that a menstrual age of less than 37 completed weeks was in agreement with sonography in only 78 percent of cases, and a menstrual age of greater than 42 weeks agreed with sonography in only 11 percent of cases.

Uncritical acceptance of sonography, however, could gloss over subtle differences in growth that may be of etiologic interest. The fundamental assumption underlying sonographic estimation of gestational age is that inter-individual differences in growth are nonexistent early in pregnancy. Recent research has suggested that this assumption is not tenable. For example, compared to menstrual dating, gestational age estimated from the fetal biparietal diameter consistently underestimated the gestational duration of girls compared to boys, and of fetuses of mothers who smoked compared to nonsmokers (Henrihsen, Wilcox, Hedegaard, & Jorgen Secher, 1995; Morin et al., 2005). This suggests that even in the first half of pregnancy, known influences on fetal growth are operative and can impact measurement of gestational age. This phenomenon has recently been reported in first-trimester sonography among pregnancies with a known date of conception (due to in-vitro fertilization). In that study (Bukowski et al., 2007) a fetus whose sonographic estimate of gestation was even one day greater than the known time of conception was less likely to be undergrown, or even preterm, at birth. This suggests that growth differences are present even in the first trimester, and may be of etiologic significance.

For these reasons, the NCS will not have a single "study gestational age." Data will be collected on menstrual history, sonography, and other clinical measures of duration of pregnancy, and individual researchers will be free to explore these intriguing differences further.

Measures of fetal growth

Assessments of fetal growth are based on relative size for a given gestational age. In the NCS, linear measures of growth including biparietal diameter, abdominal circumference, and femur length will be obtained via standardized ultrasounds at approximately 22-24 weeks and 28-30 weeks of gestation. These repeated measures, as well as others obtained from the newborn infant, will enable true

growth rates to be calculated and may enable the NCS to distinguish slow growth in the first half of pregnancy from slow growth occurring later. In addition, though not a routine clinical measure, mid-thigh lean and fat mass circumferences will be obtained. Although it would be ideal to acquire additional growth measures, cost and participant burden constraints will not allow additional study-related visits for standardized ultrasounds. However, if additional clinical scans are performed, relevant growth data from those scans will be collected. At birth, birth weight, length, head and abdominal circumferences, and triceps and subscapular skin folds will be measured on each infant. Birth weight may be compared to external size-for-gestational age standards, such as those of Alexander, Himes, Kaufman, Mor, and Kogan (1996), Zhang and Bowes (1995) or Kramer et al. (2001). Long-term impacts of size at birth may not, however, be limited merely to the smallest percentiles of size-for-dates, but rather may operate continuously across of broad spectrum of relative size (Innes et al., 2003), and therefore the complete continuum of size will be evaluated on the NCS.

8.1.3 Assessment of Congenital Anomalies

Clinical management of pregnancy offers the opportunity for congenital anomaly assessment of several types and at various stages, depending on maternal and familial risk factors. Women more than 35 years old or with a relevant genetic history will generally be offered chorionic villus sampling at 10-12 weeks gestation or, more commonly, amniocentesis around 16 weeks. Early ultrasounds primarily used for confirming gestational age can also be used to ascertain nuchal fold thickness, if performed between 10 and 14 weeks gestation; increased nuchal translucency is associated with increased risk of Down Syndrome, other chromosomal abnormalities, and some cardiac defects. Serum triple or quad screen is generally performed between 16-18 weeks of pregnancy to assess risk for neural tube defects or Down Syndrome. A fetus may also receive one or more anatomic surveys by ultrasound, depending on maternal risk factors and perceived fetal growth. Though commonly performed around the 20th week of pregnancy, anatomic scans are also obtained both earlier and later to assess for structural defects.

Case definition and ascertainment

The NCS will rely primarily on recording clinical diagnosis of major structural anomalies, rather than performing specific diagnostic tests as part of the Study protocol. NCS involvement in diagnosis of anomalies may be confusing to a pregnant woman, who is receiving principal medical care and advice from her clinician. In addition, the NCS is not in a position to provide the necessary counseling and follow-up if tests show positive or even equivocal results. Although the NCS ultrasounds are focused on measuring fetal growth, procedures for referral and follow-up, if abnormalities are noted, will be included in the manual of operations.

The prevalence of congenital anomalies depends heavily on the period of ascertainment, prenatal diagnosis, elective terminations, and the data sources reviewed. For example, the percentage of structural defects diagnosed by ultrasonography increases by the trimester of pregnancy (Withlow, Chatzipapas, Lazanakis, Kadir, & Economides, 1999). In addition, a pregnancy may be terminated before the time of viability because of a serious or lethal defect, and among spontaneous pregnancy losses and fetal deaths the prevalence of structural malformations declines as pregnancy progresses (Dimmick & Kalousek, 1992). Thus, although the NCS will rely primarily on existing clinical diagnosis, records from every spontaneous abortion, stillbirth, and elective termination will be obtained whenever possible to determine the presence of a structural defect in the fetus. Should a Study- or clinically-obtained sonogram detect an anomaly and the pregnancy not be terminated, birth records will be abstracted to determine the ultimate diagnosis; terminations because of defects detected sonographically will be confirmed by review of medical and/or pathology records whenever possible. Since the vast majority of pregnancies in the

NCS will have been ascertained in the first trimester, such complete surveillance will be technically feasible.

Review of the maternal and infant charts at birth, as well as maternal questionnaires during pregnancy, will be used to ascertain prenatal diagnosis of congenital anomalies. After birth, a standardized observational infant exam will record any major anomalies. Selected morphologic measurements, such as intercanthal distances and anogenital distance, also will be made at that exam. A standardized digital facial photograph will be taken at birth and stored for later analysis. Digital photographs of external anomalies will also be taken. Questionnaire data regarding medical diagnoses and information recorded in the child's health care visit log after birth will be used to identify birth defects not recorded at the birth visit. The accuracy of questionnaire reports of birth defects, however, has been called into question (Rasmussen, Mulinare, Khoury, & Maloney, 1990; Romitti, Burns, & Murray, 1997). Therefore, potentially major birth defects identified through questionnaire will be further investigated. To the extent possible, review of extant medical data, including physical examinations, operative notes, autopsy records, cytogenetic and metabolic studies, and/or imaging studies, will be used to identify and characterize major congenital anomalies accurately. Furthermore, a considerable fraction of all defects are not apparent at birth but only become known over time. This is particularly the case for defects of the internal organs, such as the heart and kidneys. Parents/guardians of all Study children will be interviewed on multiple occasions during the first 5 years of life and on each occasion will be asked about any diagnosis of birth defects as well as any operations or significant medical procedures. If these screenings suggest the presence of any defect, relevant records will be obtained for confirmation whenever possible, following procedures similar to those used by active birth defects surveillance programs (Correa et al., 2007).

Case classification

Researchers studying birth defects recognize that application of current knowledge of embryology and pathophysiology is essential when classifying these conditions. It is inappropriate simply to classify defects as any versus none, or even by organ system (e.g., all heart defects, all limb defects, etc.). Such classification ignores the etiologic heterogeneity present in these defects. Furthermore, researchers distinguish between infants with an isolated defect, a known malformation syndrome, a sequence (i.e., multiple defects that are the result of a single primary defect), or other complex sets of defects. Etiology can reflect malformations (i.e., a localized error in morphogenesis), deformations (alteration of an otherwise normally developing structure, usually by mechanical forces), disruptions (destruction of a normally formed structure, usually by vascular, mechanical, or infectious insults) or dysplasias (lack of normal organization of cells into tissues).

Distinguishing between these subtleties requires a thorough review of all available information on each child in order to classify defects in an etiologically homogeneous manner. As noted above, the information collected by the NCS will enable detailed, specific review of the information on each case by a group of experts in the relevant field, for example, a pediatric cardiologist, a clinical geneticist, etc. While such review may not necessarily be done in "real time," appropriate data will be collected to enable future research to make classifications based on the most current science.

8.1.4 Assessment of Other Pregnancy Outcomes

Information on the occurrence of miscarriage or stillbirth will be ascertained via maternal questionnaire. At each regular contact with an enrolled pregnant woman, the woman will be asked how the pregnancy is progressing. If the woman indicates she is no longer pregnant, she will be asked for

further information regarding when the loss occurred. When possible, additional diagnostic information, including evidence of chromosomal malformations or birth defects, will be obtained from medical or post-mortem records. A standardized procedure for examination of stillbirths similar to that used by the NICHD Stillbirth Collaborative Research Network may be possible in some Study sites, but will be difficult to implement universally due to the number and diversity of medical care systems involved in the NCS.

8.1.5 Assessment of Related Factors

Experiences during pregnancy, particularly maternal medical status, have been linked to adverse pregnancy outcomes. Suboptimal thyroid function in pregnancy is associated with risk for preterm birth. Impaired glucose metabolism during pregnancy is associated with a variety to congenital anomalies, including malformations of the heart, central nervous system, and musculoskeletal system. Maternal infection, and thus fetal exposure to mediators of inflammation due to maternal infection, has also been implicated in preterm birth. The NCS will assess maternal medical status and other maternal exposures repeatedly during pregnancy.

8.2 Neurodevelopment and Behavior

8.2.1 Definition

Children's achievement of age-normative levels of developmental functioning, and non-normative deviations from those developmental milestones, will be of great concern on the NCS. The domain of neurodevelopment and behavior, which includes neurocognitive and motor functioning, attentional abilities, social functioning, and behavior regulation, will be assessed at multiple time points. Identification of both symptoms of disorder, and of specific developmental, behavioral, or mental health disorders—conditions such as autism spectrum disorders, attention deficit-hyperactivity disorder, anxiety disorders, depression, or schizophrenia—will be identified using a number of modalities, as discussed below. Details about neurodevelopment and behavior measures appear in Appendix F.1.

8.2.2 Assessment of Developmental and Mental Health Problems and Disorders

Diagnoses of specific behavioral or mental health conditions in clinical research are generally based on a patient's history, patient-provider interaction, and the use of condition-specific diagnostic tools, such as the Autism Diagnostic Observation Schedule and Autism Diagnostic Interview-Revised for autism. Actual diagnosis of specified conditions is currently defined by the International Classification of Diseases-Clinical Modification of the World Health Organization; the Diagnostic and Statistical Manual of Mental Disorders-IV of the American Psychiatric Association; or, the Diagnostic Classification of Mental Health and Development Disorders of Infancy and Early Childhood Zero-to-Three. Criteria for these diagnoses can be ascertained by any of the above methods. Additionally, targeted clinical studies can employ laboratory procedures such as functional imaging (e.g., positron emission tomography scans or functional magnetic resonance imaging), electroencephalograms, or other techniques to measure brain function. While potentially powerful, those imaging modalities are not appropriate for inclusion in the core protocol of a broad-based longitudinal study. Consequently, the NCS will rely on a combination of screening instruments and diagnostic information, including records of health care provider diagnoses, to identify developmental and mental health disorders.

Specific conditions of interest to the NCS include: learning, sensory, and motor disabilities; autism-spectrum disorders; attention and conduct problems (e.g., ADHD); depression and anxiety disorders; and schizophrenia. Importantly, the NCS will not only attempt to capture conditions meeting diagnostic criteria, but will also use instruments that capture relevant symptoms in order to identify subclinical manifestations operating below diagnostic thresholds. Early identification of symptoms will be dependent primarily upon reliable and valid parental report screening tools. Direct diagnostic assessment of the child will be used whenever possible. Between birth and age 1, the conditions of primary concern to the NCS are sensory and motor disabilities, as well as serious developmental delays. Early screening for autism and for early precursors of mood and behavioral disorders will be added to these domains between ages 1 and 2.

Diagnoses will also be confirmed, whenever possible, through the child's medical records of documented clinical diagnoses by the child's pediatrician or other health care providers. The American Academy of Pediatrics (AAP) issued a policy statement in 2006 instructing child health care providers to engage in a program of developmental surveillance, screening, and diagnosis (Council on Children with Disabilities et al., 2006). Specifically, the AAP recommended that surveillance take place throughout infancy and early childhood, and that regular developmental screening tests be administered at well-child visits at 9, 18, and 30 months. Positive screens should then be followed by full diagnostic evaluations and referrals to early intervention. By obtaining confirmation through the child's medical records when possible, the NCS should be able to track provider diagnoses of developmental and behavioral disorders. Using a combination of screening instruments for symptoms and diagnoses from health care providers, the NCS will track not only the onset of neurodevelopmental, behavioral, and mental health symptoms and disorders, but the course of the disorders across development. Through repeated assessments over time, the NCS will be able to examine trajectories of children with diagnoses, including precursors of disorder and responses to early intervention efforts. This will permit a better understanding of the stability and course of disorder as children develop and are exposed both to treatment and to new and different environmental influences.

Assessment of learning, sensory, and motor functioning and disabilities

Some sensory and motor difficulties are evident very early in the child's life, and such disorders are usually more severe than those identified later. Other sensory and motor disorders can often be identified by age 2, whereas learning disabilities are often not identified until children enter school. Routine infant hearing screening is recorded in the hospital chart at birth, which will be abstracted by the NCS. Screening for sensory and motor disabilities on the NCS will begin before the neonate has been discharged from the hospital. During a pre-discharge examination, the infant's neurological status will be assessed using the NICU Network Neurobehavioral Scale (NNNS)(Lester & Tronick, 2005), a direct examination of neuromotor and neurobehavioral functioning. The NNNS is an effective screen for problems in early neurobehavioral functioning and has been shown to be sensitive to in utero substance exposure (Lester et al., 2002).

The NCS will continue to track children's developmental status during infancy with regard to cognitive, motor, and language delays using multiple assessment strategies. At 12 months, the NCS will administer three of the Bayley III Scales of Development: Cognitive, Motor, and Language (Bayley, 2006) to all enrolled children to assess the achievement of developmental milestones within these domains. The Bayley is a widely used and extensively normed assessment of developmental functioning long recognized as a standard in the field of developmental assessment (Albers & Grieve, 2007; Sattler, 2001). Low scores can be interpreted as indicating developmental delay (Bayley, 2006).

In addition to the administration of the Bayley III, actual diagnosis of learning, sensory, and motor disabilities will be confirmed whenever possible through the child's medical records, including the diagnoses and treatment plans of their medical providers. The child's health care visits will be reviewed at every contact with the parents, including both in-person contacts at 6 and 12 months and phone contacts at 3, 9, 18, and 24 months, and will continue to be assessed regularly after that. Throughout childhood and adolescence, the child's developmental status with regard to cognitive, language, and motor functioning will continue to be assessed periodically through direct testing by the NCS, and diagnoses confirmed whenever possible through health care provider diagnoses.

Assessment of autism-spectrum disorders

Autism-spectrum disorders, including but not limited to autism, Asperger's syndrome, and other pervasive developmental disorders, are not generally diagnosed until the child's second year or later (Robins & DuMont-Mathieu, 2006). Autism is a developmental disorder of great concern because of increased prevalence and unknown etiology in most cases. Symptoms related to the autism spectrum include deficits in social behavior and communication, and repetitive and stereotyped behavior, and often extend to cognitive impairments or motor abnormalities (Diagnostic and Statistical Manual of Mental Disorders IV-Text Revision, 2000). The NCS will begin screening for autism spectrum disorders when the child is 18 months old and continue to screen for symptoms periodically through the toddler and preschool period. The screening instrument the NCS will use is the Modified Checklist for Autism in Toddlers (M-CHAT)(Robins, Fein, Barton, & Green, 2001), a parental report instrument that has excellent psychometric properties. The M-CHAT, however, is a screen for risk of autism and does not yield a diagnosis of autism or autism spectrum disorders. For diagnostic information, the NCS will rely on diagnostic assessments conducted by the children's health care providers and abstracted from medical records whenever possible. This will include not only private pediatrician contacts, but also diagnoses received through specialty clinics for developmental disorders or through early intervention programs.

Assessment of depression, anxiety, and attention and conduct problems

Behavioral, attention, and mood disorders are rarely diagnosed in infants. Although infants display variability in their moods, conduct, and attentional abilities, configurations of individual differences in these domains are not usually sufficiently stable to warrant diagnoses at this age (Zeanah, Boris, & Scheeringa, 1997). Little is known, however, about precursors of adult mental illness, so during the infancy period the NCS will assess social and cognitive behaviors that may be precursor symptoms to later problems. At 12 months the parent will be asked to complete the Brief Infant-Toddler Social and Emotional Assessment (BITSEA)(Briggs-Gowan, Carter, Irwin, Wachtel, & Cicchetti, 2004) a validated screening instrument which assesses risk for mood problems, behavior problems, and self-regulatory deficits. The BITSEA, or an age-appropriate modification of the BITSEA, will be repeated through the toddler and preschool period to track risk for problems over time. As the children age, other similar screening instruments will be used, such as the well-validated and widely used Strengths and Difficulties Questionnaire (Bourdon, Goodman, Rae, Simpson, & Koretz, 2005; Goodman, 1997), which assesses conduct problems, emotional problems, hyperactivity and inattention problems, and relationship problems, and can be completed by parents, teachers, and in the teen years by the adolescents themselves.

Early diagnoses of disorders will be confirmed whenever possible through the children's health care providers' records. Later in childhood, measures and diagnostic interviews such as the Preschool Age Psychiatric Assessments (PAPA)(Egger & Angold, 2004) interview or the National Institute of Mental Health Diagnostic Interview Schedule for Children (NIMH-DISC-IV)(Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000) may be used to supplement diagnostic information from the

health care providers and assure diagnostic information on children who do not visit health care providers regularly.

Assessment of schizophrenia

Schizophrenia, a psychotic disorder believed to have both genetic and environmental etiology (Walker, Kestler, Bollini, & Hochman, 2004), will also be of interest to the NCS. Schizophrenia, however, is rarely diagnosed before late adolescence or early adulthood (Hafner, Maurer, Loffler, & Riecher-Rossler, 1993). Because screening and diagnostic procedures for schizophrenia will likely continue to evolve before the NCS children reach that life stage, no specific screening or diagnostic tool will be identified at this point. Instead, the best tools available at the time will be evaluated for use on the NCS.

8.2.3 Assessment of Related Factors

There are many exposures that have the potential to exacerbate or buffer the aspects of children's neurodevelopmental and behavioral functioning that will be assessed on the NCS. This includes exposures such as prenatal infection and inflammation, child and parental exposure to organophosphate pesticides, social relationships, and child and family engagement with social institutions such as child care, schools, and religious organizations. Environmental and social exposures will also be investigated as they interact with genetic factors, such as genetic alleles for paraoxonase-1 or variations in the 5-HTT genetic alleles, to produce risk or protective factors for child neurodevelopment and behavior outcomes. See Section 9.5 for details.

8.3 Child Health and Development

8.3.1 Definition

Identification of neurodevelopmental, behavioral, and mental health disorders, as discussed in Section 8.2, is essential to the mission of the NCS. Nonetheless, the tracking of normative developmental trajectories—normal growth in functioning across domains that occurs with age—is equally important. Although there is overlap in the domains of functioning between the outcomes of neurodevelopment and behavior and child health and development, there are important conceptual and practical distinctions that warrant the separate consideration of these outcomes. Child health and development is concerned not with disorder or symptoms of disorder, but with individual differences in trajectories of normal, healthy adaptation over time. Age-appropriate development in social, cognitive, and behavioral and emotional health domains is usually defined either through age-normed benchmarks from standardized testing, or identification of patterns of adaptive functioning in a particular domain. For example, cognitive abilities, temperament, and social competence can be assessed along a continuum and tracked longitudinally among all children participating in the NCS. Details of child health and development measures appear in Appendix F.1.

8.3.2 Assessment of Developmental Trajectories

One challenge in assessing developmental trajectories is that behaviors indicative of normal development at one age—clinging to a caregiver who is trying to leave, for example—may be a marker of maladaptation later in development (Sroufe, 1979; Sroufe & Waters, 1977). At each phase of

development, decisions must be made regarding the most appropriate assessment tool to capture the construct of interest and close attention must be paid to interpreting the information in a developmentally appropriate fashion. A great deal of effort has been expended to find efficient, valid, and reliable assessments that will enable effective measurement of the same domains across developmental stages. The NCS will assess multiple domains of child development using standardized and frequently used instruments. One criterion for these assessments is their ability to connect to later developmental measures of the same constructs. Areas to be covered include cognitive processes, language, and social and emotional development.

Assessment of development and behavior in children is challenging, whether in clinical or research settings. For younger children, in particular, reports of child functioning are often dependent upon subjective parental or other third party reports, which can lead to reporting biases (Fiske, 1987). Determining the best assessment modality for child functioning requires careful planning and consideration. In addition, administration of direct assessments to a child requires attention to the child's comfort in the testing situation, whether at home or in a clinical setting, as variations in feelings of ease or distress can affect children's responsiveness to the examiner and cause dramatic variations in testing outcomes. All examiners who administer assessments to children on the NCS will be extensively trained in developing rapport with children.

Assessment of cognitive and language abilities

Trajectories of cognitive and language development are important indices of developmental progress and problems as the child's experiences and exposures can compromise healthy trajectories of cognitive and language development (Cicchetti, Rogosch, & Toth, 2000). The NCS will begin tracking these milestones during infancy and continue tracking them through adolescence. At 12 months, the NCS will administer the cognitive and language subtests of the Bayley III Scales of Development (Bayley, 2006), a direct child assessment of achievement of developmental milestones within these domains. The Bayley is a widely used and broadly normed "gold standard" assessment of developmental functioning (Albers & Grieve, 2007; Sattler, 2001). The cognitive subtest assesses sensorimotor development, exploration and manipulation, object relatedness, concept formation, memory, play, and other aspects of cognitive processing. The language subtest includes both expressive and receptive language ability. Also at 12 months the child's parent will be asked to complete the MacArthur-Bates Communicative Development Inventory Short Form (CDI)(Fenson et al., 2000). The CDI was developed to supplement direct assessment of child language by obtaining information about the parent's broad knowledge of the child's communication skills, thus incorporating information more representative of children's everyday language use than what can be obtained in a relatively short direct assessment. Both the vocabulary checklist and the actions and gestures communication subtests of the CDI will be administered.

During the preschool years and beyond, measures of cognitive and language abilities will reassess similar constructs, such as concept formation, memory, and expressive and receptive language ability. This may include repeating the Bayley III scales, or at older ages using developmentally appropriate standardized assessments of achievement and intelligence such as the Woodcock-Johnson Test of Achievement (McGrew & Woodcock, 2001) and the Kaufman Brief Intelligence Test (Kaufman & Kaufman, 2004), both of which will also be used to assess parental abilities. This will permit an examination of the ways in which cognitive abilities and achievement can change over time, as the child responds to experiences and exposures in the environment. Other assessments will focus on additional developmentally relevant abilities such as executive function and attention, and will use a combination of parent and teacher report and direct testing of the child.

Assessment of social and emotional development

Social and emotional development covers several important domains of child functioning, both intrapersonal and interpersonal. Infants begin this trajectory with basic temperamental qualities and with the formation of their first relationships: parent-child relationships. They face subsequent challenges in learning to regulate their emotions and behavior and to navigate the increasingly complex reciprocity of relationship interactions. Social and emotional development in the first two years will be assessed on the NCS through a combination of parental report and direct observation.

Assessing temperament early in development is important, as temperamental qualities not only exert direct influence on children's adjustment but also influence parental reactions to the infant's signals and needs and thus affect subsequent development indirectly. When the infant is 6 months old, the NCS will collect maternal reports of child temperament using three subscales of the Rothbart Infant Behavior Questionnaire-Revised (IBQ-R)(Gartstein & Rothbart, 2003; Rothbart, 1981), including activity level, fearfulness, and positive anticipation of and approach to novelty. The IBQ-R asks the parent to report on specific, recent infant behaviors, a technique that minimizes parental bias in the report of child temperament (Rothbart & Goldsmith, 1985).

Also at 6 months, the NCS will conduct its first videotaped observation of mother-child interaction. This will entail videotaping the mother and infant for 15 minutes as they engage in a semistructured play session with a set of toys provided for them during the visit. This technique has been used on many studies, including the NICHD Study of Early Child Care and Youth Development, and the associated coding scheme taps elements of parent-child interaction such as parental sensitivity and cognitive stimulation (National Institute of Child Health and Human Development Early Child Care Research Network, 2003). Observation is considered the "gold standard" of assessment in the domain of parenting (Zaslow et al., 2006).

At 12 months the child's social and emotional development will be assessed using parental report on the BITSEA (Briggs-Gowan et al., 2004). The BITSEA, a well-established brief measure of infant and toddler problems and strengths, assesses effective behavioral, and emotional self-regulation as well as social competence skills, including compliance, attention, mastery motivation, imitation/play, empathy, and prosocial peer relations. At the 12-month visit, a parent-child interaction observation will be repeated, but this time with the child and the child's alternate caregiver (it is anticipated that this will most often be the child's father), giving an expanded view of the child's functioning within the social network.

During the toddler and preschool years, the same constructs will be assessed again, using the same procedures where appropriate, or assessments that are age-appropriate measures of these constructs such as the Strengths and Difficulties Questionnaire (Bourdon et al., 2005; Goodman, 1997), which assesses prosocial behavior and relationship skills.

As the child ages and begins to spend time in the broader social contexts of school and community, assessments of developmental trajectories in social and emotional competence will be tailored to include these normative changes. This will involve assessing family, peer, and eventually romantic relationship qualities in addition to child functioning and adaptation across multiple contexts, such as home and school. It is anticipated that although parents are the primary reporters on child behavior in infancy and early childhood, eventually both teachers and the children themselves will serve as respondents. It is also anticipated that direct observation of parent-child interaction will continue to be assessed periodically throughout development.

8.3.3 Assessment of Related Factors

Numerous factors may influence a child's developmental and behavioral health trajectories. Prenatal exposures, such as exposure to tobacco, alcohol and maternal infection, that potentially have broad health effects will be collected through a variety of mechanisms. Ascertainment of prenatal and postnatal exposure to environmental chemicals with potential impact on the child's developmental trajectories is also outlined in Section 9.1

Questionnaire data will be used to ascertain parental cognitive function and literacy (e.g., Kaufman Brief Intelligence Test; and Woodcock-Johnson III Letter-Word Identification and Passage Comprehension subscales), maternal depression during pregnancy and several times after delivery (Center for Epidemiological Studies Depression Scale), family history of psychiatric diagnoses, and measures of family process and parenting style. Information concerning child care environments and, later in life, school environments will also be collected through maternal questionnaire, direct observation in child care settings, or interview of providers. Information collected on prescription medication use and from the health diary may also be used as sources of conditions of interest in either the mother or the child.

8.4 Asthma

8.4.1 Definition

Asthma is a complex respiratory disease characterized by episodic, reversible, inflammation-mediated constriction of small and large airways. The resulting airway obstruction leads to air trapping and clinical manifestation of the disease: wheezing, dyspnea, and hypoxia. Severe untreated attacks can be fatal. Data from gene association studies indicate a complex inheritance pattern involving perhaps hundreds of genes governing the expression of varying asthma and atopy phenotypes (Ober & Hoffjan, 2006). Asthma phenotypes that emerge from the first through sixth year of life have been predictive of persistent asthma symptoms and long-lasting decrements in lung function in cohort studies (Stein & Martinez, 2004). Childhood "asthma" can be categorized into three phenotypes: (1) airway obstruction which begins in the first two years of life but does not persist to school age, often referred to as early onset transient airway obstruction; (2) early-onset airway obstruction that persists past school age, or early-onset persistent asthma; and (3) recurrent airway obstruction that begins after the first few years of life, or late-onset asthma (Martinez & Helms, 1998; Stein & Martinez, 2004). Prospective data are needed to examine risk factors for the development of these phenotypes and for persistence of early airway obstruction into later childhood and adulthood.

8.4.2 Assessment of Asthma

The NCS will be able to assess the effect of timing of exposures, particularly during critical windows of vulnerability (e.g., specific trimester of pregnancy, early vs. later postnatal periods, etc.), on the development of childhood asthma. This will include distinguishing the effects and interactions of biologics, air pollutants, and genetics with other potential causative factors (e.g., social and economic status, health care access, diet, stress). Accurate exposure and phenotypic data are needed to assess the significance of various asthma and allergy genotype-complex exposure interactions that result in several different asthma phenotypes (Bel, 2004; Taussig et al., 2003). Identification of children at risk for developing severe forms of asthma would have clear public health impact.

Diagnosis

There is no single test that provides an unequivocal diagnosis of asthma. In young children, the clinical diagnosis of asthma is based on relevant history and pulmonary auscultation. Chest x-ray and pulse oximetry are often used in the initial diagnosis and to monitor disease. When the child is old enough to cooperate, approximately 5 to 7 years old, spirometry or more detailed pulmonary function tests can be used for objective assessment of pulmonary status, though this is rarely necessary for clinical diagnosis. Spirometry or peak flow monitoring can be used to follow disease status and progression.

In population-based research, diagnosis of asthma generally is based on combinations of reported symptom history, reports of physician-diagnosed disease, and medical records. Clinical research studies use pulmonary function tests, often in combination with provocation tests such as methacholine or exercise challenges, to attempt to obtain objective measures of lung function. Recent advances in passive pulmonary function assessment have enabled those measures to be obtained in children as young as several days old; however, the equipment is expensive, results are operator dependant, and the procedures would most likely not meet the "minimal risk" criterion.

Assessment of asthma in the NCS

Information on symptoms, signs, and other factors related to asthma will be collected throughout the course of the NCS using multiple methodologies. Questionnaires will assess a child's history of asthma symptoms (using questions based on the International Study of Asthma and Allergies in Childhood) starting with the six-month visit and continuing through adolescence. Questions regarding recent or "ever" diagnosis of asthma in a medical setting will also be asked, and information on the use of asthma-related medications will be collected. Confirmation of physician diagnoses related to any office visits, emergency department visits, and hospitalizations will be obtained whenever possible. Attempts to measure lung function via spirometry will begin at the 36-month clinic visit, although the difficulty of obtaining consistent results at this age is recognized. These measures will be repeated at subsequent clinic visits. In addition, the NCS may ask for periodic peak flow measures to be taken at home and entered into the child's health care visit log beginning at approximately age 5. NCS field staff and medical professionals will assess allergic sensitization and allergic and nonallergic rhinitis and asthma. Immune system function can also be evaluated through such measures as lymphocytes, cytokines, IgE, and interlukins. This approach of multi-method, repeated measures of asthma over time will permit investigation of the ways in which symptoms are ameliorated or exacerbated as the child faces new environmental exposures and attempts new treatment strategies.

The NCS will collect biological samples that could be the source of DNA for traditional genetic analysis of candidate genes and for gene discovery based on genome-wide association scans. The anticipation of chip-based genotyping of all participants based on current technology, or complete sequencing of all individuals at some point in the future, will provide extraordinary detail about genetic variation of nuclear DNA. Along with the planned definition of cases based on asthma phenotypes, this provides an opportunity to use the efficient nested-case control design for subsets of the sample, or the proportional hazard design for the entire sample, to evaluate effects of genes, environments, and their interaction. In addition, the Study will collect biological samples at multiple points over time, which provides the opportunity to evaluate epigenetic changes proposed to be the molecular mechanisms of some gene-environment effects (Hanson & Gluckman, 2005). The epigenetic assays for environmental effects of methylation and chromatin status are rapidly evolving, and current methods are sure to improve rapidly as a result of extensive current work (Callinan & Feinberg, 2006) and anticipated future work.

Two overlapping study populations will be used to address asthma hypotheses. For questionnaire items and other data available for the entire cohort, we will analyze the full NCS sample data. For genetic data and other items that require laboratory processing, the study population will consist of a nested case-control study design with sampled cases such as those with wheezing/asthma symptoms and a matched cohort of controls. Matching factors might include date of birth, regional location of birth, race, gender, and socioeconomic status.

Assessment of upper and lower airway disorders

It should be noted that with disorders associated with the upper and lower airways, there exist numerous definitions that can be employed at different developmental stages depending on the availability of subjective (parental report) vs. objective (spirometry, sensitization) criteria. Below are potential outcomes that may be used in the NCS at different child ages depending upon data available at each interview or clinical exam.

- *IgE antibody quantification*: Total and allergen-specific IgE antibodies (RAST) can be measured from serum levels. One advantage of the RAST test is that it does not have to be performed in a physician/hospital office and can be done instead at the child's home. The disadvantage is that it requires a venipuncture and therefore may be less acceptable to children and their primary caregivers. Sensitization status can be ascertained by the measurement of specific IgE to mite, cat, dog, grasses, foods, etc., in serum levels of infants and children (Simpson et al., 2005).

- *Rhinitis:* Rhinitis presents with a constellation of symptoms, including: nasal congestion; sneezing; rhinorrhea; itchy nose, mouth, throat and/or ears; itchy, watery, and red eyes. Symptoms are present without a cold and last for a minimum of one month (American Academy of Allergy, Asthma, & Immunology, 2005).

- *Wheeze:* Diagnosis of wheeze symptoms consistent with the airway obstruction associated with asthma is dependent on a clinical history reported by a parent, and may not be predictive of current or future development of childhood asthma. Wheezing is likely to be the primary outcome until the child can be tested using objective measures such as pulmonary function testing. Wheezing can be defined as an episode of wheezing or whistling in the chest without a cold for a certain number of times over a specified time period. The presence of wheeze without a cold is a common definition used to identify potential asthma in young children. There are additional related questions for defining more serious wheezing in the young child, such as: total number of attacks; if the child's sleep is disturbed with wheezing; if the child sounds wheezy after exercising; and if the child has a dry cough apart from a cold. Inquiry into these symptoms will be standardized.

- *Asthma:* Diagnosing asthma in young children can be difficult, and under- or over-diagnosis is a problem. As the child ages, a diagnosis of asthma may be confirmed by pre- to post-bronchodilator forced expiratory volume in one second (FEV_1) in children who meet certain predetermined criteria such as: wheezing symptoms in the previous 12 months; physician treatment for "asthma" in the previous 12 months; or an increased exhaled nitrous oxide (eNO) of greater than 10 parts per billion. Confirmatory tests would allow for additional outcome definitions to be assessed, such as allergic asthma disease, nonallergic asthma disease, allergic asymptomatic airway reactivity (AR), and nonallergic asymptomatic AR.

- *Eczema:* Despite the fact that eczema is not an airway disease, the NCS may examine atopic dermatitis as an outcome since it is one of the earliest allergic diseases in childhood, and it is associated with asthma. The clinical criteria for childhood eczema can be determined by the parent questionnaire and/or a physical examination.

8.4.3 Assessment of Related Factors

In addition to measures related to ascertaining asthma in the child, relevant information regarding risk and exposures will be collected. For example, family history of asthma and atopy will be obtained via the T1 questionnaire. We will examine maternal psychosocial stress during pregnancy, including stress life events, social isolation, racism/discrimination, anxiety, depression, cortisol in saliva, urinary catecholamines, and low socioeconomic status. Other potential gestational factors are prematurity and the child's birth weight. History of early life infections will be collected through the child's health care visit log as well as by the six-month questionnaire. The relation between the risk of developing early onset transient airway obstruction and the diet during pregnancy and early childhood will be explored. The dietary variables include consumption level of antioxidant and other micronutrients, fresh fruits and vegetables, and vitamin intake. Other dietary factors of interest include breast feeding and its relation to the risk of early onset persistent wheezing, and obesity and its relation to the risk of late onset asthma. Indicators of socioeconomic status include household income, educational level, location of residence, household composition, housing characteristics, neighborhood and community characteristics (age, race/ethnic composition, population density, housing quality), neighborhood resources (community organizations, schools, recreational facilities, public services, commercial outlets, religious organizations), and neighborhood processes (neighborhood cohesiveness, crime levels, political activity, police activity, family's perceptions of neighborhood). These factors can be associated with exposure to social, physical, psychological, and environmental factors related to the risk of asthma. Exposure to allergens early in life will be measured through dust samples as well as specific questionnaire items (e.g., presence of pets). Air pollution data will be obtained by questionnaire or biomarker (e.g., tobacco), use of ambient air pollution monitors, and home-based air quality measurements at periodic home visits. Child care and school exposures will also be obtained by the NCS, either by direct measurement, when possible, or by other sources, as necessary.

8.5 Obesity, Body Composition, and Growth

8.5.1 Definition

The ongoing increase in childhood obesity and overweight in the United States gives rise to numerous questions regarding both the antecedents of overweight and adiposity and their long-term health effects (Flegal & Troiano, 2000; Ogden, Fryer, Carroll, & Flegal, 2004; Ogden et al., 2006). Obesity appears to remain consistent from childhood into adulthood (Serdula et al., 1993), and childhood obesity is directly related to the same adverse health outcomes generally associated with adult obesity (Freedman, Khan, Dietz, Srinivasan, & Berenson, 2001; Haji et al., 2006; Orio et al., 2007). Although the cause of overweight in an individual is ostensibly obvious—energy intake greater than expenditure—elucidation of reasons behind the population-level trends in obesity and overweight is necessary to enable appropriate interventions.

Most frequently, obesity is defined simply in terms of the relation of weight to height. For example, children who are ages 2 through 19 years are considered above the range of a healthy weight if their body mass index (BMI, an index of weight in relation to height) is above the 85th percentile compared to other children in their age and gender group (Centers for Disease Control, 2007). For adults, a BMI of greater than 25 is considered above the range of a healthy weight. BMI is correlated with overall

body fat and directly associated with adverse outcomes. However, it is neither a true measure of adiposity nor a measure of the relative distribution of central (visceral) and peripheral fat, characteristics that may be the true risk factors for adverse outcomes (Arner, 1998; Bergman et al., 2006). To the extent possible within the overall structure of the diverse Study protocol, the NCS will strive to obtain measures of obesity, body composition, and growth that go beyond simple relations between weight and height.

8.5.2 Assessment of Overweight and Obesity

Maternal and paternal measures

Assessment of maternal size and body composition will start with the first home visit, either before pregnancy or in the first trimester of pregnancy and continue throughout pregnancy until birth. Initial measurements will include height, weight, waist and hip circumferences, and triceps and subscapular skin folds. Segmental heights (e.g., knee height) will be obtained as there are established associations between those measures, prepubertal (and possibly intra-uterine) nutrition and growth, and later cardiovascular outcome (Gunnell et al., 2003). The subscapular-triceps skinfold ratio is among the commonly used estimates of fat distribution, often used in combination with the waist-to-hip ratio and BMI (Stein et al., 2007). Similar measures will be obtained from the father during the first trimester home visit.

Weight and skinfolds will be obtained from the mother at each subsequent pregnancy visit, because of potential associations between maternal weight gain and adiposity, in utero growth, and subsequent metabolic and cardiovascular outcomes (Barker, 1992). The assessment of maternal body composition by anthropometry during pregnancy is complicated by the attendant changes in body water, including skin and subcutaneous accumulation (Huston Presley, Wong, Roman, Amini, & Catalano, 2000; Stevens-Simon, Thureen, Barrett, & Stamm, 2001). Skinfold measures during pregnancy, however, are often used and remain a reasonable choice within the context of the NCS and the focus on subsequent child growth and health.

Child measures

In the NCS, assessment of the child's body habitus and composition will begin in utero with the second and third trimester ultrasounds. Along with routine linear measures of growth, measures of mid-thigh lean and fat mass area and abdominal wall thickness will be obtained (Bernstein & Catalano, 1991; Bernstein, Goran, Amini, & Catalano, 1997). Though not generally part of a routine fetal ultrasound, those measures are used in a growing number of studies, can be obtained with reasonable accuracy and precision, and will provide fetal analogues to the postnatal anthropometric estimates of peripheral and central fat distribution described below.

At birth, anthropometric measures will include weight, length, head circumference, body segment lengths, and triceps and subscapular skinfolds. These measures will be repeated at each home visit, until the three-year clinic visit. At that time, and at later clinic visits, DXA and BIA will be attempted, in concert with the ongoing anthropometric measurements. Depending on their availability during the Study period, other measures of body composition, such as air displacement plethysmography (Winsley et al., 2005) or MRI (Pietrobelli, Malavolti, Fuiano, & Faith, 2007) could be used on all or on a subset of the NCS population.

The correlation between the anthropometric and other body composition measures is variable and depends on the age of the participant, the measures used, the participant's body habitus, and

perhaps even gender (Semiz, Ozgoren, & Sabir, 2007; Winsley et al., 2005; Wright et al., 2007). However, there is evidence that, at least later in childhood, the anthropometric measures do correlate with metabolic measures of interest to the NCS, providing confidence in the use of those measures as a cornerstone of body composition assessment (Freedman, Serdula, Srinivasan, & Berenson, 1999).

Interpretation of measures of growth and body composition

An individual's growth can be quantified two ways. The first is by comparison with published references, such as the CDC BMI and BMI percentile curves (http://www.cdc.gov/nccdphp/dnpa/bmi/childrens_BMI/about_childrens_BMI.htm). However, population references do not exist for many of the measures the Study will use. In those instances, and even when external references do exist, internal NCS distributions can also be created and used. The probability-based sample of the NCS should provide a strong basis for quantification and, perhaps, serve as an external reference for future studies. Skin fold thicknesses can be analyzed as absolute measures, as ratios, or as terms in any of a number of equations that can be used to estimate percentage body fat. The NCS will report the absolute measurements, not the results of estimating equations, giving the analyst maximal flexibility in the definition of obesity or body composition.

8.5.3 Assessment of Related Factors

A child's energy expenditure early in life will be assessed primarily through parental report of activity and activity diary completion. In late childhood and in the assessment of parental activity, questions will be based on the International Physical Activity Questionnaire (IPAQ), both to enable comparison with other studies and to permit the estimated quantification of metabolic equivalence of task (MET) expenditure. Though the use of the IPAQ in adults is well validated (Craig et al., 2003), its performance among adolescents is less predictable and may vary depending on the study population (Arvidsson, Slinde, & Hulthen, 2005; Craig et al., 2003). As the NCS progresses, additional measures of physical activity, either questionnaire-based or activity-based (e.g., accelerometers), may be employed.

Fetal exposure to maternal glucose will be estimated by several HgbA1c measurements during pregnancy, as discussed in Section 9.4.3. Fasting blood samples to be analyzed for glucose, insulin, and lipids will be collected from at least some of the women during the third trimester clinic visit. Maternal diet will be assessed throughout pregnancy and after birth through a combination of food frequency questionnaires, food diaries, and recalls. Infant feeding practices, including breast and bottle feeding, and subsequent child diet will be collected via questionnaire. Family process, physical activity, and the child's exposure to television and other media will also be collected by questionnaire early in life. Neighborhood characteristics that may be conducive to physical activity, or lack thereof, will be assessed by parental report and by community-level observations and data. See Chapter 9 for details on these measures.

Potentially relevant laboratory analyses can include assessment of ghrelin, leptin, adiponectin, and other "adipocytokines" to determine if those compounds are causally related to increased weight and adiposity or are an intermediate phenotype. These can be measured in maternal blood as well as in the child from infancy onward. When the child is older, it may be possible to obtain fasting blood tests for assessment of glucose and insulin, although that will be determined at a later point in the study. Lipid profiles can be assessed starting with 12 month visit, though fasting samples are necessary for triglyceride assessment.

8.6 Injury

8.6.1 Definition

Injuries are a leading cause of childhood death and disability. After age 1, they are the single leading cause of death among children and adolescents in the United States. Injuries are generally classified by both the external cause (e.g., car crash, poisoning, fall) and the intent. Of the 23,636 injury deaths to U.S. children (ages 0-21 years) in 2004, 15,871 (67 percent) were unintentional ("accidents") (Centers for Disease Control and Prevention, National Center for Injury Prevention and Control [CDC NCIPC], 2007). Intentional injuries (interpersonal violence, child maltreatment, and self-inflicted injuries) and injuries of undetermined intent accounted for the remaining 7,765 fatalities. The leading causes of injuries vary with the age of the child, with intentional injuries taking their greatest toll in adolescence (CDC NCIPC, 2007).

The World Health Organization injury surveillance guidelines (Holder et al., 2001) define injury according to the guidelines of Baker, O'Neill, Ginsburg, and Guohua (1992): "Injuries are caused by acute exposure to physical agents such as mechanical energy, heat, electricity, chemicals, and ionizing radiation interacting with the body in amounts or at rates that exceed the threshold of human tolerance…In some cases (for example, drowning and frostbite), injuries result from the sudden lack of essential agents such as oxygen or heat." (Baker et al., 1992, p.4). According to this definition, an injury from a motor vehicle crash would be due to exposure to mechanical energy, a scald burn due to exposure to thermal energy, and a poisoning due to exposure to a chemical agent.

It is expected that, of the 100,000 children enrolled in the NCS, more than 1,600 children will die from an injury and more than 8,000 will be hospitalized (Rivara & Villaveces, 2001) with many more seeking care from an emergency department or other health care provider. Virtually all children experience minor injuries (e.g., cuts and bruises). A challenge for the NCS is to use a definition that identifies the subset of injuries that are serious enough to potentially compromise health and future development. Although many studies include only those injuries for which medical care is sought, this definition is biased in a way that would preferentially identify injuries among those with greater access to health care. Thus, comparable to definitions used in the International Study of Health Behavior in School Children (Currie et al., 2004; Molcho et al., 2006; Pickett et al., 2005; Pickett et al., 2006), in the NCS an injury will be defined as physical damage to an individual that results in medical care or at least one day of limitations in activity.

8.6.2 Assessment of Injury

Beginning in early life and continuing through early childhood, ascertainment of injuries will be based on parental report. Later in childhood and adolescence parent or caregiver reports will be supplemented with self-reports from the child. Health visit logs will be provided to study participants for documentation of medical visits and will include a place for documentation of key data elements about injuries that result in medical care. Activity diaries may help identify injuries that result in limitations in activity, and specific activities associated with increased risk of injury.

Studies have shown that recall for injury events declines with time and severity of the injury (Cummings, Rivara, Thompson, & Reid, 2005; Harel et al., 1994). In one study, approximately 3 months following the event, parents were able to recall 88 percent of major injuries, 86 percent of minor injuries seen in an emergency department, 81 percent of minor injuries seen in an urgent care setting, and 58 percent of minor injuries seen in a clinic. At 6 months, recall of major injuries was 80 percent, but dropped to 56 percent by one year (Cummings et al., 2005). Throughout the Study, parents, caregivers,

and, when appropriate, children will be asked about injuries that have occurred during the interval period. Thus, from birth through age 1, information about injuries will be ascertained every 3 months and from 1 through 3 years every 6 months. Contact periods for later years have not yet been determined. For those reporting an injury, additional information will be sought about the external cause of the injury, the physical harm to the child (i.e. the nature of the injury), treatment received, and any lasting sequelae.

Traumatic brain injury is of particular interest to the Study both as a primary Study outcome and as a confounder with significant potential to impact trajectories of development and thus multiple other Study outcomes. Consequently, when a head injury is reported additional questions will be asked about changes in level of consciousness. Although even minor traumatic head injury can be identified through diffusion MRI or other advanced structural and functional imaging technologies (Suh, Davis, Hopkins, Fajman, & Mapstone, 2001), the expense and participant burden of such technologies are not appropriate for general use in the NCS population. At this time, biomarkers for measurement of head injury are not available for use in an epidemiologic study. However, it is recognized that this is an area of active research that may have implications for future protocol development or use of stored biospecimens (Berger et al., 2006; Berger & Kochanek, 2006).

Methods are being explored to supplement the self-reported data with data collection from medical records for the most severe injuries. This effort will likely be limited to those injuries that result in hospitalization or death. Like self-report data, data abstracted from medical records would include information about the external cause of the injury, the nature of the injury, treatment received, and any lasting sequelae.

8.6.3 Assessment of Related Factors

Numerous factors may be related to child behavior that increases subsequent risk of injury. Areas of interest include prenatal and early life exposures to neurotoxic chemicals, including metals and organic pesticides, family process and parenting behaviors, and home, child care, and school environments (see Chapter 9). Examination of the genetic contribution to aggressive behavior, and thus risk of injury, will be examined both for direct genetic influence on behavior, and for the interactive effects of specific environmental exposures (e.g., chemical, socio-emotional) and genotype on aggressive behavior.

Factors with potential direct relation to injury risk, in addition to temperament and activity, include the physical characteristics of a child's home and neighborhood environments. These will be assessed by questionnaire and by direct observation by Study personnel. Medication use by the child may provide additional information on either conditions associated with injuries (e.g., seizure disorder, ADHD) or medications that might have a direct influence on risk of injury (e.g., sedating antihistamines).

8.7 Reproductive Development

8.7.1 Definition

Development of the reproductive system begins early in gestation and continues through infancy, childhood, adolescence, and into adulthood. A number of adverse outcomes can occur as the result of interference with development of this complex system, which includes the reproductive organs, the endocrine system, and the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes that control their development and function. Early outcomes include birth defects such as hypospadias and cryptorchidism in boys (Pohl et al., 2007), as well as hormonal changes, such as hypothyroidism, in

boys and girls which interfere with optimal reproductive health. Later outcomes from the same exposures may include alterations in growth, timing, and progression of puberty (Herman-Giddens, 2006), and disease states such as polycystic ovary disease (PCOS) (Azziz et al., 2004) and endometriosis (Eskenazi et al., 2001) in females, and testicular dysgenesis syndrome in males (Skakkebaek et al., 2001).

Such changes in reproductive structure and development may be cumulative, that is, adverse outcomes at early ages may predispose an individual to be at greater risk for additional adverse effects, e.g., cryptorchidism and later changes in fertility (Lee, 2005) or early menarche and breast cancer (Vihko & Apter, 1986). In addition, recent studies in animal models suggest that certain exposures are associated with adverse reproductive development outcomes that are transgenerational, that is, effects that are carried into subsequent generations because of changes in DNA methylation patterns that are transmitted in the male germline to the next generation (Anway, Cupp, Uzumcu, & Skinner, 2005; Chang. Anway, Rekow, & Skinner , 2006).

8.7.2 Assessment of Reproductive Development

Evaluation of children at birth, at six month intervals during the first two years of life, and at regular intervals beyond that time, will allow assessment of birth defects and anthropometric measures. Health outcomes related to reproductive development can be assessed in relation to the timing of anthropometric measures and the physiologic development of reproductive organs and other regions of the body that respond to reproductive hormones. The NCS protocol will include physical examination for genital development and, where possible, will use medical record review for information on further diagnosis and/or surgical intervention. Following standard anthropometric measurement of weight, height/length, head circumference, and skin-fold thickness, a detailed observation of the body, particularly of the breasts and genitals, will be performed. Birth defects, such as hypospadias and cryptorchidism in boys or altered genital formation in girls, can be assessed at birth by direct observation by a medical professional or via medical record review when possible. Cryptorchidism (undescended testes) is determined by palpating for the testes in the scrotum. Surgical procedures may be required to fully diagnose and/or repair undescended testes. Hypospadias (abnormal opening of the urethal meatus along the ventral aspect of the penis) is diagnosed by direct observation. Location and severity of the urethral opening will be noted, and surgical repair may reveal more detailed diagnosis. Anogenital distance may also be measured at birth (Swan et al., 2005).

Measures of puberty onset (e.g., onset of breast development in girls, genital growth in boys, and pubic hair development in boys and girls), and stage of sexual maturation can be assessed used Tanner scales by self-assessment or examination by a medical professional (Marshall & Tanner, 1969; 1970). Validation of the best procedures to use for this assessment are likely to advance by the time children in the NCS reach age 6 or 7. Other key outcomes are age of menarche in females and spermarche in males. Menarche can be assessed through questionnaire or medical record abstraction, when possible, while spermarche can be determined through questionnaire and by examining for sperm in urine samples (Nielsen et al., 1986). In addition, hormone levels in both males and females obtained from blood samples throughout childhood will allow for the assessment of hormonal, thyroid, and pituitary gland status and function. It may also be possible to collect semen samples from participating male children when they reach age 18 to assess semen quality, sperm production, and morphology.

Serial assessment of reproductive outcomes at birth, in childhood, at puberty, and in adulthood, and collection of information from medical records, whenever possible, on disease states such as polycystic ovary disease (PCOS) and endometriosis in females and testicular dysgenesis syndrome in males, can be applied successfully to study reproductive development in the NCS. In addition, collection

of maternal breast milk and maternal and child blood and urine samples at multiple time points and serial questionnaires to assess pathways of exposure will provide the necessary data to evaluate both exposures and the links between exposures and reproductive development.

8.7.3 Assessment of Related Factors

Exposure to environmental agents that are hormonally active agents (HAAs; also called endocrine disruptors) has been shown to affect the reproductive system in a number of ways in both animals and humans. A variety of environmental chemicals have been cited in the literature as potential HAAs, including insecticides and herbicides (e.g., DDT, atrazine); pharmaceuticals (drug estrogens); chemicals associated with consumer goods/household products (e.g., bisphenol A, phthalates, nonylphenol, polybrominated diphenyl ethers [PBDEs], perfluorinated compounds [PFOA, PFOS]); industrial chemicals (e.g., polychlorinated biphenyls [PCBs], dioxins, polycyclic aromatic hydrocarbons [PAHs]); heavy metals (e.g., arsenic, lead, mercury, and cadmium); and natural hormones such as the phytoestrogens (Ashby, Tinwell, Stevens, Pastoor, & Breckenridge, 2002; Ceccatelli, Faass, Schlumpf, & Lichtensteiger, 2006; Eriksson, Fischer, & Fredriksson, 2006; Fenton, Hamm, Birnbaum, & Youngblood, 2002; Gray, Ostby, Cooper, & Kelce, 1999; Howdeshell, Hotchkiss, Thayer, Vandenbergh, & vom Saal, 1999; Kuriyama, Talsness, Grote & Chahoud, 2005; Lilienthal et al., 2006; McDonald, 2005; Rubin, et al., 2001; Schonfelder et al., 2002; Talsness et al., 2005; Wolf et al., 1999). Recent studies of environmental agents suggest that PCBs (Blanck et al., 2000) or organochlorine pesticides (Krstevska-Konstantinova et al., 2001) may accelerate pubertal development in girls while PAHs (Den Hond et al., 2002) or lead (Selevan et al., 2003; Wu et al., 2003) have been associated with delays in pubertal development. Data on the effects of HAAs on age at puberty in boys are fewer (Den Hond et al., 2002) but indicate an association between PCB and polychlorinated dibenzofuran (PCDF) exposures with delayed puberty and decreased penile length (Den Hond & Schoeters, 2006). These observations are concordant with laboratory data on the effects of HAAs. Because there are only limited data on specific critical windows for chemical exposures in relation to timing of puberty, the entire prepubertal period, including in utero growth and development and the peripubertal period, should be considered critical times for exposures. Environmental samples and biological specimens will be collected to allow measurements for a number of chemical exposures. These exposures will then be examined to look for associations with alterations in reproductive development.

Obesity, diet, and nutrition measures are important related factors for reproductive development. Higher percentage of body fat increases the risk of precocious puberty; later onset in underdeveloped nations is often attributed to poor nutrition (Anderson, Dallal, & Must, 2003). In addition, obesity and precocious puberty have been associated with conditions such as neurofibromatosis, hypothyroidism, PCOS, etc. (Cesario & Hughes, 2007). Delayed puberty has been associated with several conditions such as sickle cell disease, thalassaemia, Celiac disease, Gaucher disease type I, Cushing's disease, and other endocrine deficiencies. Anthropometric data, hormonal changes, and medical record abstraction, when possible, will be included in the NCS to examine these potential relations.

The prenatal and postnatal smoking status of parents may reduce the age of onset of puberty (Windham et al., 2004). Urine cotinine will be measured to examine active/passive smoking exposures.

Generally the mother's menstrual history is considered the biggest predictor of age of puberty, and some of this effect may be seen in ethnic differences (Blanck et al., 2000). There are genetic components for hypospadias, cryptorchidism, spermarche, and semen quality that may be related to the father's reproductive history (Pohl et al., 2007). In addition, genetic factors such as 5-alphareductase type 2 gene mutations (Silver & Russell, 1999) and androgen receptor mutations (Silver, 2000) are risk factors

for hypospadias. Maternal reproductive history and, when possible, paternal reproductive history will be collected using questionnaires and possibly medical records. Blood samples will be collected to determine genetic factors that may be involved.

There is some evidence that a younger gestational age at birth is associated with greater incidence of hypospadias and cryptorchidism (Pohl et al., 2007) and is a predictor of an earlier age at menarche; however, evidence points to small-for-gestational-age as the predictor of precocious puberty (Adair, 2001). Gestational age at birth and growth and development will be recorded in the NCS.

Maternal alcohol consumption may (Carbone et al., 2007) or may not (Blanck et al., 2000) be related to an increase in hypospadias and/or a delayed onset of puberty and can be monitored by measuring blood alcohol levels. In addition, the impact of stressful sociological factors may be related to precocious puberty (Cesario & Hughes, 2007). Questionnaire data on socioeconomic status and stress will be collected in the Study.

9. RATIONALE FOR EXPOSURE MEASURES

The primary purpose of the National Children's Study exposure measurements is to enable the epidemiological analyses of relations between the priority exposure areas and the priority outcomes, not to provide a comprehensive assessment of all a child's environmental exposures from all pathways. Thus, measures have been selected that reflect the Study's priority areas, can be measured consistently through relevant time periods of the child's life, and are suitable for a large-scale population-based study. Similar to the outcome measures, the collection of the array of exposure measures will allow the examination of exposure-outcome relations to be more inclusive than those specified in the NCS hypotheses. It will also facilitate the investigation of mediating pathways.

Because the NCS seeks to establish relations between environmental exposures broadly defined as chemical, physical, psychosocial, and biological, and various health and developmental outcomes at specific points in a child's life, the exposure assessment for the Study must consider how to measure exposures of varying kinds during the child's different developmental phases. Environmental effects, both adverse and beneficial, could result from exposures either prior to, or concurrent with, the outcome. Further complicating the evaluation of the relations between exposure and outcome is the fact that there may be times in children's development when they are differentially susceptible to the effects of the environmental exposure. Because of the longitudinal nature of the NCS, the Study can examine both the overall effects of exposure and susceptibility within each life stage. Given the multiple hypotheses and their related exposures and outcomes it will be important to measure environmental exposures throughout the child's life as primary exposures in some instances may be key covariates or confounders in others.

Aspects of the NCS that have important implications for the collection of exposure measures include the Study's geographic dispersion and the varied socioeconomic, demographic, and urban vs. rural nature of the study population. These characteristics present challenges to the collection of exposure measures, necessitating consideration of a number of factors, including: the stability of biological and environmental samples; acceptability of data collection processes to various segments of the study population; and, availability of local environmental data or information sources.

The NCS will store many of the collected samples and immediately analyze only those critical to the NCS priority areas and those that are subject to degradation in storage. Many of the environmental samples and biospecimens will be collected, aliquotted, and stored so they can be analyzed later in NCS subpopulations or in nested case-control studies (Sections 9.2, 9.5, and 9.6). This practice maximizes the efficient use of finite samples for future analyses that will be driven by the evolution of research questions, advances in analytic techniques, and availability of funding.

This chapter is subdivided into five major sections that represent different aspects of exposure: demographic, chemical, physical, psychosocial, and biological. Though the characterization of some potential exposures is obvious (e.g., infections as biologic exposures), others are less so (e.g., medication use is listed under chemical exposures). Some broad categories of exposure span two sections (e.g., some aspects of neighborhoods are identified under physical exposures, while other aspects are outlined under psychosocial). The classification of potential exposures here is just one possible organization and cannot reflect the overlapping and extensive nature of the spectrum of exposure assessment measures within the NCS.

9.1 Demographics/Culture

In the context of epidemiologic studies such as the NCS, demographic data refer to individual-level characteristics that can be used to define sub-populations of people within a larger population. Examples of attributes commonly classified under the demographic umbrella are: age; gender; measures or estimates of social position such as education, occupation, and income; and indicators of race, ethnicity, and culture. These data are important to the NCS not only because of the strong association many of them have with the Study's priority outcomes and exposures, but also because of the Study's charge to elucidate causes of existing health disparities among U.S. children.

In the NCS, as in most studies, demographic data will be collected by self-report from the parents with initial proxy reporting for the child. Though the Study's intent is to obtain information pertaining to the biological father and other "father figures" directly from the individual, proxy reporting from the mother will be used if necessary.

Standardized measures of race, ethnicity, education, family income and structure, religion, employment, public program participation, health insurance, financial security, and food sufficiency will be collected on all study participants. Baseline demographic information will be collected from NCS participants beginning in pre-pregnancy for women in the pre-conception cohort, and at the first in-person interview for women who are enrolled after they become pregnant. Key demographic measures will be updated for both cohorts during all face-to-face visits, throughout pregnancy and after birth. Family structure will be established at the first in-person visit by obtaining a roster of household members. The gender, age, race, and ethnicity of each family member, and their relationship to the index female, will be collected. Changes in household composition will be tracked during subsequent face-to-face visits, including visits following the birth of the child. Measures chosen to assess family structure include those used on other large-scale studies (Census 2000; National Health Interview Survey [NHIS]; Survey of Income and Program Participation [SIPP]).

Employment status, educational level, and income will be measured for the mother and father at the initial face-to-face visit using standard questions from SIPP, Census 2000, and the American Community Survey [ACS]. This information will be updated at in-person visits during pregnancy and following the birth of the child. Changes to the mother's employment status will be captured during selected phone contacts during preconception and pregnancy. At the pre-discharge visit, the mother will be asked about her plans to return to work. Information on the parents' religious affiliation and attendance at religious services will be collected at the first pregnancy visit using standard questions from the National Study of Youth and Religion and then not again until after the child's birth.

Information on the mother's and father's country of birth, languages spoken in the home, acculturation, and connection to other cultures will also be measured. During pregnancy and infancy, these factors relate to the behavior and parenting practices of the parents; as the child grows, these factors relate to the ability of the child to acculturate and integrate into the American culture and to perform well in school. Information about cultural practices will also be updated if there are changes in primary caregivers (e.g., new father figure). Changes in these parameters will be charted longitudinally (e.g., the mother marries someone from another culture or ethnicity). Standard items from Census 2000 and the Early Childhood Longitudinal Study-Birth Cohort [ECLS-B] are among the measures used to collect this information.

9.2 Chemical Exposure Measures

Exposure assessment is the process of estimating or measuring the magnitude, frequency, and duration of exposure to an agent, along with the number and characteristics of the population exposed (Needham et al., 2005; Zartarian, Bahadori & McKone, 2005). The primary purpose of exposure assessment in the NCS is to support epidemiological analyses of relations between exposures and outcomes. The exposure assessment framework for chemical agents draws on work of the NCS Exposure to Chemical Agents Working Group (NCS Chemical Agents Workgroup, 2004) and of investigators from the Children's Environmental Health Centers (Kimmel, Collman, Fields, & Eskanazi, 2005). The framework is composed of three concepts:

(1) Core measures will be obtained for the entire cohort, and validation sub-samples will be considered for more intensive exposure measurements. The most precise or detailed chemical measures are often the most intrusive and costly. Targeted sub-sampling efficiently uses those tools, decreases their use in settings in which chemical concentrations would be below detectable limits, and increases the utility of survey-based and indirect measures (e.g., community-based measures applied to individuals) among participants who do not receive those intensive measurements.

(2) A hierarchical approach will be implemented that relates measures obtained at different geographical levels (e.g., individual, residential, neighborhood, and region). Using air pollution as an example, individual-level exposures can be estimated from neighborhood and residential monitoring and other data (e.g., time-activity questionnaires). In some communities, depending on the availability of local ambient monitoring stations and spatial variability of pollutants, the NCS researchers may need to collect neighborhood-level samples for some media (e.g., air, water).

(3) Multiple exposure assessment approaches will be used, including information from environmental and biological samples, questionnaires, diary reports, and physical and visual assessments. In general, biological measurements will be used where biomarkers of exposure are available, especially for persistent chemicals for which there are relatively consistent exposure patterns, and for which knowledge of the route of exposure is not critical. For other chemicals, such as non-persistent pesticides, environmental samples will also be collected at home visits. These results will be combined with questionnaire answers, observations, and neighborhood-level monitoring to estimate total exposure. Where feasible, some environmental samples will be collected prospectively and stored for later analyses to help with the interpretation of biological measurements. Figure 9-1 demonstrates how these assessments come together to yield the "true" level of exposure.

Domains, sub-domains, and example target chemicals were identified in the Study hypotheses either as the primary agents of interest or as potential covariates. Approaches for measuring these chemicals or their metabolites in environmental and biological samples, or for identifying questionnaire- or observation-based surrogates, were identified. Selection of specific methods to estimate exposure depends on the relative importance of environmental and biological measurements, the pathways of exposure, and the timing of related (hypothesized) outcomes by life-stage at each visit/contact. Temporal and spatial variability, along with developmental changes in children's physiology and behavior, were considered in selecting the combination of measures and questionnaire items. For example, in pregnancy, environmental measures with greater temporal stability are combined with short-term biomarkers and questionnaire responses on the frequency of source use to characterize both chronic and intermittent exposures. In contrast, environmental measures and questions are not included when the agent was unlikely to have an effect on the fetus (e.g., potential relation to birth outcome) independent of

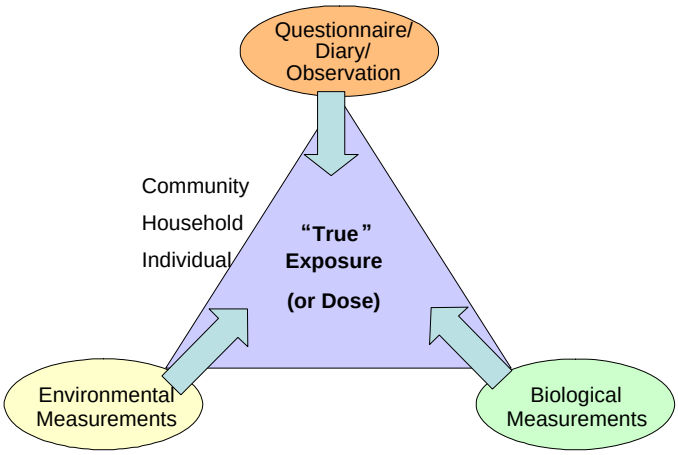


Figure 9-1. True Exposure as a Combination of Multimethod Assessments

that represented by a biomarker taken from the mother. The combined exposure assessment approach is summarized in Table 9-1, with specific measures by contact provided in Appendices F.1, G, and H. Approaches to assessing exposures for the major classes of chemicals are discussed below.

Persistent organic chemicals (POCs) include organochlorine pesticides, polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), polyfluorinated biphenyls (PFBs), perfluorinated chemicals (PFOA/PFOS), brominated flame retardants (PBDEs), and dioxins/furans. Chemicals in this class usually have half-lives of months or years in the environment. POCs are readily absorbed into the blood supply by passive diffusion and distributed into the fatty portions of organs, tissues, and breast milk. During pregnancy, POCs may also distribute in the fetal compartment. Assessment of maternal POC burden, and thus an indirect measure of fetal exposure, can be obtained from maternal blood taken before or during pregnancy and maternal blood, milk, or adipose tissue taken soon after parturition. Fetal and early life exposure to POCs have been hypothesized to be associated with numerous health outcomes, including neurodevelopment and thyroid function, later type 1 diabetes, and reproductive health at puberty.

Because valid biomarkers exist for POCs and are considered the gold standard for measuring persistent compounds (Needham et al., 2005), these will be the primary means of exposure assessment in the NCS. POCs will be measured in the mother's blood and urine in samples taken prior to and during pregnancy. POCs will also be measured in samples of breast milk, cord blood, and the child's blood and urine during early childhood. (See Appendix G for details of samples to be collected by contact.)

The purpose of testing at multiple time points is to determine when the exposure(s) occurred. Because POCs are persistent, it is difficult to determine if elevated levels in biological samples are due to past high-level exposures or recent lower-level exposures, yet this distinction can be important in understanding exposure-dose-outcome relationships. Thus, dust samples will be collected from the homes of all study participants that can be measured later for organochlorine pesticides and other POCs in the subset of mothers/children with high levels in biological samples. Collection of water samples in homes in rural communities, or where community water supplies are reported to have organochlorine

Table 9-1. Summary of NCS Chemical Exposure Assessment Approaches

Approach	Types of samples / Questionnaire domains	Target chemical/agent class (measures) / Topic areas (for questionnaires)
Biomarkers	Blood	PCBs, persistent and non-persistent pesticides, PBDE, perfluorinated compounds, PBDE flame retardant; perchlorate; lead, mercury, cadmium; bisphenol A
	Urine	PFBS, alkyl phenols, Hg(inorganic), As(speciated), perchlorate, halogenated phenols (PCP), phthalates, atrazine, OPs, carbamates, pyrethroids, EBDC/ETU,cadmium
	Breast milk	Dioxins/furans; organochlorine pesticides; PCBs
	Meconium	Cotinine, organophosphate metabolites
	Nails	Mercury (organic, inorganic)
	Hair	Cd, cotinine, mercury, nicotine
Environmental measurements	Indoor Air (Residence, child care locations)	Particulate matter (PM10), NO2, O3, CO VOCs, aldehydes and ketones,
	Outdoor air (community-level)	PM2.5, NO2, NOx, SO2, O3
		Pollen
	House dust	Allergens, endotoxin, mold, metals, pesticides (plus archives for future analyses)
	Potable water	Disinfection byproducts (BBPs), metals, coliforms, nitrate, perchlorate, pesticides
	Soil	Metals, pesticides
	Food	Metals, pesticides
Questionnaire, diary, or observation	Visual assessment	Housing, neighborhood characteristics
	Housing characteristics	Building age, renovations; heating/cooling systems/usage, clothes dryer, vaporizers, air cleaners, stove use, water for drinking and cooking, ozone sources, vacuum cleaner use, garage location and use, gasoline exposure, noise
	Occupational/hobby exposures	Types of jobs, activities, exposures
	Product use	Creams/lotions that are widely applied; cleaning products
	Pets and pesticide use	Type, method, frequency of application, and use protective equipment; number and types of pets, and exposure to flea/tick treatments
	Time and activity	Time spent at home, work/school, in-transit for work and non-work days
	Diet	Food-frequency questionnaire; three-day checklist; infant feeding/intake; eating behaviors (child)
	Related domains/topics	Environmental tobacco smoke, take home exposures, physical activity, household composition and demographics

contamination, will allow assessment of residual levels of organochlorine pesticides in drinking water. Methods will be comparable to those used in the U.S. Department of Housing and Urban Development / U.S. Environmental Protection Agency's First National Environmental Health Survey of Child Care Centers and the National Cancer Institute's New England Study of Environmental Health (NESEH).

Because dietary intake is another important exposure route, information on diet and food preparation will be collected at multiple times throughout the study (before and during pregnancy and during early childhood). Sample food items may also be collected based on those foods identified as being consumed most often. The mother's food frequency questionnaire, three-day checklist, and child's milk and food feeding forms (as used in the National Health and Nutrition Evaluation Survey [NHANES]) will be linked to national environmental contaminant databases, including the U.S. Department of Agriculture's Pesticide Data Program and Food and Drug Administration's Total Diet Study, as well as to a community database of environmental contaminants to be developed for the NCS. The use of flame retardant clothing for the child will also be assessed through questionnaires.

9.2.2 Non-persistent Organic Compounds

Non-persistent volatile organic chemicals (VOCs) include compounds in the air such as formaldehyde, benzene, vinyl chloride, other aldehydes, acrolein, ketones, and disinfection by-products in drinking water such as trihalomethanes (e.g., chloroform) and haloacetic acids.[1] Concentrations of these chemicals vary during short periods of time, depending on use of VOC-emitting products, smoking, ventilation in the home, and treatment and storage of community water supplies. Exposure to VOCs in utero and postnatally is hypothesized to increase risk of asthma, to reduce neurobehavioral and cognitive skills, and to impact the endocrine system and type 1 diabetes.

Although VOCs can be measured in biological samples such as expired air, blood, and urine, VOCs are rapidly metabolized and excreted so measurements made at a specific time will only address the exposures that occurred in the prior few hours. Multiple biological samples taken during pregnancy and childhood can be costly to the Study, burdensome to the participant, and logistically difficult to collect and store. Thus at least initially, biospecimens will not be collected for VOC analysis in the NCS.

Indoor air and drinking water samples will be collected for VOC analyses using methods that have been employed by studies such as The National Human Exposure Assessment Survey (NHEXAS), NHANES, and NESEH. Because of the temporal nature of VOC exposures, week-long average air measurements will be made multiple times during the Study, including pre-pregnancy, during pregnancy, and early childhood. Water collections will be made at homes served by a community water supply during pregnancy and early childhood; samples of the community supply also will be collected.

In addition, questionnaires will address use of VOC-emitting materials and products in the home and elsewhere, occupational and hobby related exposures, traffic exposures, smoking, and home ventilation factors. Observations in and outside the home will also identify sources of VOCs and ventilation.

Non-persistent semi-volatile organic chemicals (SVOCs) include organophosphate and carbamate pesticides, herbicides (including atrazine), polycyclic aromatic hydrocarbons, phthalates, halogenated phenols, alkyl phenols, and environmental tobacco smoke. Like exposure to VOCs, exposure to SVOCs in utero and postnatally is hypothesized to increase the risk of asthma, reduce neurobehavioral

[1] Haloacetic acids are not generally considered to be VOCs, but they are included in this document because it is a class of disinfection by-products of interest, along with trihalomethanes.

and cognitive skills, and impact the thyroid system. Non-persistent nonvolatile organic chemicals (NVOCs) include pyrethroids and other pesticides and phytoestrogens. They are hypothesized to increase the risk of compromised neurobehavioral and cognitive skills, to impact the thyroid system, and to increase risk of type 1 diabetes.

Though SVOCs and NVOCs are generally rapidly metabolized and excreted, there are valid biomarkers for some of these compounds; they must, however, be measured at multiple times. The mother's urine will be collected before and during pregnancy and at birth, the father's urine will be collected during pregnancy, and the child's urine will be collected during early childhood. Organophosphate pesticide metabolites also will be measured in the meconium. Since potentially non-toxic pesticide metabolites may be present in environmental samples, bio-specimens tested for those metabolites may be positive even without exposure to the actual pesticide (Morgan et al., 2005). Thus, environmental samples can be analyzed for both the pesticide and the metabolites to help interpret positive bio-markers.

To assess exposure to environmental tobacco smoke, nicotine and cotinine will be measured in the hair and urine of the mother before, during, and after pregnancy; of the child during infancy; and of the father. The analysis of hair and urine at multiple times will allow for characterization of recent and chronic exposure of the child to environmental tobacco smoke. Interview questions addressing parental tobacco use in the home will be asked at multiple visits, and nicotine may also be measured in house dust during early childhood to help differentiate between inhalation and dermal exposures.

Pesticides will be measured in air, dust, water, and soil before birth and during early childhood, since usage patterns may change during pregnancy and after the birth of a child. To reduce the number of samples that are likely to have non-detectable measurements, air samples for the semi-volatile and non-volatile pesticides will be collected only when a recent application is reported, or in agricultural areas. Dust samples will provide a long-term indicator of potential for exposure. Water and soil samples for pesticides will be collected only in rural areas. Polycyclic aromatic hydrocarbons will be collected in air samples during early childhood.

SVOC questionnaire and observation items will elicit the types and frequency of insecticide use, including flea control products and lice or scabies treatment, smoking, combustion sources, product use, and dietary consumption and sources of foods. The mother's time and activity patterns, including commuting patterns, will be assessed during pregnancy. Questions about the child will include teething and pacifier use, and mouthing of hands and objects which may result in greater exposures to contaminants in dust or on surfaces and toys. Combining these measures with the biologic and environmental samples should allow for identification of both acute and chronic exposures.

9.2.3 Inorganic Chemicals

Bioaccumulative inorganic chemicals include lead, mercury, and cadmium. These metals persist in the environment. Because they are slow to metabolize and excrete, they accumulate in the body as the element itself or as organometallic compounds. Exposure to bioaccumulative inorganic chemicals in utero and postnatally is hypothesized to impact the endocrine system, timing of puberty, neurodevelopment, and type 1 diabetes in the child. Valid biomarkers exist for bioaccumulative metals (Needham et al., 2005) and will be used for exposure assessment in the NCS. Lead, mercury, and cadmium will be measured in the mother's blood before and during pregnancy, cord blood, and in the child's blood during early childhood. Cadmium and inorganic mercury will be measured in the mother's urine before and during pregnancy and in the child's urine during early childhood. In addition, inorganic and organic mercury will be tested in the mother's nails during pregnancy and the child's nails during

early childhood. Cadmium and organic and inorganic mercury will be measured in the mother's hair pre-pregnancy and during pregnancy, the father's hair during pregnancy, and the child's hair during early childhood. Hair and nails account for a longer term exposure and are easy and cost effective to obtain and to store. These specimens will be collected at visits throughout childhood.

As with the persistent organic compounds, relevant environmental samples will also be collected and stored so that those with high biomarker concentrations (and a sample of others) can be analyzed to help determine whether the high concentrations are due to current exposures. Lead and cadmium will be measured in air and soil samples several times before, during, and after pregnancy. Lead also will be measured in house dust and drinking water during pregnancy and during early childhood. Methods will be comparable to those used in NHEXAS, the National Survey of Lead and Allergens in Homes, and NESEH. Mercury measures in the environment are not included as core Study measures; however, this could be done as an adjunct study in areas where this is of concern.

Non-bioaccumulative inorganic chemicals include arsenic, chromium, manganese, nitrate, and perchlorate and can be measured in air, dust, water, food, and soil. These chemicals are readily absorbed into the body; some distribute to various tissues and others are rapidly excreted. Exposure to non-biaccumulative inorganic chemicals in utero and postnatally is hypothesized to impact the child's thyroid system, neurodevelopment, and type 1 diabetes. In general, measurements of these chemicals in hair will offer a longer-term dosimeter for exposure, while urine will provide an assessment of more recent exposure.

Perchlorate, manganese, and other metals will be measured in the mother's breast milk. Arsenic (speciated) and perchlorate will be measured in the mother's urine before and during pregnancy and at birth, in the father's urine during pregnancy, and in the child's urine during early childhood.

Manganese will be measured in air samples taken at multiple times during the Study, since inhalation may be the most toxic route of exposure. Arsenic will be measured in house dust and drinking water during pregnancy and during early childhood, in soil samples around the house and near CCA-treated wood during early childhood. Nitrate and perchlorate will be measured in drinking water only in rural areas.

Questionnaires and observations will focus on the home's age, renovations, and source of drinking water. Observations and geographic information system (GIS) data will also identify nearby industrial sources. Occupational and hobby questions will highlight metals exposure at work or brought home, as well as other occupation-related exposures. Dietary ingestion is likely to be an important exposure route for most metals. Thus, as for persistent organic chemicals, dietary consumption information will be collected before and during pregnancy and during lactation for the mother, and after birth for the child. Food preparation information during pregnancy also will be collected.

9.2.4 Criteria Air Pollutants

Criteria air pollutants include particulate matter (PM), carbon monoxide (CO), nitrogen oxides, and ozone. Air pollutant exposure depends on ambient concentrations, season, traffic patterns, and indoor combustion and ventilation. Biomonitoring currently has a limited role in the assessment of exposure to criteria pollutants. While CO can be measured in blood or expired air, this is burdensome and reflects only short term exposures. Exposure to air pollutants during pregnancy, infancy, and childhood is hypothesized to increase the risk of asthma and wheezing.

Active and passive sampling and measurement using direct reading instruments are the most common form of environmental assessment tools for these air pollutants. In general, biomonitoring has a limited role. While personal sampling better reflects an individual's exposure, the logistical and burden demands of personal sampling have led the NCS to adopt indoor sampling in rooms or areas where the participants spend the most time. Particulate matter (<10 micron) samples will be collected before, during, and after pregnancy to assess the variability in exposures over time and season. Nitrogen oxides and CO will be measured during pregnancy and during early childhood. Ozone will be measured starting in early childhood and only in homes with ozone sources present. Methods will be comparable to those used in NHEXAS.

PM measurement and some gaseous air pollutants may also occur at the community level, where existing monitoring sites fail to provide coverage of the selected communities or where spatial variability is high due to local sources. Adjunct studies may also explore person-level exposures for various pollutants, e.g., traffic-related exposures, to help evaluate and adjust for measurement error in assessments based on measures of locations by accounting for individual differences in proportions of time.

Questionnaire items and home observations will focus on heating and cooking fuels and use patterns, exhaust ventilation of the home, and ozone sources. Additionally, observational and GIS data will identify sources of air pollutants in the neighborhood. Activity patterns on working and non-working days will be collected, including time spent in different locations and information on in-vehicle exposures.

9.3 Physical Exposure Measures

As children mature their physical environment, and thus many environmental exposures, expand from being primarily the home to a broader array of locations, including childcare settings, schools, neighborhoods, and recreational facilities. The NCS will collect data describing the physical exposures at a number of these locations through the child's life. Resource and participant burden considerations will limit data collection activities at each of these locations. Since even up to age 5 the average child spends the majority of time indoors at home [up to 16 hours daily (Hubal et al., 2000)], the child's home environment(s) receives the greatest consideration early in the Study.

The NCS will adopt many of the basic questionnaire items and observations from already established surveys, such as the American Housing Survey, but in the interest of participant burden, it will limit questions to factors related to chemical and biological exposures in the environment, injuries to children, opportunities for recreation and physical activity, and access to services such as health care and shopping. A summary of assessment approaches for the physical environment exposures appears in Table 9-2 below, and detailed information appears in Appendix H.

9.3.1 Housing Characteristics and Condition

Housing characteristics generally describe the physical configuration and condition of the residential structure. The physical configuration includes such information as the date of construction; materials used for the initial construction and subsequent renovations; the heating, ventilation, and air conditioning systems; the electrical and plumbing systems; floor covering; the presence of major appliances such as stoves and fireplaces; and aspects of the land on which the residential structure is built. Housing condition refers to the existing state of maintenance of the house, including structural integrity, presence of functioning electrical supply, integrity of painted surfaces, and the coverage of the site by

grass, dirt, or other material. Additional housing-related physical exposures include indoor temperature and humidity, and noise.

Table 9-2. Summary of NCS Physical Exposure Assessment Approaches

Approach	Domains	Topics within domain
Questionnaire or observation	Housing characteristics and condition	HVAC, structural integrity, integrity of painted surfaces, insects/rodents, floor covering, presence of major appliances, fireplaces and exhausts
	Visual assessment of neighborhood	Type and condition of housing, presence and type of businesses, industries, recreational areas, graffiti, safety, traffic
Maps and databases	Geographic information systems data	Superfund and brownfield sites, Superfund Amendments and Reauthorization Act (SARA) reporting sites, nearest hospitals/medical care, recreational facilities, traffic
	Aerial photography	Geographical details (rivers, mountains, highways)
	Census data	Population density

Household characteristics and condition will be assessed through a combination of participant questionnaires and direct observations by Study personnel (see Appendices F.1 and H). To minimize burden, questions for the participant will be limited to those that cannot be easily observed, e.g., date of construction and use of special filters on the ventilation system. Most housing characteristics will be recorded via direct observation. Observation forms will parallel those used in many other studies, including HUD/NIEHS National Survey of Lead and Allergens in Housing (Vojta et al., 2002), HUD/EPA's First National Environmental Health Survey of Child Care Centers (Marker, Fraser, & Viet, 2001), HUD/EPA's American Healthy Homes Survey, and the Cincinnati Children's Environmental Health Center–HOME Study.

Most housing characteristics and conditions are relatively stable in the short-term and therefore require only periodic updating. In consideration of participant and study burden, assessments will focus on the characteristics and conditions most relevant to the current life stage of the Study children. For example, recording condition of paint on walls and sills, or recent home renovations, will occur throughout the Study since potential lead or other exposures are germane during pregnancy as well as childhood. However, home safety assessments potentially related to childhood injury (e.g., burns, falls, poisoning, drowning) will be initiated after the birth of the child.

In addition to the physical assessment of the child's home, the NCS plans to collect similar data from sites of child care and, as the Study progresses, schools. The extent to which these assessments will be obtained through direct observation by Study personnel, through self-assessment by the child care site, or through other means, has not yet been determined and will be influenced by a number of factors including cost and ability to gain access to the various child care sites.

9.3.2 Neighborhood Characteristics

Neighborhood characteristics extend the description of the child's environment beyond the boundaries of the structure in which they live. The influence of a child's neighborhood environment on his or her development may arise from both physical characteristics (e.g., amenability to outdoor activity or proximity to a hazardous waste site) and social factors (e.g., community cohesion and collective efficacy). Although the physical and social aspects of a neighborhood are related, this section

concentrates on the assessment of the neighborhoods' physical characteristics and leaves the description of social factors to Section 9.4.

Features of the physical or built environment of interest to the NCS include conditions that influence physical activity, safety, access to nutritious foods, and exposure to chemicals. Numerous aspects within each of these four features will be measured to ensure comparability of findings from the NCS to previous studies that examined health effects related to the physical or built environment. Aspects of these four features frequently overlap or are inter-related. These aspects include, but are not limited to: population density; residential density; neighborhood vegetation or green space; land use mix; safe walking/cycling locations; high speed traffic; heavy traffic; proximity to intersections of major highways or railroads; intersection density (connectivity); lack of crosswalks and sidewalks; access to trails; density of bus and subway stops; urban sprawl; dependence on motorized transportation; and street connectivity.

The physical or built environment is of increasing interest primarily because of its linkages with physical activity and obesity. Residential and population density promote mass transit usage and walking as a means of transportation (Frank, Andresen, & Schmid, 2004; Frank, Engelke, & Schmid, 2003; Ross & Dunning, 1997; Saelens, Sallis, & Frank, 2003). Street connectivity, or the density of intersections, affects the ease with which individuals can walk in a direct path to their destinations (Frank et al., 2003). Mixed land use is a good predictor of walking because areas with a higher mix of commercial and nonresidential destinations facilitate walking as a means of accomplishing daily activities, which reduces the risk of obesity (Frank & Pio, 1995; Handy, 1996; Sallis, Saelens, & Kraft, 2004). Access to leisure facilities including public gymnasiums, swimming pools, and soccer fields is related to recreational walking, but not to walking as a means of transportation (McCormack, Giles-Corti, & Bulsara, 2007). Access to commercial food sources are consistently related to walking for transportation, however, the type of store is equally notable as convenience stores often stock unhealthy foods (Sallis, 2007). Urban sprawl, as measured by a number of different indices, has been consistently associated with risk of obesity among U.S. adults (Ewing, Schmid, Killingsworth, Zlot, & Raudenbush, 2003; Lopez, 2004) and adolescents (Ewing, Brownson, & Berrigan, 2006). The underlying mechanism of urban sprawl's effects (decreased population density, low street connectivity) may originate from greater dependency on motorized transportation, decreased ability to walk to destinations, and environmental degradation such as more greenhouse gas emissions and reduction of open spaces that facilitate walking and physical activity (Lopez, 2004).

Different aspects of the built environment may interact with or confound each other when predicting likelihood of physical activity and obesity. Low urban sprawl (more sidewalks, greater population density) may facilitate walking for exercise, but may be associated with higher crime rates, which deters walking. Therefore, the inter-relations among aspects of the built environment are complex and require the assessment of multiple characteristics of the built environment.

The built environment has also been linked with outcomes other than obesity. Children living in census tracts that faced intersections with highways or railroads had a 60 percent increased risk of developing asthma compared to children who did not live in census tracts facing intersections, after adjustment for individual- and neighborhood-level covariates (Juhn et al., 2005). Neighborhood physical environment characteristics may be associated with chemical exposures, including the presence of industrial facilities such as incinerators, recycling facilities, chemical manufacturing or mining operations, and hazardous waste sites.

The methods by which aspects of the built environment will be obtained in the NCS include parental report interview, direct observation by Study personnel, and the examination of existing maps and databases. In the NCS, the majority of objective information concerning the neighborhood physical environment will be obtained via direct observation using standardized tools. Items to be recorded as

observed from the dwelling structure are presented in Appendices E and H. Though standardized and relatively objective assessment of the built environment is a comparatively recent development, numerous instruments have been developed (e.g., SPACES, Irvine-Minnesota Inventory). These and other similar instruments will be considered for use on the NCS. The selected instrument must be well-validated in several different settings and must capture a range of built environment characteristics.

To obtain information that cannot be observed from the home, Geographic Information Systems (GIS) may be used to identify industrial areas and facilities in the neighborhood and may be integrated with aerial photography (where available) and local census data to measure aspects of the built environment objectively such as intersection density, street connectivity, population density, residential density, and land use mix. This secondary type of community-level data will be linked to the NCS data.

In addition to observational and GIS data, the NCS will also collect respondents' perceptions of their neighborhood via questionnaires. Although objective measures obtained by GIS and by measures of individuals' perceptions of the built environment have poor agreement, each is independently associated with physical activity (McGinn et al., 2007). Thus, it is important to address both objective and perceived measures of the built environment in the NCS.

9.4 Psychosocial Exposure Measures

Psychosocial and behavioral factors have broad-reaching effects on children's health and well-being, and are linked to key Study outcomes. The identification of psychosocial domains to be included in the NCS is based on the exposures and covariates named in the core hypotheses, on developmental white papers commissioned by the Study, and on workshops held on specific topics (e.g., parenting, racism/discrimination, media exposure, prenatal stress, gene-environment interactions). The measures have been selected or adapted from established, well-validated, standardized instruments utilized in other (primarily epidemiologic) studies.

An important requirement for the selected measures is that they have the ability to measure the same construct (e.g., parenting practices) reliably through time and to capture changes in exposures to the individual child through different developmental stages. They should also have the ability to track societal changes (e.g., changes in racism/discrimination) or trends across time. The most reliable way to document these influences on child development is to measure the same construct at each participant contact. Given the breadth of exposure domains in this large, complex study, and the broad scope of psychosocial factors (family influences, child care, media, neighborhoods, socioeconomic status, school environment, etc.), repeating all domains at every visit would create interviews that far exceed a reasonable participant burden. The schedule for administration of psychosocial measures has therefore been designed to provide data primarily at critical time points for each domain. Decisions were based on careful deliberations and the advice of many outside experts. Criteria used in the evaluations of measures included sound psychometric properties, logistical feasibility (time, burden) for testing in the home, and flexibility of administration modalities to minimize respondent burden and cost. Other criteria used for selection of measures were that they be relatively easy to administer and score, be unbiased (e.g., against low-income, minority or cultural groups), and be sensitive to individual variations. An important consideration also included suitability for translation into multiple languages.

As with the chemical exposures, these measures also involve hierarchical data. There will be community level data (e.g., crime levels, percent of inhabitants on welfare), neighborhood effects (e.g., collective efficacy), school characteristics, and individual exposures. These factors will interact with each other in creating a child's psychosocial environment and can be dealt with analytically through hierarchical analyses (see the Statistical Analysis Plan, Chapter 10). A summary of assessment

approaches for the psychosocial exposures appears below in Table 9-3; a list of questionnaire, interview, and direct assessment measures at each visit up to age 2 can be found in Appendix F. Most of the psychosocial domain will involve interviews with relevant respondents, observations in the home, and in some cases, verification with biological specimens (e.g., catecholamines and cortisol for stress).

Table 9-3. Summary of NCS Psychosocial Exposure Assessment Approaches

Approach	Domains	Topic areas
Questionnaire or observation	Demographics and culture	Household composition, age, ethnicity, country of origin, languages spoken in the home, income, education, religious affiliation, employment, resources
	Family process/environment	Family structure, parenting, dyadic relationships, home environment, domestic violence
	Maternal depression	Prenatal, postnatal depression
	Psychosocial stress	Prenatal life events, perceived chronic stress, racism/discrimination
	Social support	Emotional support, instrumental support, network support
	Neighborhood and community	Collective efficacy, social cohesion
	Health behaviors	Smoking, alcohol consumption, physical activity, substance abuse
	Child care and schools	Structural and qualitative aspects
Biomarkers	Saliva	Cortisol (diurnal variation)
	Urine	Cotinine
Extant databases	Neighborhood and community	Examples reviewed for possible use: Subsidized households; Neighborhood Change Database; School District Data Book; Uniform Crime Reports; Office for Civil Rights Census of Schools; Census of Agriculture; County Business Patterns; occupational employment statistics; American Housing Survey Area Resource File; Behavioral Risk Factor Surveillance Survey; Bureau of Economic Analysis: data on per capita income; Gardiner Tobacco Data Health Care Finance Administration File; NCHS Compressed Mortality File; NCHS Vital Statistics Data and Death Index; state and local employment and unemployment rates; State and Metropolitan Area Data Book; etc.

9.4.1 Family Process/Environment

Family environment has a consistent and enduring influence on a child's social, emotional, and cognitive development. The security of attachment the child has with parents or primary caregivers influences their relationships with teachers and friends (Shonkoff & Phillips, 2000), memory processes (Belsky, Spritz, & Crnic, 1996; Kirsh & Cassidy, 1997), self-concept (Verschueren, Marcoen, & Schoefs, 1996), and conscience development (Kochanska, 1995, 1997). Parenting practices and home environments are also an important source of cognitive stimulation for literacy and numeracy skills, as

well as for language development (Snow, 1993; Ginsburg, Klein, & Starkey, 1998; Bradley et al., 1989). In addition, the family environment strongly influences a child's learning of self–regulation (Cummings & Davies, 1994) and conflict resolution (Thompson, 1988). Parents act as managers of their children's environment and influence them through multiple pathways, including parent-child interactions, parenting knowledge and attitudes, cognitive stimulation, and stress modulation.

The family environment as it is defined in the NCS includes household structure, the quality of relationships among household and family members, media use, domestic violence, division of labor, parenting behaviors, and parental mental health and cognition. Due to flexible family configurations tied to divorce, remarriage, and non-married cohabitation, the family environment requires re-assessment at regular intervals. Parenting ability can also vary at different developmental stages and should be measured longitudinally. The measures described below are those that have been selected for pregnancy and early infancy. For time periods when each is measured see Appendix F.1. These constructs will be measured throughout childhood and adolescence, although the specific instruments may vary according to developmental stage.

Family environment will be measured in early childhood with the Infant/Toddler Home Observation for Measurement of the Environment (IT-HOME), an observational instrument designed to describe the attributes in a young child's environment that contribute to social and cognitive development. It is designed for infancy from birth to age 3 and has six subscales: parental responsivity, acceptance of child, organization of the environment, learning materials, parental involvement, and variety in experience. It has been used extensively in multiple longitudinal studies (National Institute of Child Health and Human Development [NICHD] Study of Early Childcare; National Survey of Child and Adolescent Well-being; National Longitudinal Survey of Youth). The HOME can be used at multiple stages of child development.

The quality of the marital or partner relationship impacts parenting competence, and interacts with parental mental health and domestic violence in predicting child outcomes. The Dyadic Adjustment Scale (DAS-7) will be used to assess the quality of the parental relationship. This short version scale, validated by Hunsley, Best, Lefebvre, and Vito (2001), provides three relationship subscales: dyadic consensus, dyadic cohesion, and general satisfaction. It will be administered early in pregnancy and periodically throughout childhood.

Domestic violence will be measured with the Modified of Abuse Assessment Screen (AAS), a clinical instrument that measures frequency and severity of abuse of women. Test-retest reliability is high, and it has been validated in ethnically and socioeconomically diverse samples. This measure will be administered to the mother early in pregnancy and at later stages of her child's life in a way that allows her to respond accurately even if the husband is present in the room.

Division of child care responsibilities within the family will be assessed using the "My Time as a Parent" measure, which has been validated in major longitudinal studies (NICHD Study of Early Childcare). This will be measured after the child is born, and at age 6 months as the duties of both parents increase.

Parenting practices and behaviors will be assessed in both mothers and fathers throughout childhood. In early childhood, specific questions will be similar to those used in the Early Childhood Longitudinal Study–Birth Cohort (ECLS-B). Domains will include wantedness of the child, parenting activities and practices involving decisions, looking after the child, meals, and attitudes about being a parent. Observations of standardized parent-child interactions will also be videotaped for later coding. The Three Boxes Task is a semi-structured activity completed by the parent and child in interaction. Parental sensitivity, parental intrusiveness, cognitive stimulation, parental positive regard, parental

negative regard, and parental detachment are assessed. Three scales assess child behavior: engagement with the parent, sustained attention, and negativity toward the parent. The Three Boxes Task has one of the few coding systems that can be used in large-scale studies, has good psychometric properties, and produces robust scores predictive of later development in both cognitive and socioemotional domains. It has been validated in several large-scale studies (NICHD Study of Early Childcare; Early Childhood Longitudinal Study–Birth Cohort [ECLS-B]; Early Head Start Research and Evaluation project).

Media use will be assessed by questions to the mothers throughout childhood beginning when the child is six months old. Topics will cover the amount of time the television or radio is on; how often the child watches television or movies, plays video games, or listens to music; the extent of exposure to books and other reading material; and the content of the media exposure.

Parental mental health and cognition will be measured in both mothers and fathers. Domains include intelligence, literacy, depression, and anxiety. Parental depression, which influences not only parenting behavior but is also a potent stressor during pregnancy (Lundy et al., 1999) will be measured using the Center for Epidemiological Studies–Depression scale (CES-D)(Radloff, 1977) during and after pregnancy. Anxiety, also an important influence on parenting, will be assessed with the Spielberger State-Trait Anxiety Inventory (STAI) scale.

To measure potential genetic and cognitive influences of parental IQ on the child, the Kaufman Brief Intelligence Test, Second Edition (KBIT-2) will be used. It has the advantage of including a non-verbal scale (in addition to a verbal one) which is relatively invulnerable to SES factors and language background. It yields scores similar to other intelligence tests, with a mean of 100 and a standard deviation of 15, making it possible to compare KBIT-2 scores with other measures of IQ. The Woodcock-Johnson-III Tests of Achievement Letter-Word Recognition subtest, which measures the individual's word decoding skills, will also be administered.

9.4.2 Psychosocial Stress and Social Support

Psychological stress is the distress experienced by individuals who feel overwhelmed and unable to cope with the demands in their lives. These demands may arise from varying or multiple sources (work, partner relations, family responsibilities, financial insecurity, social isolation, neighborhood issues, racism, etc.) but the emotional experience of distress is the mediator of the detrimental physiological and behavioral responses that occur. It is important to understand that the outside demands that may be a source of distress for one person will not necessarily be stressful for another. It is the experience of distress that starts the cascade of physiological reactions known as the "stress response." For these reasons, we will measure both the participants' global experience of stress, and the sources that may be affecting it. To qualify for the NCS, measurement of these exposures must be germane to pregnancy and/or parenting, or, in the child, to developmental outcomes. They must have high validity across varying ethnic groups, socio-economic levels, urban/rural settings, and religions; and include good psychometric properties and low subject burden. Interview questions will be taken from already validated measures and biological measures of stress hormones (cortisol in saliva). To understand why one person tolerates more demand than another, factors that can serve as buffers against stress (e.g., social support) will also be assessed. Due to the changing nature of stressful situations and the increase in effect when they become chronic, these measures will be administered to parents and children at repeated time points throughout the Study, starting in pregnancy.

Global perceived stress will be measured with Cohen's Perceived Stress Scale (PSS) during pregnancy and after birth. The questions are general and are relatively free of content specific to any sub-population group.

Racism/discrimination, a possible source of stress in some populations, will be measured in the mother during pregnancy and in early childhood with the Experiences of Discrimination (EOD) questionnaire, modified for the Coronary Artery Risk Development in Young Adults (CARDIA) Study. This questionnaire was chosen because it has been validated in other national studies (e.g., the National Study of Youth and Religion) and because it also allows for the measurement of discrimination based on sexual orientation or disabilities. As the child develops, measures of discrimination will include other (e.g., school) environments relevant to the child. While empirical studies of discrimination have been done on African Americans, little research has been done to address systematically how prejudice and discrimination affect other racial/ethnic minority groups (Cain & Kington, 2003). Given the existing health disparities in this country, a measure of discrimination will be pertinent not only as an independent predictor of family influences on child development, but also as a covariate in other hypotheses related to socioeconomic status.

Prenatal life events refer to stressful life events that have happened to the respondent or a spouse or a partner since the respondent became pregnant. Prenatal life events have shown associations with birth weight and birth outcomes (Lobel, 1994). The Prenatal Life Events Scale (PLES) was developed for use in pregnancy and adapted from Epidemiological Catchment Area studies.

Parenting stress will be measured in early childhood with a validated short form of Abidin's Parenting Stress Index (PSI) (from the NICHD Study of Early Child Care). This instrument is designed to identify parent-child systems that are under stress and at risk for development of dysfunctional parenting. The PSI has good psychometric properties and is appropriate for use with parents of infants.

Family/work stress will be assessed with the Work and Family Conflict Scale, which measures strains associated with combining work and family. This scale has been validated in other large scale studies (NICHD Study of Early Childcare). It will be administered in early childhood and periodically throughout childhood.

Financial stress questions have been adapted from several large studies (Fragile Families; ECLS-B; U.S. Department of Agriculture Food Security Scale) and include owning a home, having a bank account, being able to pay the monthly bills, and having food security. These questions will be administered several times throughout the Study.

Social support will be assessed with Sarason's Social Support Questionnaire (SSQ) Short-Form. An extensive program of research using the SSQ, both Long-Form and Short-Form, shows the SSQ to be valid and highly internally consistent. The SSQ is a quantitative and qualitative measure of social support. Social support in the mother will be measured early and late in pregnancy and again during early childhood. Later, it will be measured in the child.

9.4.3 Neighborhood and Community

This section outlines the methods to be used in the assessment of a child's neighborhood's social characteristics (e.g., social cohesion, collective efficacy, safety, social capital, crime statistics, and average SES level) that may influence health and development. Assessment of the neighborhood's physical attributes was described in section 9.3.2.

Multiple sources will be utilized for neighborhood measures in the NCS. Extant databases can be used to provide data such as crime statistics, unemployment rates, average income and education, and disparity measures. The participant's subjective evaluation of relevant neighborhood characteristics will be assessed by interview. A specific measure that may be used is an adaptation of the Neighborhood

Environment for Children Rating Scales, used in the Project on Human Development in Chicago Neighborhoods (Coulton, Korbin, & Su, 1996). Examples of the neighborhood attributes described by this tool include the participant's evaluation of social capital (e.g., community organizations), collective efficacy, extent of institutions and social services (Coleman, 1988; Sampson, Raudenbush, & Earls, 1997).

9.4.4 Child Care/Schools

For children receiving non-parental child care, the potential influence of that care on their development may be through one, or both, of two broad areas. The structural aspects of child care outside the home and schools include the amount of time a child spends in care outside his or her home; whether it is home-based or center-based care; the training and experience of the child care providers; the ratio of children to caregivers; and the age ranges of the other children. Alternatively, qualitative aspects of the care received by the child may include activities providing cognitive stimulation; discipline techniques; and stressors (e.g., noise, bullying, violence, racism/discrimination) inherent in the child care or school environments. Normal developmental progress of the child, changes in child care arrangements, and school advancement necessitate repeated measures as structural and qualitative aspects of care change as the child moves from context to context over the course of development. Structural aspects of a child's early child care experience can be collected through a variety of methods, including parental report, reports from care providers at the facility, or through direct observation of the environment by Study personnel. Qualitative aspects can also be assessed through those modalities; however, the "gold standard" for assessing qualitative aspects is by direct observation using a structured instrument.

In the NCS, both structural and qualitative aspects of child care will be ascertained through maternal report. A number of large studies in the U.S. have collected information about structural and qualitative aspects of child care, including the National Child Care Survey, the National Household Education Survey, the Early Childhood Longitudinal Study-Birth Cohort, and National Longitudinal Survey of Youth. The NCS will use similar instruments to those used in these large studies.

As described in Section 9.3.1, the NCS plans to collect direct observations from at least a sub-sample of participants' child care settings. The Study of Early Child Care and Youth Development, and the Early Childhood Longitudinal Study – Birth Cohort, both used direct observation to collect a combination of structural and qualitative data. A tool will be adopted from those instruments used in these studies.

9.5 Biological Exposure Measures

A child's biologic environment covers a swath of potential exposures, from in-utero interaction with maternal physiology (e.g., maternal glucose metabolism, thyroid hormone levels, or response to infection) to direct contact (primarily, but not solely, after birth) with allergens or infectious agents. As will be the case with all NCS data collection, a balance must be struck between relying on biologic samples and tests considered "diagnostic" or "gold standard," particularly in a medical or clinical research setting, and those appropriate for use in a large, diverse, population-based epidemiologic study. Discussion of maternal glucose metabolism assessment in relation to the potential association with serious structural birth defects (Section 9.5.3) elucidates some of the trade-offs faced in collection of biologic exposures. In addition to the biospecimens, information relevant to biologic exposures will be collected via other modalities. For example, history of recent infectious disease can be obtained through a questionnaire or a health diary.

The broad implications of an individual's genetic characteristics, and their interaction with environmental exposures, including chemical, psychosocial, and biologic, are discussed below. A summary of the assessment approaches for biological exposures appears in Table 9-4, and in detail in Appendix I.

Table 9-4. Summary of NCS Biological Exposure Assessment Approaches

Approach	Types of samples / Questionnaire domains	Target analytes (measures) / Topic areas (for questionnaires)
Biomarkers	Blood (maternal, child, or cord)	Cytokines and chemokines, immunoglobulins, Hgb A1c, fasting glucose and insulin, lipids, adipokines, thyroid studies, corticosteroid studies, estrogens, progesterone, dietary antioxidants, folate, CBC, lymphocyte subsets, DNA, RNA
	Urine (maternal)	Infection (PCR)
	Breast milk	Cytokines and chemokines, immunoglobulins, macro and micro nutritional components
	Placenta, umbilical cord	Histology for inflammation and infection, cytokines and chemokines, immunoglobulins, DNA
	Saliva (maternal)	Cortisol, periodontitis-specific IgA
	Vaginal swabs	Gram stain, cytokines and chemokines, metalloproteinases
Environmental measurements	House dust	Endotoxin, pollens, molds, other allergens
Questionnaire, diary, or observation	Housing characteristics	Mold, pet-related and other allergens
	Health behavior and status (maternal or child)	Recent illness or fever, chronic conditions, mental health, dental health, reproductive history, health care use, stress, sleep, physical activity, diet and nutrition, medication and supplement use
	Family medical history	Child's parents, siblings, grandparents, aunts, uncles

9.5.1 Allergens

The development of asthma in particular, and atopy in general, may be strongly influenced by early-life antigenic exposure. The appropriate development of antigen-specific immune response, and the general evolution to a mature TH-1 inflammatory response, is likely influenced by the interplay between timing of initial infection with viral or other infectious agents and contact with microbial or other antigens. Differences in the timing of initial exposure to allergens, the nature of the specific antigenic exposure, and whether the exposure was preceded by a viral or other infection may help explain contradictory findings suggesting that early infections can be both protective for asthma (hygiene hypothesis) and associated with an increased risk of asthma. Elucidating the contributions of allergic exposure, infection, and inflammation to asthma and other inflammatory-related conditions will be an important challenge for the NCS.

Common allergens of interest to the NCS include cat, dog, mouse, rat, cockroach and mite antigens, and multiple varieties of pollen and molds. In addition, the TH-1 inducing effects of lipopolysaccharide endotoxin suggests that, when not associated with sepsis or an overt bacterial infection, the health-related effects of endotoxin exposure are more closely related to "allergic" response than to any potential infection from the endotoxin-producing organisms.

Biomarkers for allergen exposures include specific immunoglobulin measures in the mother during pregnancy and at birth and in the cord blood at birth. Because the child's blood may not demonstrate specific antigenic response in infancy, and because blood will not be drawn from the child until age 1, environmental samples are important for assessing early allergen exposure. Many of the above allergens, including endotoxins, will be measured in household dust samples. Methods will be comparable to those used in HUD's National Survey of Lead and Allergens in Homes, HUD/EPA's First National Environmental Health Survey of Child Care Centers, and NHANES. Assessment of pollen exposure will rely on established monitoring data in conjunction with regional pollen studies, where supplemental data is needed.

A panel of 36 mold species can be measured in the mold dust samples using a mold-specific Quantitative Polymerase Chain Reaction (QPCR) method developed and licensed by the EPA. This method was selected in lieu of traditional culture methods because of its quantitative nature and simplicity of sample collection. Only 5 mg of sieved vacuum dust is required for analysis, and samples can easily be stored and analyzed later.

Relevant questionnaire items will focus on recent home renovations, activities used to control allergens in the home, the infant's bedding and sleeping environment, and the presence of dogs and cats in the home. Household observations will include assessment for mold sources both in and outside the home.

9.5.2 Infections and Inflammatory Mediators

Maternal or early childhood exposure to different microorganisms (manifest bacteria, viruses, and fungi) has tentatively been implicated in the development of several health outcomes of interest to the NCS, including neurodevelopment and psychiatric disorders, asthma, and type 1 diabetes mellitus. In addition, links between maternal genital tract infection and preterm birth are well-recognized. In contrast to the immediate and direct suppurative effects of infection, such as the cognitive and hearing loss associated with bacterial meningitis, some of the association between infection and the above outcomes is thought to be due to the distal influence of host inflammatory mediators produced in response to infection, as well as to the influence of infection on the maturation of a child's developing immune system.

Identification of infection and inflammation in a medical or a clinical research setting generally involves microbiologic or biochemical analysis of biospecimens. Current or recent infection or colonization with specific organisms can be identified through culture, through molecular fingerprinting (e.g., PCR DNA amplification), or through direct visualization (e.g., Gram stain and microscopy). Host immunoglobulin response to specific organisms can identify recent or historic infection. Non-specific inflammatory response is diagnosed using combinations of up and down regulating cytokines as well as other non-specific markers such as C-reactive protein (CRP).

The NCS will obtain multiple biospecimens from the mother and the child, at multiple times, to enable assessment of infectious and inflammatory exposures. Specimens will be collected, processed, and stored in such a manner that all the laboratory modalities listed in the paragraph above will be possible, with the exception of culture. The logistic difficulties of assuring standardized handling of multiple samples in a field setting and the variety of culture media and techniques needed to grow the plethora of organisms of potential interest make culture untenable for the NCS. Examples of biospecimens to be obtained include maternal blood and urine (once before pregnancy from women in the pre-pregnancy cohort and at several times during pregnancy for all women); vaginal swabs during

pregnancy for Gram stain and for cytokine identification; cord blood and neonatal heel stick blood; breast milk to enable analysis of immunoglobulins or other maternal factors transferred directly to the child after birth.

In addition to the biospecimens, other modalities will be used to collect indirect data relevant to infectious and inflammatory processes. Questionnaires will assess history of infection and fever in the mother and child. Information collected on family composition, particularly siblings, and on child care and school attendance can be used as proxies for viral exposures. The health care visit log will enable tracking of physician visits related to infections. Dust samples collected from the home prior to birth and during infancy provide estimates of early life exposure to endotoxins.

9.5.3 Maternal Glucose and Glucose Metabolism

An increased rate of structural birth defects among children born to women with type 1 diabetes mellitus is generally interpreted as demonstrating the teratogenic effect of fetal exposure to high levels of glucose. Studies also suggest, though not with unanimity, an association between fetal exposure to maternal diabetes and later obesity or insulin resistance. Fetal response to high maternally-derived glucose load, transient increases in in-utero insulin production, and subsequent permanent changes ("programming") in fetal and child metabolism are the presumed factors driving this association. Most research examining fetal exposure to elevated maternal glucose levels examined populations of women with pre-existing diabetes or gestational diabetes. It is interesting that among pregnant women without pre-existing diabetes, glucose metabolism appears to become more efficient in early pregnancy before deteriorating as pregnancy progresses. The challenge facing the NCS is to examine whether sub-clinical impaired glucose metabolism, perhaps even with clinically normal maternal glucose levels, is associated with adverse child health.

Using criteria promulgated by the American Diabetes Association, clinical diagnoses of diabetes and of "impaired glucose tolerance" are made using a combination of fasting glucose levels, casual glucose levels, or the results of a two-hour oral glucose tolerance test (OGTT). Gestational glucose intolerance or diabetes is commonly assessed at approximately 24 weeks gestation using an oral glucose tolerance screen, with follow-up as indicated. Though powerful as diagnostic tools, the above measures cannot assess subtle alterations in glucose and insulin metabolism which might result in normal serum glucose levels. The "gold standard" assessment of insulin resistance used in targeted clinical studies, the euglycemic clamp, is clearly not suitable for the NCS due to issues of participant burden. The ability to obtain fasting serum insulin and glucose levels early in pregnancy is also questionable because the initial NCS contacts will occur in the home environment. It will be difficult to schedule visits around an eight hour fast, and the capacity to separate and refrigerate the samples rapidly will be sporadic. In addition, those measures provide only a cross-sectional snapshot of a woman's glucose status during a period of metabolic change.

In the NCS, biochemical measurement of maternal glucose metabolism early in pregnancy will be estimated by collection of serum for hemoglobin A1c. Hemoglobin A1c provides an integrated measure of maternal glucose levels over 6-10 weeks and will reflect exposures in the periconceptional period as well as during early embryogenesis. The analyte is stable for several days at room temperature, and thus is suitable for collection in the field. It does not, however, allow for assessment of subclinical impairment of glucose metabolism. In addition, the possibility of obtaining fasting specimens for glucose and insulin analysis exists for at least a sub-sample of the NCS population, depending on the characteristics of the individual study sites.

In addition to the biochemical measures obtained directly by the NCS, clinical reports of maternal OGTT results and fasting glucose tests will be obtained during the perinatal chart review. Maternal and family diagnoses of diabetes will be obtained via questionnaire.

9.5.4 Endocrine Markers

Two endocrine exposure measures are of specific interest to the NCS: maternal thyroid hormone, and cortisol in both the mother and the child.

Maternal hypothyroidism is associated with sub-optimal neurodevelopment in exposed offspring. The potential influence of subclinical hypothyroidism, especially as it relates to maternal exposure to hormonally active compounds (primarily some of the persistent organic chemicals discussed earlier), on subsequent health is not known.

Blood for assessment of maternal thyroid stimulating hormone (TSH) and thyroxine levels will be obtained prior to pregnancy in the pre-conception cohort, enabling periconceptional estimation of fetal exposure. Thyroid measures will be obtained from all women at the first trimester home visit and at the third trimester clinic visit. Cord or neonatal heel stick blood will be available for late fetal assessment of thyroid status.

Maternal stress or response to stress, as measured through cortisol, may influence the development of the fetal immune system and lead to persistence of the TH2-type response associated with asthma and atopy in childhood. Whether this is due to the central effect of stress on the maternal HPA axis or to other mechanisms (e.g., maternal and fetal response to placental CRH) is not certain.

Cortisol measures may be performed in blood, saliva, or urine. Blood measurements of cortisol reflect total cortisol, including protein-bound cortisol. In saliva or urine, cortisol measures are believed to more accurately reflect the free, biologically active fraction of cortisol. Therefore measurements of cortisol are performed using multiple measures in saliva in a day or using a 24 hour urine collection.

The NCS will obtain multiple daily saliva specimens from the mother twice during pregnancy to capture the diurnal patterns in cortisol that enable characterization of stress response (see Appendix G). Maternal and paternal samples will be obtained at 6 months as a biological indicator of parental stress and depression, to be used in conjunction with assessments of child development, family process, and related domains. In early childhood, saliva samples will be attempted at the 6- and 12-month visits for evaluation of HPA activity. At each collection, three to four samples will be collected at specified times (on awakening, mid-day, evening) to check for diurnal patterns. Urine samples can be collected as an alternative to saliva if needed.

Maternal stress will be assessed via questionnaires at several time points during and after pregnancy and periodically throughout childhood (see Section 9.3.3) to enable comparison between reported or perceived stress and biologic measures of stress that may be affecting the fetus or affecting parenting efficacy after birth.

9.5.5 Parental Medical History

The mother's past and current medical history will be obtained during the first interview. Past history will include ascertainment of chronic disease, such as asthma or diabetes; frequent acute

disease, such as urinary tract infections; and mental health, such as depression or anxiety disorders. There will be a focus on factors potentially related to pregnancy outcome, including the mother's birth history (preterm birth, birth weight, plurality, prior pregnancies, and outcomes), and reproductive history (age of first menstruation, menstrual cycle, doctor visits, and normal health care providers). Additional information about the current pregnancy (due date, hospital, pregnancy-related conditions, and illness during the periconceptional period or early pregnancy) will be obtained and updated throughout the pregnancy. Data on use of fertility services will also be collected early in pregnancy. Maternal family history, including the histories of parents and siblings, will be obtained as well, with a focus on chronic and mental diseases.

The identified biological father will also complete a medical history and a family medical history early in pregnancy.

Information about maternal doctor visits, diagnoses, and other medical events will be collected in a diary prior to and throughout pregnancy, and in a health care visit log. After birth, the focus will shift to the child's medical conditions, doctor visits, injuries, and use of car seats. Structured information regarding contacts with the health system will be recorded in a health care visit log.

9.5.6 Health Behaviors and Status

Starting in pregnancy, measures of health behavior and health status in the NCS will be taken or adapted from those commonly used in other epidemiologic studies. The domains include use of tobacco products, alcohol consumption, substance abuse, diet, and physical activity. In addition to these, car safety seat use, maternal sleep habits, maternal douching during pregnancy, the presence of breast implants, eating disorders, dental health, parental and child health history, and documentation of clinical encounters (for unexpected events) will be recorded. Multiple longitudinal measurements of many of these exposures are required due to their variability between time points and the cumulative effect of behaviors on long-term health of the child.

Diet: Within the NCS, dietary intake of the mother during pregnancy in conjunction with the child's diet is considered the major factor influencing nutritional status and is considered a potential source of chemical (primarily pesticide and metals such as mercury) exposure. If issues of burden and cost were not considerations, a minimum of four 24-hour diet recalls or two sets of four-day food records would be collected on the mother or child at each measurement point, following the precedent of more focused studies (U.S. Department of Agriculture, Rhodes et al., 2004). These methods also include coding for food preparation, an important source of toxicant exposure. The 24-hour method underreports less than other methods (Subar et al., 2003) and minimizes recall bias. However, a single 24-hour report is not representative of an individual's total diet and should not be used to estimate actual diet (Research Council, 1986). As a result, recall over multiple days is needed to assess an individual's usual intake.

For these reasons, the NCS has selected a self-administered Food Frequency Questionnaire (FFQ) as the primary method of collecting dietary exposure data for mother and child. This approach is the most commonly used assessment method in large epidemiological cohort studies. FFQs ask respondents to report their usual frequency of consumption of each food from a list of foods for a specific period. Information is collected on frequency and sometimes portion size. The FFQ's major strength is its ability to estimate usual intake of foods during a long period of time (e.g., past week, month, or year). Because it is self-administered, it is relatively inexpensive and does not have to be completed during a Study visit. The FFQs will be augmented by a three-day checklist, and, for the child, will be supplemented with breastfeeding and formula questions at 6 and 12 months.

Of the validated FFQs used in epidemiological research, the NCI Diet History Questionnaire (DHQ) has been chosen for the NCS because it is a public use instrument; the paper questionnaire completed by the participant can be optically scanned; it can be modified to add additional foods and questions of interest to NCS; and it can be linked to the major exposure databases (Total Diet Study; USDA/EPA Pesticide Database Program; Dietary Exposure Assessment Module; Dietary Exposure Potential Model). The DHQ will be administered to NCS mothers at preconception, twice during pregnancy, and at one month after birth for lactating mothers. The NCS will also use the Harvard Service Food Frequency Questionnaire (a proxy form, generally completed by the mother) to collect information on children at 18 months and 36 months (Blum et al., 1993; Gilman, ongoing; Welsh et al., 2005).

As an adjunct to the FFQ, a self-administered three-day checklist will be used to collect information about the current diet before and during pregnancy, and the early post-natal period. For the child, a three-day checklist will be used at 6, 12, 18, and 36 months. Other dietary instruments include feeding forms for children at 1, 6, and 12 months. These self-administered forms collect information about frequency and quantity of breast and formula feeding, and types of formulas. They also include questions on initiation of solid foods, preparation of formula and bottles, and use of commercial baby foods. They have been adapted from the FDA Infant Feeding Practices Study II (http://www.cdc.gov/ifps/). The child's diet will continue to be measured longitudinally throughout development.

Physical activity of the mother will be measured using the International Physical Activity Questionnaire (IPAQ)–Short, Last Seven Days. This instrument allows the calculation of total physical activity in metabolic equivalents (METS), which can then be used to compare different levels and types of activity within the NCS, and also with the numerous other studies that use the IPAQ. The IPAQ is recommended for monitoring population levels of physical activity globally for those ages 18-69. The mother's physical activity will be assessed prior to pregnancy and again early in pregnancy.

Information allowing the estimation of the child's physical activity will be collected at 6 and 12 months through the questions used to assess his or her usual activities and developmental status. Time-activity diaries will also be employed starting at 12 months. Starting at 36 months, the use of accelerometry may be attempted, though the protocol to be used for those measurements has not yet been determined. Physical activity will be measured throughout development and the specific measures will be determined starting two years before each wave of data collection.

Tobacco use: Maternal use of products containing tobacco will be ascertained throughout pregnancy and updated after the child is born. Questions about tobacco use, including prior and current usage and type of product used, are adapted from NHANES and the National Survey of Family Growth. Tobacco use will also be assessed through diary entries completed by women prior to and throughout pregnancy. In addition, cotinine will be analyzed from urine, hair, or blood samples drawn from the mother before and during pregnancy. Paternal use of tobacco and tobacco use by other household members will be also be ascertained during pregnancy and infancy by similar methods. Maternal tobacco use during pregnancy has been reported in association with Attention Deficit Hyperactivity Disorder.

The child's actual exposure to tobacco in utero and after birth will be estimated by measuring cotinine in the cord blood or heel stick, and in urine at 6 and 12 months. In addition to the questionnaire and biologic samples, house dust collected at the home visits can be analyzed for nicotine to allow further categorization of potential tobacco exposure.

Measures of environmental tobacco smoke will be made throughout childhood, and the child's own use of tobacco in adolescence will be carefully investigated.

Alcohol use and abuse: Use of alcohol by the mother will be ascertained by questionnaires before and during pregnancy. Relevant questions were adapted from other major epidemiologic studies including NHANES, World Health Organization–ASSIST, and the Coronary Artery Risk Development in Young Adults (CARDIA) Study.

Questions on alcohol use will include amount, frequency, and type of alcohol, both with regard to the year prior to the time woman knew she was pregnant, and with regard to current use (during early pregnancy and late pregnancy). Excessive use by the mother will also be assessed after child's birth.

Use of prescription drugs in ways other than those prescribed by a doctor will be obtained for the year prior to the time woman knew she was pregnant, and during pregnancy.

Medications and supplements: Use of prescription medicines, over-the-counter medicines, supplements, and alternative medicines will be assessed prior to and throughout pregnancy by direct observation of medicine bottles by the NCS data collector during in-person contacts. This technique has been used by many epidemiologic studies (including NHANES) and provides an accurate inventory of medications in use. Prior to the visit, the participant is asked to gather bottles of all medicines she is currently taking. This can easily be done when the visit occurs in the home; for clinic visits, the participant will be asked to bring the bottles to the study clinic. By reviewing the bottle, the interviewer is able to reliably record the name, strength, dosage, and form of each medication.

Use of over-the-counter and prescription drug, dietary and pharmaceutical supplements, and herbal and alternative medications will also be ascertained via the maternal and child questionnaires. Prescription information will also be collected in the health care visit logs. An important source of fetal exposure to medication will be the abstraction of the maternal prenatal, labor, and delivery records at the birth hospital.

Abuse of drugs that are prescribed by a doctor will be ascertained by questionnaire using a single measure which groups classes of drugs (e.g., sedatives, tranquilizers, analgesics, etc.) During pregnancy, two time periods are covered – the year before the woman knew she was pregnant and currently. This measure was taken from the Composite International Diagnostic Interview (CIDI), drug module.

Illicit drugs: Self-report of illicit drugs will be obtained from the mother before and during pregnancy and after birth. A single measure has been selected to ask about major categories of street drugs, including amphetamines, marijuana, cocaine, inhalants, hallucinogens, and opioids. This measure has been used previously for CIDI and (World Health Organization— Alcohol, Smoking, and Substance Involvement Screening Test [WHO-ASSIST]). Drug screening of biologic samples (blood, cord blood, and urine) can also be performed. Use of illicit drugs by the child will also be assessed.

9.5.7 Other Health-related Behaviors and Status

The dental health assessment by questionnaire includes questions adapted from NHANES regarding routine cleanings, gum health, past dental procedures, dental problems, and use of dental rinse products. Dental health questions will be asked prior to and throughout pregnancy. Performance of a clinical diagnostic periodontal and dental examination was initially considered for inclusion, but is not practical given the NCS visit schedule and geographic distribution of the study population.

Maternal and child sleep habits will be assessed prior to pregnancy, throughout pregnancy, and during early childhood. Questions include amount of time sleeping at night, amount of time sleeping

during the day, and sleep apnea during the past week (from the National Heart, Lung, and Blood Institute, Assessing Child and Maternal Sleep in the Early Years).

Other maternal health related behaviors to be collected by interview include current and past eating disorders, information about current and past breast implants, and frequency of douching and type of douche product (prior to pregnancy and during early pregnancy).

9.6 Genetic Measures

The longitudinal design and scope of the NCS provide vital resources to help answer many questions related to the role of genetics and genomics in the health of our nation's children. The size of this cohort, and the fact that exposures are measured during pregnancy and pre-pregnancy (in a sub-sample of the cohort), will also provide a unique opportunity to investigate the combined effect of genotype and exposure on structural and functional properties of the brain and on other organ systems during development. In this context, the ability of the study to investigate fetal/mother interaction during pregnancy will be especially important.

Venous whole blood samples will be collected from the mother and father to obtain genomic DNA and to extract and store peripheral blood mononuclear cells (PBMCs) for later transformation into cell lines. Genomic DNA from whole blood is the gold standard for most genetic studies, especially those involving genomic variation of candidate genes, and will be obtained in the NCS. Genomic DNA can also be utilized for whole genome genotyping studies (linkage or association), sequencing, epigenetic studies, and assessing change in genetic material over time (National Children's Study Workshop, 2004; Wallace, 2007). Cord blood will be collected at birth to obtain a sample of germ line DNA and RNA, and to extract and store PBMCs for later transformation into cell lines. A heel spot will also be obtained from the child at birth to confirm the purity of the cord blood sample. In the cases where blood cannot be drawn, saliva will be collected to extract DNA. A summary of assessment approaches for genetics appears in Table 9-5.

Table 9-5. Summary of NCS Genetics Assessment Approaches

Approach	Types of samples	Target components (measures)
Biomarkers	Whole blood	Genomic DNA: SNPs & haplotypes, gene expression, DNA adducts, nucleotide sequences (if/when economically feasible) Epigenetics RNA Mitochondrial DNA: haplogroups, somatic mutations
	Peripheral blood mononuclear cells	Cell lines, epigenetics
	Cord blood	Imprinting, epigenetics
	Saliva	DNA (if blood draw refused)

9.6.1 Genomic DNA

The HapMap Project has shown that approximately 80 percent of recombination occurs in about 15 percent of the genome; the project has reduced the task of measuring millions of single nucleotide polymorphisms or SNPs by using linkage disequilibrium to identify a reduced set of tag SNPs that captures variation throughout the genome (Gibbs & Singleton, 2006). The NCS will obtain genetic

samples from multiple family members across a broad sample of participants, and will therefore give investigators the chance to perform both linkage and association studies which use both population and family based study designs (Laird & Lange, 2006). Genome wide-association studies (GWAS) have emerged as a robust and unique approach to identifying multiple interacting disease susceptibility genes and their respective pathways (Keith, 2007; Yeager et al., 2007). Studies of genomic variation in NCS children can contribute information not only about the relation of genetic variants to disease risk (e.g., susceptibility, severity, prognosis) and the interaction of genotype with environmental risk factors (e.g., the 5-HTTLPR serotonin transporter gene, early life stress and alcoholism), but also about the response to therapeutics (Duff, 2006; Keith, 2007; Kelsoe, 2004; Laird & Lange, 2006; Laird, 2005; Reich & Patterson, 2005; Wallace, 2007; Yeager et al., 2007). The size of the NCS cohort helps protect linkage and association methods from population stratification, allowing case control association studies to be more successful and giving NCS investigators a variety of avenues for research.

9.6.2 DNA Modifications

Many DNA modifications and alterations have been shown to be caused by environmental exposures (Flato, Hemminki, Thunberg, & Georgellis, 1996; Kiyohara & Yoshimasu, 2007). If these alterations are not repaired appropriately by the body's DNA repair mechanisms, genetic instability and mutations can result that contribute to increased disease risk (Flato et al., 1996; Kiyohara & Yoshimasu, 2007). Genetic variations in DNA repair genes have been shown to impact DNA repair capacity, ultimately impacting disease susceptibility (Kiyohara & Yoshimasu, 2007). In addition, chemical compounds can attach to DNA molecules to form adducts which are often studied as molecular measures of exposure (Verdina, 2006). Whether a DNA adduct has biological significance depends on several factors including the type of adduct formed and the rate of DNA repair. Modifications are time dependent, and thus the multiple, repeated blood collections planned in the NCS will help to identify possible genetic modifications resulting from environmental influences and exposures (Flato et al., 1996). The identification of molecular biomarkers such as DNA adducts, in combination with high-throughput genotyping techniques to identify polymorphisms in DNA repair and other genes, will facilitate the characterization of exposures mediating disease pathways and related outcomes in the NCS sample.

9.6.3 Epigenetics and Epigenomics

The design of NCS is ideal for studying epigenetic effects, such as DNA methylation and histone modifications, which result in changes in gene expression that (though maintained through meiosis and/or mitosis) do not involve alterations in DNA sequence (Rodenhiser & Mann, 2006). Epigenetics is defined as the study of heritable changes in gene expression and function that occur through alterations in the chromatin structure, ultimately impacting transcriptional control of genes (Rodenhiser & Mann, 2006). The collection of genetic samples from the mother, father, and child trio, and possibly from other family members, will also provide a strong opportunity to study epigenetic modifications to genomic DNA (Laird & Lange, 2006). Epigenetic changes can provide insight into how aspects of the environment, such as chemical or psychosocial exposures, affect gene regulation (Anway & Skinner, 2006). The epigenetic influence of some exposures is time dependent, having a stronger influence at certain stages of development than at others. The multiple measures of exposures, biospecimens, and outcomes in the NCS will facilitate investigation of these critical exposure windows on the epigenome. Epigenomics promises a unique perspective of the genome due to the ability to identify and detect quantitative modifications and alterations outside of genes. Emerging high-throughput technologies, such as microarray analysis, will facilitate a reproducible and quantitative approach to epigenomic analyses (Callinan & Feinberg, 2006). A good example of the type of epigenetic change relevant to the NCS would be dietary influences on gene expression, such as folate deficiency, which can

influence DNA methylation in pregnant mothers, predisposing their children to several complex diseases including anemia (Donnelly, 2001).

9.6.4 Mitochondrial DNA

Disturbances in mitochondrial DNA (mtDNA) metabolism have been implicated in developmental delay, mental retardation, dementia, seizures, neuro-psychiatric disturbances, migraines, strokes in the young, and movement disorders (Naviaux, 2000). The spectrum of diseases associated with mitochondrial dysfunction or variation in mtDNA is expanding into disorders such as autism (Graf et al., 2000) and diabetes mellitus type II (Mogensen et al., 2007; Weijers & Bekedam, 2007; Fuku et al., 2007), and may play a role in susceptibility to some environmental exposures.

Several characteristics of mtDNA are distinct from nuclear DNA and make mtDNA an interesting biomarker of disease and exposure. Mitochondrial DNA is exclusively maternally inherited. Therefore, mtDNA sequences are not altered by recombination as passed from generation to generation, but through the accumulation of mtDNA mutations along female lineages (Brandon et al., 2006). In the NCS, because DNA will be obtained from both mothers and children, it will be possible to track some of the lineage.

In addition to maternal inheritance, and unlike inheritance of nuclear DNA that occurs in an all-or-none fashion, the frequency of transmission of mutated mtDNA is stochastic and may occur in a range of 0-100 percent transmission. This results in a mixture of normal and mutant DNA (Brandon et al., 2006; Gropman et al., 2004; Wallace, 2005; 2007). The percentage of mutated mtDNA transmitted may be associated with distinct phenotypes (Wallace, 2007). The large sample size of the NCS makes it well suited to examining the association of different percents of mutant mtDNA with disease phenotypes.

Moreover, mtDNA has a high mutation rate. mtDNA is particularly susceptible to DNA damage in comparison with nuclear DNA (Marcelino & Thilly, 1999; Masayesva et al., 2006; Yakes & Van, 1997) due to the lack of histones protecting the DNA and reduced efficiency of DNA repair (Kujoth, Bradshaw, Haroon, & Prolla, 2007; Penta, Johnson, Wachsman, & Copeland, 2001) when compared with nuclear DNA. The mitochondria produce a large amount of reactive oxygen species (ROS), which can damage DNA. As a result, mutations in mtDNA accumulate in post-mitotic cells of the body with age (Kujoth et al., 2007; Wallace, 2005). Because the NCS will obtain specimens at different times during development, it will be possible to track potential changes in mtDNA.

Mitochondrial DNA will be isolated from whole blood of NCS participants and obtained several times during development. In addition to isolating mtDNA from blood, in some studies it has been detected in urine (Fliss et al., 2000) and in saliva (Fliss et al., 2000; Masayesva et al., 2006). Both will be collected in NCS, and may be used for ancillary studies. Examination of changes in mtDNA will be performed by DNA sequencing or using genotyping technology. Another approach for high throughput sequencing of mtDNA, which will be explored by NCS, is the use of the MitoChip, an oligonucleotide microarray for rapid sequencing of the entire mitochondrial genome (Sui et al., 2006; Jakupciak et al., 2005).

9.6.5 RNA

Some recent studies examined the use of RNA obtained from whole blood or peripheral blood mononuclear cells (PBMCs) for use of expression profiling (Lampe et al., 2004; Whitney et al., 2003). In these studies, using both whole blood and isolated PBMCs, variation of gene expression profiles

were observed among individuals (Debey et al., 2004; Whitney et al., 2003) The variation of gene expression in healthy subjects was much smaller than the variation observed in individuals with cancer or bacterial infection (Whitney et al., 2003). This suggests that gene expression profiling of RNA obtained from blood is a possible biomarker of disease. Furthermore, gene expression patterns from isolated PBMCs may be altered by exposure, as suggested by the observation of a gene expression signature associated with tobacco smoking (Lampe et al., 2004). In the NCS, whole blood and PBMCs will be collected for studies of gene expression. RNA obtained from whole blood specimens represents several cell types, while PBMCs are only one type of cells. Therefore, these RNA sources may be used to address different questions relating to gene expression (Debey et al., 2004; Whitney et al., 2003).

One of the greatest challenges to this type of RNA analysis both from whole blood and PBMCs is that the samples tend to degrade quickly during collection and storage. Preserving RNA is vital since the stability of the RNA affects the analysis of gene expression, consequently preserving RNA with RNAase inhibitors is imperative. Commercially available blood collection tubes that reduce RNA degradation and additives to stabilize RNA exist but are expensive (Chai, Vassilakos, Lee, Wright, & Young, 2005; Pahl & Brune, 2002; Rainen et al., 2002). To improve stability in the NCS, RNA isolation from whole blood is planned at the central repository prior to long-term storage. RNA may also be isolated from cryopreserved PBMCs; one study observed high quality RNA extraction from PBMCs which were frozen for 15 months (Marteau, Mohr, Pfister, & Visvikis-Siest, 2005). Many issues related to stability will undoubtedly be solved during the next few years, but issues with respect to long term storage will be a challenge.

9.6.6 Cell Lines

Generating cell lines from collected specimens will provide a valuable resource for future studies. This is an expensive process, but if cell lines are generated, an almost unlimited supply of genetic material will be available to investigators for many types of future studies, including genetic and biochemical assays (Beck, Beiswanger, John, Satariano, & West, 2001; Hayes, Smith, Huang, Read, & Kopp, 2002). Cell lines and PBMCs also provide a source for the development of phenotypic assays. Such assays allow exploration of the function of entire biological pathways to determine if reduced efficiency of a particular pathway is associated with disease. These assays either examine enzyme activity or expression of particular proteins.

To utilize samples cost effectively, the NCS plans to isolate and cryopreserve the PBMCs within 30 hours of collection. PBMCs will then be transformed into cells in the future when relevant cases necessitating such transformation have been identified. Previous studies suggest that cryopreserved PBMCs may be stored for two years or more prior to transformation with high transformation efficiencies (Beck et al., 2001; Hayes et al., 2002; Kleeberger et al., 1999).

10. STATISTICAL ANALYSIS PLAN

10.1 Introduction

10.1.1 Study Design

The design for the National Children's Study (NCS) is based on a nationally representative sample of about 100,000 births to be sampled in 105 geographic areas, called either primary sampling units (PSUs) or Study sites. The pregnancy status of all eligible women of child-bearing age in these areas will be monitored for 4 years, and prepregnancy survey data will be collected for those trying to get pregnant. All women living within the Study sites who become pregnant during the 4-year period will be enrolled in the Study as early in pregnancy as possible in order to measure in utero exposures. The pregnant women will be followed through birth; their children will be followed for 21 years. Throughout this period, the NCS will collect extensive data on a variety of health outcomes and environmental measures and social, demographic, economic, and neighborhood characteristics.

10.1.2 Objectives

The NCS is designed to address hypotheses developed over several years by a variety of stakeholders following a review of the current state of the art in the many areas related to child development and environmental exposures. These primary or "core" hypotheses relate to multiple diseases and developmental outcomes, including asthma, physical and neurological development, diabetes, adverse pregnancy outcomes, obesity, and behavior and mental health problems, such as autistic spectrum disorders.

The NCS will collect data on the children's exposure to chemical, physical, biological, psychosocial, and behavioral environments and their communities, child care, and schools. It will also collect data about the parents' workplaces concerning exposures that might affect their children and data on the children's health from their physicians. Thus, there will be multiple levels of data collection: individual, household, immediate neighborhood, community (e.g., community air quality), and county (e.g., schooling and sociodemographic characteristics).

The study will have the power to examine gene-environment interactions from a developmental perspective in a way that has not previously been done. The NCS will provide a rich source of data with which to investigate the genetic mechanisms associated with rare diseases such as autism; the quantitative contribution of genetic variation to common conditions such as obesity; and the impact of gene and environment interactions on complex diseases and conditions, such as asthma and depression. Multiple gene-environment and gene-gene interactions will play a key role, creating the need for highly complex, computer-intensive forms of analysis. An important goal of the NCS is to provide data to support such analyses.

10.1.3 Overview of the Chapter

This chapter describes statistical methods that will be employed in analyzing NCS data and important issues for these analyses. One primary consideration, of course, is the sample size and power that can be expected in the NCS. This is discussed in Section 10.2. In Section 10.3, we discuss a number of issues relevant to all statistical analysis of NCS data, such as design-based versus model-based

analysis, confounding, measurement error, and missing data. Section 10.4 illustrates the range of methods that will likely be used in analyzing NCS data, and Section 10.5 discusses analysis of genomic data.

10.2 Sample Size and Power

10.2.1 Overall Sample Size and Key Subgroups

As noted earlier, the overall sample size for the NCS is about 100,000 sampled children at birth. However, this number is expected to decrease by about 2 percent per year so that, for example, the sample size at age 18 will be reduced to about 69,000 children remaining in the study. Furthermore, the sample size will be smaller for some endpoints. For example, for schizophrenia, the sample size will be reduced because the postulated analyses require placental data and serum from early in pregnancy that are assumed to be available for only 80 percent of the sampled children.

In addition, some hypotheses apply to selected subgroups, defined by characteristics such as sex, race, ethnicity, living area, genotype, or combinations of these characteristics. Examples include the following outcomes: age at puberty, which requires separate analyses for boys and girls; asthma among breast-fed children; and IQ score among "at-risk" children. Subgroup sample sizes are often small, leading to substantially less power.

10.2.2 Impact of Complex Sample Design

The sample design for the NCS is a complex clustered design involving unequal selection probabilities, stratification, and multi-stage sampling. Complex sample designs, particularly clustered designs, have a substantial impact on standard errors and power. The impact of the complex design is measured by the design effect for a given survey estimate. A design effect greater than 1.0 indicates the estimate is less precise than the corresponding estimate computed from a simple random sample of the same size.

Much empirical research has shown that design effects for complex clustered sample designs are generally lower for analytic statistics, such as odds ratios and regression coefficients, than for descriptive statistics, such as means and proportions (see, for example, Kish 1995). The design effects for regression coefficients are discussed in Scott and Holt (1982). The estimates of power for the odds ratios presented in Section 10.2.3 incorporate an allowance for estimated design effects associated with the complex NCS sample design. For most of the calculations, the homogeneity of the exposures in the PSUs is assumed to be modest. However, for hypotheses relating to infant mortality and rate of developmental disabilities, the exposures are assumed to be highly homogeneous within PSUs. The reason for the high level of homogeneity in these cases is that the exposures of interest are neighborhood or community characteristics and policies that will be the same for all children in the neighborhood or community.

10.2.3 Power for Subgroups/Primary Objectives

In hypothesis-driven studies, there are two types of errors. A type I error (generally denoted as α) occurs when the null hypothesis is true but is rejected; a type II error (generally denoted as β) occurs when the null hypothesis is false but is not rejected. For example, if the null hypothesis is that a given factor is not associated with an outcome, then a type I error occurs when there is in fact no association but the study concludes that there is one. Type II error, failing to reject the null hypothesis when a given factor is actually associated with an outcome, is the complement of statistical power; thus,

the higher the power, the smaller the chance of making a type II error. While there are no universally accepted error rates, the values of $\alpha = 0.05$ and $\beta = 0.20$ (i.e., power = 80 percent), respectively, are most frequently used when designing studies.

A range of medically important outcomes will be used here to illustrate the ability of the NCS to test exposure-outcome associations involved in the primary hypotheses with power of 80 percent. These outcomes exhibit the range of prevalence that NCS outcomes are likely to have. While some outcomes are common, most are rare and some are very rare. Many of these outcomes are relevant for a single primary hypothesis, but some are relevant for more than one. For example, several hypotheses address different possible predictors of childhood asthma, including environmental factors, exposure to bacteria and microbial products, maternal stress during pregnancy, and diet. For each outcome, a set of different exposures is considered. In each case, power has been calculated for exposure prevalence of 1.0 percent, 2.5 percent, 5 percent, 25 percent, and 50 percent (this range is based on hypotheses developed for the NCS).

Using cerebral palsy (CP) as an example, the results on power displayed in Table 10-1 can be interpreted as follows: Since CP has a prevalence of about 0.2 percent in the general population, that is the rate to be expected in the NCS. Table 10-1 gives the odds ratio (OR) that can be detected with 80 percent power for exposures (i.e., risk factors) with a 5 percent significance level and a prevalence ranging from 1 percent to 50 percent. For very rare exposures (e.g., 1 percent), only those that have a dramatic impact on the occurrence of cerebral palsy (OR greater than or equal to 5.0) can be reliably detected in the NCS. However, for more common exposures, such as those with 5 percent prevalence or greater, factors with more modest effects (OR greater than or equal to 2.6) can be detected with 80 percent power.

Two simplifications were made in these power calculations. First, the analyses consider only the simple bivariate relationships between the exposures and outcomes without addressing the need to control for confounders. The inclusion of confounders likely results in a reduction in the power for detecting the effects of exposures, but often the reduction will be modest. Second, all outcomes and exposures are assumed to be dichotomous variables. This assumption is again made to simplify the table. In fact, most of the NCS outcomes and exposures will be continuous variables. As a result, the power estimates in the tables are likely to be conservative since dose-response analyses with continuous outcome and/or exposure variables would likely lead to greater power.

Table 10-1 displays the magnitude of the minimum odds ratios that can be detected with 80 percent power for the selected outcomes and the range of exposures for analyses. The sample sizes for Table 10-1 assumed to be the full sample for which data are available. As noted above, the sample available is reduced through attrition and, for some outcomes like schizophrenia, by availability of special data required for analysis. As Table 10-1 shows, the magnitude of the detectable odds ratio depends on the prevalence of both the outcome and the exposure. For a given outcome, the closer the prevalence of the exposed group is to 50 percent, the smaller the detectable odds ratio and the greater the power. Similarly, in general, the closer the prevalence of the outcome is to 50 percent, the smaller the detectable odds ratio, i.e., the detectable odds ratios are small when the exposure prevalence is reasonably high. All the ratios are less than 2 when the exposure prevalence is between 25 percent and 50 percent. The bold line in the tables separates the detectable odds ratios into those above and those below 2.

Table 10-1. Detectable Odds Ratio When Analyzing the Total Sample

Outcome	Age	Prevalence of outcome (%)	Prevalence of exposure				
			1%	3%	5%	25%	50%
Infant mortality*	1	0.7	6.01	3.87	2.95	1.97	1.94
Type I diabetes	18	0.2	5.71	3.72	2.86	1.93	1.89
Musculoskeletal defects	1	0.2	5.00	3.33	2.60	1.80	1.75
Cerebral palsy	1	0.2	5.00	3.33	2.60	1.80	1.75
Schizophrenia#	18	0.3	5.06	3.36	2.62	1.81	1.76
Nervous system defects	1	0.3	4.09	2.82	2.25	1.62	1.58
Metabolic syndrome	18	0.4	4.03	2.78	2.23	1.61	1.56
Autism spectrum disorder	4	0.4	3.66	2.57	2.09	1.54	1.49
Heart defects	1	0.6	3.03	2.21	1.84	1.42	1.38
Type 2 diabetes	18	1	2.75	2.05	1.73	1.36	1.32
Major birth defects	1	3.5	1.76	1.47	1.33	1.16	1.14
Adolescent aggressive behavior	18	4	1.82	1.50	1.35	1.17	1.15
Chronic physical aggression (CPA)	10	4	1.76	1.47	1.33	1.16	1.14
IQ score less than 75	18	5	1.73	1.45	1.31	1.16	1.14
Asthma	4	7.5	1.53	1.33	1.23	1.11	1.10
Neurocognitive development	12	8	1.55	1.34	1.24	1.12	1.10
Depression	18	8.3	1.57	1.35	1.25	1.12	1.11
Asthma	7	8.5	1.51	1.32	1.22	1.11	1.10
Neurodevelopmental disabilities	18	10	1.52	1.32	1.22	1.11	1.10
Preterm birth < 37 weeks	0	12	1.41	1.26	1.18	1.09	1.08
Asthma	18	12.5	1.47	1.29	1.20	1.10	1.09
Adverse pregnancy outcomes	0	15	1.38	1.23	1.16	1.08	1.07
Developmental disabilities*	18	17	1.92	1.54	1.37	1.18	1.16
Developmental disabilities	18	17	1.41	1.25	1.18	1.09	1.08
Obesity	12	17.1	1.39	1.24	1.17	1.08	1.07
IQ score less than 100	18	50	1.32	1.20	1.14	1.07	1.06

* The exposure for this hypothesis is a community rather than an individual level characteristic.
# This analysis is restricted to children for whom placental data and serum from early gestation are available (assumed 80 percent).

To illustrate the increase in the magnitudes of detectable odds ratios for subgroup analyses, Table 10-2 presents results comparable to those in Table 10-1, but with the sample size reduced to a 20 percent subgroup. The results in this table could be applied to case-control studies or other analyses based on subsets of the overall NCS sample. It is assumed that the geographic distribution of the subgroup is proportionate to the general population, which would generally be true in case-control studies and other subset analyses. The detectable odds ratio remains below 2 when the outcome prevalence is 3.5 percent or higher and the exposure prevalence is 5 percent or more, but for rarer outcomes and exposures, it exceeds 2. Many subgroups of interest will comprise less than 20 percent of the population and will thus have larger detectable odds ratios.

Table 10-2. Detectable Odds Ratio When Analyzing a 20 Percent Subsample

Outcome	Age	Prevalence of outcome (%)	Prevalence of exposure				
			1%	3%	5%	25%	50%
Infant mortality*	1	0.7	17.91	10.13	7.08	4.32	5.35
Type I diabetes	18	0.2	16.13	9.42	6.68	4.12	4.99
Musculoskeletal defects	1	0.2	13.44	7.96	5.69	3.50	3.93
Cerebral palsy	1	0.2	13.44	7.96	5.69	3.50	3.93
Schizophrenia#	18	0.3	13.73	8.08	5.77	3.54	3.99
Nervous system defects	1	0.3	10.23	6.19	4.51	2.81	2.93
Metabolic syndrome	18	0.4	10.04	6.07	4.42	2.76	2.86
Autism spectrum disorder	4	0.4	8.77	5.39	3.97	2.51	2.55
Heart defects	1	0.6	6.73	4.26	3.22	2.11	2.08
Type II diabetes	18	1	5.87	3.78	2.89	1.94	1.90
Major birth defects	1	3.5	2.96	2.15	1.79	1.39	1.35
Adolescent aggressive behavior	18	4	3.13	2.24	1.85	1.42	1.38
Chronic physical aggression (CPA)	10	4	2.97	2.16	1.80	1.39	1.35
IQ score less than 75	18	5	2.89	2.10	1.76	1.37	1.33
Asthma	4	7.5	2.35	1.80	1.55	1.27	1.24
Neurocognitive development	12	8	2.40	1.83	1.57	1.28	1.25
Depression	18	8.3	2.46	1.85	1.59	1.29	1.25
Asthma	7	8.5	2.30	1.77	1.53	1.26	1.23
Neurodevelopmental disabilities	18	10	2.33	1.78	1.54	1.26	1.23
Asthma	18	12.5	2.21	1.71	1.49	1.24	1.21
Preterm birth < 37 weeks	0	12	2.04	1.62	1.43	1.21	1.18
Adverse pregnancy outcomes	0	15	1.95	1.56	1.39	1.19	1.17
Developmental disabilities*	18	17	3.79	2.46	1.96	1.44	1.39
Developmental disabilities	18	17	2.07	1.62	1.43	1.21	1.18
Obesity	12	17.1	2.00	1.59	1.40	1.20	1.17
IQ score less than 100	18	50	1.90	1.49	1.33	1.15	1.13

* The exposure for this hypothesis is a community rather than an individual level characteristic.
# This analysis is restricted to children for whom placental data and serum from early gestation are available (assumed 80 percent).

10.3 Statistical Inference

10.3.1 Design-Based vs. Model-Based Inference

Statistical theory provides the basis for drawing inferences about a population based on a sample taken from that population. One approach to statistical inference that could be applied when analyzing NCS data is based on the sample design (design-based inference), i.e., the randomized procedures used to select the sample. An alternative approach is based on a statistical model that the underlying data are assumed to follow (model-based inference). Design-based inference provides the basis for most published descriptive estimates. However, model-based inference is often used when statistical methods are more complex. This section discusses these two analytical frameworks.

10.3.1.1 Design-Based (Randomization) Inference

In design-based inference, a randomly selected sample is used to estimate parameters that would have been obtained had all members of the population under study been included in the sample and provided data. These parameters may be termed census parameters (see, for example, Chambers & Skinner, 2003; Kalton, 2002). A given individual's data are considered fixed, however. The randomization comes about through the sampling used to select the individual. The statistical theory for the designed-based approach to inference from population-based survey data was developed in the late 1940s and discussions of this topic are available from many sources (e.g., see Cochran, 1977; Kish, 1965).

In regression analysis, the census parameters to be estimated consist of the census regression coefficients and the census squared multiple correlation coefficient. If the regression model is correctly specified and the population is large, the census parameters would be virtually the same as the model-based parameters. However, when the model is not correctly specified, the census parameters will differ from the model-based parameters. In this case, the model-based parameters are problematic, but the census parameters are still interpretable (at least under mild misspecification). The census parameters provide the best fitting model of the given structure for the population under study. In that sense, the design-based approach is somewhat robust.

There are two distinctive features of design-based inference: the need to use sampling weights when analyzing the data to estimate the census parameters and the need to take the sample design into account in estimating the standard error of estimates derived from sample data. The weights reflect the unequal selection probabilities with which sample units are selected and also weighting adjustments to compensate for nonresponse and noncoverage and to calibrate the sample to conform to known population distributions. Standard errors, p-values, and confidence intervals for survey estimates must be calculated using special procedures that reflect both sampling weights and any stratification and clustering used in the sample design.

The robustness gained from using weights in making sample estimates of census parameters comes at a price of a loss in precision as compared with correctly specified model-based estimates. With large samples and limited variation in the weights, that loss of precision is generally acceptable. There are, however, cases when the loss of precision is very large, such as when units are sampled with very unequal selection probabilities. In such cases, alternative estimation approaches may be required, for example, incorporating the sample design features into the analytic model (see, for example, Korn & Graubard, 1999, with examples from health surveys, and Chambers & Skinner, 2003).

10.3.1.2 Model-Based Inference

Model-based inference assumes a model for the population data *Y* as a function of a set of parameters θ. One version of this approach is superpopulation modeling (Royall, 1970; Thompson, 1988) where values of θ are considered fixed, and the observed population values are assumed to be drawn from a superpopulation whose distribution is given by $f(Y \mid \theta)$. Inferences about θ are based on the joint distribution of *Y* and the sampling mechanism *S*.

An alternative modeling procedure is Bayesian population inference (Little, 2004). As in design-based approaches, Bayesian population inference focuses on population quantities of interest $Q(Y)$. However, inference is made about $Q(Y)$ by considering the marginal posterior predictive distribution (Ericson, 1969; Holt & Smith, 1979; Skinner et al., 1989), which requires postulating a prior

distribution for the model parameters $p(\theta)$ in addition to the model for the data. This is similar to the missing data formulation in which all of the population not observed is considered to be missing and values are multiply imputed via the posterior predictive distribution of the data (Little & Rubin, 2002), although in practice the actual step of imputing values for the entire population can usually be avoided. Probability samples that are "noninformative" in the sense of Rubin (1987) in that the distribution of *Y* and *S* are independent (possibly conditional on fixed covariates *X*) so that the parameters θ and ϕ that govern the data and sampling mechanisms are distinct, allow inference to be made using a posterior predictive distribution based only on the model for the data. This is equivalent to the ignorable missingness assumption (see Section 10.3.4.1 for a discussion of this assumption) that allows inference about θ to be made conditional on observed data in item-missingness situations. However, to maintain the noninformative sampling assumption, the model must be formulated in a fashion that accounts for the sample design. Thus, for example, models being utilized in sample designs with unequal probabilities of selection might stratify based on the probability of selection to account for any associations between the parameters of interest and the probability of selection.

The model-based approach provides a framework in which point estimation and inference can be made in the same fashion as in other areas of statistics. As discussed in the previous section, the greatest disadvantage of the model-based approach is that, if the model is seriously misspecified, it can yield inferences that are worse—perhaps much worse—than design-based analysis. Careful model development and consideration of how and why models are likely to fail can serve as some protection against this outcome.

10.3.2 Confounding and Mediating Variables

Since the NCS is an observational study and not a randomized trial, the main challenge to making causal inferences from NCS data will be to control for confounding variables. Confounding variables are factors related both to an outcome variable and to exposure variables that are being evaluated as risk factors for that outcome, but that are not themselves dependent on the risk factors. The relationship between potential confounders and the outcome variable is not itself of analytical interest. However, the validity of estimated effects of exposures obtained from analyses depends critically on the inclusion of all the important confounders in the analysis.

When choosing potential confounders to be controlled for in an analysis, care must be taken to distinguish them from mediators. Confounders and mediators are each related to both the exposure and the outcome under study. However, confounders are causally prior to the exposure whereas mediators are on the causal path between the exposure and the outcome. Controlling for mediators will lead to a reduced or nonexistent relationship between the exposure and the outcome, thus providing a false impression of the full effect of the exposure on the outcome.

Confounders should also be distinguished from effect modifiers, sometimes called moderators. Effect modifiers partition an independent variable into subgroups where the effects of the independent variable on the dependent variable differ within each subgroup (Baron & Kenny, 1986). For example, Simons and Wood (2004) found that response to ozone exposure varies both by age and gender, with older persons (and particularly older women) experiencing less reduction in FEV_1 than younger persons. Thus, age and gender are effect modifiers for response to ozone exposure.

Appropriate control for confounders, whether by regression methods or propensity scoring, is essential with the NCS data. The NCS will collect information on a wide range of covariates that may be considered as potential confounders for a given analysis. In studying the possible effects of

environmental pollutants on asthma and wheezing, for example, there are a number of confounding variables related to environmental and genetic factors as well as to the risk of asthma and wheezing in children. In general, the approach utilized will be to review the scientific literature that describes previously observed factors associated with environmental and genetic factors and the increased risk of asthma and wheezing in order to select a set of covariates that are potential confounders for a specific analysis. In the asthma example, potential confounders in an analysis of the possible effects of environmental pollutants and genetic variation on asthma severity might include maternal gestational factors, such as premature birth and stress and infection during pregnancy; childhood infections; diet and nutrition; socioeconomic variables, such as parents' education, household composition, and housing characteristics; demographic characteristics such as race/ethnicity; access to health care; and so forth.

Sections 10.4.2.1 and 10.4.2.2 discuss linear and nonlinear regression and propensity scoring as methods for controlling for confounding variables. Matching is another method used to control for confounding variables. In matching, the individuals in the comparison group are selected to match the target group on a potentially confounding variable. This holds the effect of the confounding variable constant across groups in analyses. For example, in a study of the influence of prenatal drug exposure on children's cognitive development, the nonexposed comparison group would need to be matched to the drug-exposed target group on premature birth status to rule out a potential alternate explanation for cognitive delays in the drug-exposed group. Case-control studies, which represent a particular example of matching, are discussed in Section 10.4.6.

10.3.3 Measurement Error

10.3.3.1 Impact of Measurement Error

The role of measurement error in the analysis of epidemiologic data (both environmental and other study data) is multifaceted. As with any other types of data, there is the potential for bias and increased uncertainty in predicting outcomes when outcomes or covariates are measured with error. Measurement error in environmental exposures often results from data collection decisions. For example, there is potential bias and increased uncertainty in ecological designs where the required individual-level exposures are measured at the population or group level rather than at the individual level.

Individual measurements for each subject at each time period may contain measurement errors. The extent of these errors, such as those caused by equipment limitations, may be constant across subjects and time, but they may also vary due to collection or processing methods across laboratories or study sites, particularly for measures based on environmental samples or biospecimens. A further complication is that NCS analyses will not be restricted to estimating mean levels or correlations. Some analyses will require sampled individuals to be classified according to whether or not they have been exposed to chemical levels above certain cutoffs; other analyses will want to use continuous measurements to investigate whether threshold levels are the same at different developmental stages.

The effects of measurement error, which depend on the measurement error distribution (Carroll et al., 1995), that are possible include: (1) attenuation of a regression coefficient or other effect measure to the null; (2) hidden effects; and (3) a sign reversal in estimated coefficients. Thus, measurement errors can introduce both variability and bias into data analysis and must be accounted for.

10.3.3.2 Types of Measurement Error

The two general types of covariate measurement error are classical and Berkson error. Let X represent the true covariate measure that cannot be observed for all study participants. If X is fixed and the surrogate measure W varies due to error, then the classical measurement error model is appropriate, $W = X + U$, where U represents measurement error. For example, the biological samples of phthalates obtained from blood/urine, cord blood, infant urine, and meconium samples are potentially measured with classical measurement error. Conversely, if W is fixed and X varies due to error, the regression calibration or Berkson model is appropriate, $X = W + U$. For example, if the variable of interest is the actual amount of chemical absorbed by the body, the measurement of the chemical level in drinking water or air particles may be the fixed surrogate with the true level absorbed by the body varying as a function of the surrogate.

10.3.3.3 Assessing Measurement Error

There are several sources of data that can be used to evaluate the extent of measurement error. In some cases, study data can be validated for a subsample of cases. For example, it may be possible to obtain more accurate observations for a subset of the primary data or, at an aggregate level, from external sources. For air quality measurements, it is often too expensive to take a personal measurement for every study participant. It is more reasonable to randomly select a subset of the sample to measure personal air quality and collect more general measures such as room air quality for the entire study. The personal measurements can then be used to assess the extent of measurement error due to using room air data.

With replication or reliability assessment, multiple measures of the surrogate variable are observed via internal or external sources; the variation in the replicated measurements gives an indication of extent of variable measurement error.

Another approach uses instrumental variables. An instrumental variable must be (1) correlated with X, the covariate being measured; (2) independent of $W - X$ (i.e., measurement error: the true value minus observed value); and (3) independent of the outcome (Y) given X and any additional covariates that are measured without error (Z) (Carroll et al., 1995). For example, in the Faroese Mercury Study of a birth cohort of children, neither validation data nor replication data were available to estimate the cord blood mercury measurement error (Budtz-Jorgensen, et al., 2003). Instead, secondary exposure variables such as the concentration in maternal hair and the average number of whale dinners per month were used as instrumental variables.

10.3.3.4 Modeling Approaches

Regression calibration is essentially the replacement of the true covariate X by the regression of X on (Z, W) using replication, validation, or instrumental data (Carroll & Stefanski, 1990). It follows an algorithm of the following three steps: (1) use validation, replication, or instrumental data to estimate the regression of X on (Z, W); (2) replace the unobserved X by the estimate from step 1 and rerun the standard analysis to obtain parameter estimates; and (3) adjust the resulting standard errors to account for the estimation. In some cases, a simulation extrapolation approach can be used if validation or replication data are not available to model the calibration function. Heuristically, this approach is a self-contained simulation study that illustrates the effect of measurement error on parameter estimates (Carroll et al., 1995). There are commands available in Stata version 8 as well as macros for SAS that will fit generalized linear models when one or more covariates are measured with error.

In the context of structural equations models, it has been shown that latent variable models can be used to adjust for measurement error in the predictor and response with multiple measures on all subjects (Palta & Lin, 1999).

10.3.4 Missing Data

This section discusses types of missing data that will be encountered in the NCS, and methods that can be used either to adjust for them or to analyze data in the presence of missing data. In any survey, there are data losses due to noncoverage and nonresponse. Noncoverage occurs when individuals are missed in the listing process resulting in some members of the target population having no chance of selection. Total nonresponse (also called unit nonresponse) refers to eligible individuals who are sampled but do not provide any usable survey data. Item nonresponse refers to missing data items for eligible individuals who participate in the study and provide most of the required survey data. Partial nonresponse refers to eligible individuals who are sampled for the study but who provide only a portion of the survey data. This can occur, for example, when data collection involves multiple components (e.g., lab tests, questionnaires, etc.). Wave nonresponse occurs in longitudinal studies in which a sampled individual fails to provide data for one or more of the required waves of data collection. This type of nonresponse can be due to attrition, in which an individual who participated in early waves of data collection drops out of all subsequent waves. Wave nonresponse can also be intermittent rather than attritive, where a participant misses one or more waves of data collection but returns in a subsequent wave.

10.3.4.1 The Missingness Mechanism

When developing methods to account for missing data, it is important to evaluate the process that gave rise to the missing values. This process is called the missingness mechanism. Rubin (1976) and Little and Rubin (2002) define a typology of missingness mechanisms. Data are missing completely at random (MCAR) if the missing data are essentially a simple random sample of the underlying complete data. MCAR is unlikely to hold across an entire sample, but it may hold within strata or classes defined by race, sex, geographic location, or other variables.

The second mechanism is called missing at random (MAR). Data are said to be MAR if the probability that an observation is missing depends on the underlying complete data only through elements of the data that are fully observed. For example, if the probability that a subject drops out depends on classes defined by race, sex, or geographic location and class membership is known for all sampled persons, then the data are MAR.

The third type of missingness is the nonignorable (NI) mechanism. When data are NI, the probability of missingness depends on unobserved data even after adjusting for all observed data. With NI data, the application of standard approaches for handling missing data in the analysis are not valid. Since it is not possible to distinguish NI from MAR using observed data, the only way to identify NI missing data with any confidence is to gather the missing data from a fraction of those not responding. Thus, attempts to model NI missing data are generally speculative and have a limited role in applications.

10.3.4.2 Compensating for Missing Data in Design-Based Analysis

Design-based methods to compensate for missing data consist primarily of weight adjustment and imputation. The following sections describe methods that will be used to weight the NCS data as well as alternative methods for compensating for missing data under model-based inference. The model-based approach for handling missing data in the National Children's Study is discussed in Section 10.3.4.3.

Weighting adjustments

The primary method of compensating for unit nonresponse in survey data consists of adjusting the sampling weights. The initial sampling weight for each respondent is the inverse of the original selection probability for that respondent. These initial weights (often called base weights) can be adjusted for unit nonresponse and, in many cases, noncoverage using methods described below (see Brick & Kalton, 1996, for example, for a more detailed discussion). The NCS will use these procedures to adjust for unit nonresponse.

To compensate for nonresponse, adjustment factors are calculated within selected weighting classes formed by demographic or other data. These data must be available for both respondents and nonrespondents. The adjustment factors are then used to inflate the base weights. The weighting classes are typically based on information from the sampling frame. The underlying assumption is that the nonrespondents are missing completely at random (MCAR) within the weighting classes.

Adjustments for noncoverage are based on external data sources, typically the Census of population or, in this case, of National Children's Study birth certificate counts. The adjustment process consists of calibrating nonresponse adjusted weights so that sample estimates of key characteristics conform to the known population characteristics from the external source. This calibration compensates for noncoverage and it also reduces variance of estimates associated with the characteristics involved in the calibration. Birth certificate data are a likely external data source that can be used for making nonconvergence adjustments in the NCS. These data can, for instance, provide data on the numbers of births to mothers resident in a county and on the characteristics of those births (e.g., birth weight, APGAR results) and of the families (e.g., mother's age, race, education).

Imputation

Imputation is widely used in survey research to assign values to missing survey items and thus compensate for item nonresponse. The imputed values are derived using data from other items available for the respondent that serve as predictors of the missing values. The "hot deck" method is the simplest and probably most frequently used imputation method. In this method, missing data are assigned values from another respondent who is judged to have similar characteristics. For example, missing income data might be replaced with the income of another respondent of similar age, education, and gender. (See Brick, Kalton, & Kim, 2004, for more discussion.)

Numerous methods have been developed for imputation, ranging from the fairly simple and nonparametric hot deck to Bayesian model-based imputation (Little & Rubin, 2002). Multiple imputation is another frequently cited approach (Rubin 1987). As noted by Kalton and Kasprzyk (1986) and Brick and Kalton (1996), most of these methods fall within the general multiple regression framework.

Regardless of the method used, it is advantageous for a project like the NCS to produce filled-in, public-use data sets, as these can be analyzed by researchers without the need for sophisticated statistical modeling and missing-data adjustments.

Compensating for wave nonresponse in the NCS

Some NCS participants will fail to provide data for one or more of the survey waves. While some persons may drop out of the study at one wave and be lost for all subsequent waves (attritors), others may miss one wave but return to the study at a subsequent wave (nonattritors). The choice between weighting adjustments and imputation to handle wave nonresponse is not clear cut. In attrition nonresponse, however, weighting adjustments are usually preferred to a mass imputation of all variables for each of the missing waves.

Weighting adjustment for attrition nonresponse is relatively straightforward since this type of nonresponse is "monotone." Each successive wave adds an additional set of nonrespondents on top of those who dropped out in previous periods. Weighting adjustments at the current wave can be applied to the nonresponse-adjusted weights from the previous wave.

10.3.4.3 Compensating for Missing Data Under the Model-Based Approach

Missing data create a number of problems in statistical analysis. First, multivariate analysis methods typically assume complete data for all subjects. If some items are missing for a given subject, then the subject must be dropped from the analysis unless some form of adjustment is used (Vonesh & Chinchilli, 1997). A second issue concerns statistical efficiency since unavailability of some data elements decreases the effective sample size for statistical analyses resulting in wider confidence intervals and underpowered tests. A third issue is bias, since individuals with missing data may differ systematically from those with complete data. For example, in studies of health-related quality of life, subjects with the poorest quality of life are also most likely to be lost. Thus, analyses of the available data may be biased toward higher quality of life values.

However, a number of analysis methods can be used when missing data are present. The central tool for model-based analyses of longitudinal measurements is the linear mixed model (Diggle et al., 2002) as implemented in the SAS procedure Proc Mixed. The assumptions of MAR and parameter distinctness are sufficient to guarantee that likelihood-based analyses accomplished in Proc Mixed or similar software are correct even with substantial amounts of missing data.

Another analytic approach that avoids some of the stronger assumptions of likelihood-based analysis but still allows considerable flexibility in modeling is the generalized estimating equation (GEE) method (Liang & Zeger, 1986; Zeger & Liang, 1986; Diggle et al., 2002). With this so-called *marginal modeling* approach, one estimates a generalized linear model for the outcome variables (which can be either continuous or discrete), accounting for correlation within subjects (or larger units) by computing an adjusted variance matrix. Validity is robust to assumptions about the within-unit correlation. GEE modeling is valid under the assumption of MCAR though not generally under MAR. However, one can correct this by estimating a model for dropouts given observed data, and then weighting observations by the inverse dropout probability (Robins et al., 1995a, 1995b). A potential disadvantage is that GEE models describe only the marginal distributions of outcomes and therefore fail to capture within-subject correlation, which may be a critical feature of the phenomenon under study (Lindsey & Lambert, 1998).

Sensitivity analysis can be used to evaluate the impact of assumptions about the missingness mechanism. Pioneering work includes articles by Copas and Li (1997), Verbeke et al. (2001), and Troxel et al., (2004). A paper by Ma, Troxel, and Heitjan (2005) describes a method for local sensitivity analysis in a longitudinal model.

Another approach to handling missing observations is to impute them (as discussed in Section 10.3.4.3) and then analyze the filled-in data set as though it were complete. However, analyzing the filled-in data set without some accommodation for the imputation generally overstates precision. Rubin (1978, 1987) proposed multiple imputation (MI) as a way to avoid this difficulty. In MI analysis, one creates not one but several sets of filled-in data. The analyst then analyzes each filled-in data set separately, combining these results into a single overall analysis. Some MI algorithms are now available in commercial software, including the SAS procedure PROC MI.

10.3.5 Variance Estimation under the Design-Based Approach

The NCS is based on a complex sample design involving stratification and clustering by PSUs and by segments within PSUs. Under the design-based approach to inference, these features need to be taken into account in estimating the precision of estimates, whether they are basic descriptive statistics (means, percentages, totals in the total population or in subgroups) or analytic statistics (regression coefficients, odds ratios, etc). Failure to take account of the sample design in analysis can lead to invalid tests and erroneous conclusions (Skinner et al., 1989). This section briefly reviews the methods available for computing variance estimates for survey estimates based on complex sample designs.

Two approaches are commonly used for estimating sampling errors from complex sample designs: (1) Taylor series linearization methods, and (2) replication procedures. With the Taylor series method, estimates of variance are derived using a first-order Taylor series approximation of the deviations of estimates from their expected values. The required sums of squared deviations are then computed using the "ultimate cluster" approach described by Hansen, Hurwitz, and Madow (1953a, Chapter 6) and Kalton (1979). Replication methods, on the other hand, use subsamples of the full sample to obtain the standard errors of estimates (Rust & Rao, 1996). The subsamples, called "replicates," can take on a variety of forms including balanced repeated replicates, jackknife replicates, and bootstrap subsamples. A statistic of interest is calculated for the full sample and for each replicate, and the variability of the replicate estimates is used to estimate the variance of the statistic. An advantage of replication methods is they eliminate the need to specify complicated variance formulas (e.g., see McCarthy, 1966).

Both Taylor series and replication methods require appropriate variance stratum and variance unit codes in order to calculate the sampling errors. The variance units correspond to the actual first-stage sampling units within a stratum; thus, for the noncertainty PSUs, the variance units are the PSUs themselves, whereas within the certainty PSUs (which are, in reality, strata), the variance units are the sampled segments. (If a segment in a certainty PSU is also selected with certainty, the variance units would be the dwelling units.) Moreover, both methods require at least two variance units per variance stratum. In order to satisfy this condition, it may be necessary to collapse some of the noncertainty sampling strata for variance calculations. If collapsing is required, the resulting variances will tend to be overstated (Hansen, Hurwitz, & Madow, 1953b, page 218). To accommodate analyses that take the sample design into account, software such as WesVar, SUDAAN, and STATA can be used (LaVange et al., 1996; Research Triangle Institute, 2004; Westat, 2002).

10.4 Major Types of Analysis

10.4.1 Overview

This section discusses statistical methods that would be used as tools in the major types of analysis that will be undertaken in the NCS. The analyses are:

10.4.2 Cross-sectional exposure on an outcome;

10.4.3 Identifying causal pathways;

10.4.4 Analysis of neighborhood effects; and

10.4.5 Evaluating temporal effects.

Sections 10.4.2 through 10.4.5 discuss the specific statistical methods that will be used to achieve these analytical objectives. Before these methods are undertaken, however, certain basic, descriptive analyses will be carried out for all the variables being considered for inclusion in the planned analysis. For example, in the case of a dichotomous outcome, the prevalence of exposures (e.g., percent with detectable levels) from various sources (e.g., air, urine, blood, cord blood) will be compared between groups with and without the outcome. The distributions of quantitative exposures (continuous data) from these sources will be assessed with logarithmic or other transformations carried out as necessary to meet assumptions for statistical modeling. Finally, exposure levels measured from these sources will be characterized by the mean, standard deviation, median, and interquartile range. After these exploratory steps, detailed analysis will be done using the methods described in Sections 10.4.2 through 10.4.5.

10.4.2 Individual Cross-Sectional Exposure/Outcome Analysis

10.4.2.1 Linear and Nonlinear Regression

An important part of the data analysis in the NCS will be to investigate the association between an outcome of interest and some exposure measurement, controlling for possible confounders. For example, researchers may be interested in evaluating the association between prenatal exposure to polychlorinated biphenyls (PCBs) and cognitive and motor development in young children. Regression models, linear or nonlinear, are important analytical tools to address such scientific questions.

Linear regression methods are associated primarily with continuous outcomes. Many outcome measures in the NCS can be regarded as continuous in nature, including fetal growth, children's fine and gross motor skills, bone density, and heath care utilizations. Linear regression models can be used to study the relationships between such continuous outcomes and exposures of interest while controlling for confounders.

As an illustration, Daniels et al. (2003) examined the association between mother's prenatal exposure to PCBs and children's cognitive and motor development using data from the Collaborative Perinatal Project. The Bayley Scales of Infant Development were used to assess the infants' mental and psychomotor development at 8 months of age. PCB exposure represented the sum of 11 measured congeners from maternal nonfasting blood sampling collected at the third trimester. Possible confounders included maternal education, socioeconomic index, intelligence quotient, marital status, prenatal smoking status, prepregnancy body mass index, third trimester serum triglyceride, total cholesterol, and dichlorodiphenyldichloroethylene levels, the child's birth order, gestational age, and whether the child

ever breast fed. The investigators treated the Bayley Scales as continuous and fitted a linear regression on the exposure and the confounders.

On the other hand, many other outcomes in the NCS will be discrete. For example, congenital malformations can be categorized as present or absent. Outcomes may also take more than two levels of values. For example, a measure of activities of daily life may be coded as very good, good, bad, or very bad. Other outcome measures can be count variables, such as number of hospitalizations in a month. For these outcomes, nonlinear models are appropriate. In particular, logistic regressions (Hosmer & Lemeshow, 1989) are useful for modeling the association between a binary outcome and the exposure, controlling for the confounders. When the outcome is polytomous, some generalizations of the logistic regression can be applied. For example, when the levels of the outcome have no meaningful order, polytomous logistic models can be used (Hosmer & Lemeshow, 1989; McCullagh & Nelder, 1989). When the levels of the outcome follow a natural order, either the adjacent-category logistic model (Agresti, 1984), the proportional odds model (McCullagh & Nelder, 1989), or the continuation-ratio model may be used. When the outcome is a count measure, Poisson regression models or extensions of Poisson models are appropriate (McCullagh & Nelder, 1989).

It is likely that survival or time-to-event analysis will be appropriate for some outcomes, such as child development milestones or occurrence of childhood illnesses. The proportional hazards model is essentially a nonlinear regression model where the time to an event (e.g., infant's first steps or first holding a spoon) is the dependent variable. The potential effects of genetic and environmental factors can be modeled as predictors of delayed or accelerated development using the proportional hazards model (Marubini & Valsecchi, 1995). Variables that change over time (time dependent covariates) can also be included in the model. For example, environmental or other exposures that change with time can be included in the model.

Interaction terms can be added into both linear and nonlinear regression models when the exposure effect may vary with the level of a moderating variable. For example, if the decrease in motor development index for one unit increase in prenatal PCBs exposure is larger in breast-feeding children than in non-breast-feeding ones, then an interaction between the PCBs exposure term and the variable for breast-feeding can be added in the model. Variables such as gender, race/ethnicity, and age are likely to modify the effects of exposures in many NCS analyses.

The standard practice is to use forward or backward selection procedures in determining whether a confounder should enter the model. With these procedures, the main effects are selected first followed by the interaction terms. Likelihood or deviance measures are used to assess overall model fitting. Residuals and Pearson's residuals are used to address model diagnostics (Cook & Weisberg, 1982; McCullagh & Nelder, 1989).

10.4.2.2 Propensity Scoring

Propensity scoring provides an alternative method for controlling on confounder variables. Since its introduction by Rosenbaum and Rubin (1983; 1984), the method has become widely used for this purpose, particularly in biostatistical applications (D'Agostino, 1998).

With propensity scoring, exposure is viewed as a chance event where the chance of being exposed at a given level depends on the confounder variables. For example, breast-feeding rates differ substantially by race, socioeconomic level, and other demographic factors (Li & Grummer-Strawn, 2002). Thus, an infant's chance of being exposed to breast feeding depends on these factors, which may act as confounding variables in an analysis of true effects of breast feeding on healthy growth and development

outcomes. The first step of the analysis is to develop a model for predicting the exposure level given the confounders. In the simplest case of a dichotomous exposure (exposed vs. unexposed), a logistic model can be used. With several levels of exposure, an ordinal logistic model can be used (Joffe & Rosenbaum, 1999). An attractive feature of this approach is that a large number of potential confounders and interactions can be introduced into the models. An individual's propensity score for a particular exposure level is then given by his or her predicted probability of experiencing that exposure level.

Comparing groups at different exposure levels controlled on the same propensity scores removes the effects of the confounders included in the propensity model. Often, the propensity score distribution is divided into a number (5 to 10) of subclasses, and confounder control is carried out by performing the analysis within each subclass (see, for example, Rubin, 2007). The subclass results can then be combined in a weighted analysis. Tests of balance for each of the individual confounders can be carried out to check that the distributions of the confounders are equated across the exposure levels. Further balance can be achieved for key confounders by additional calibration procedures (see, for example, Judkins et al., 2006).

Propensity scoring bears a close resemblance to survey weighting. Survey weighting aims to achieve a weighted sample that mirrors the total population of inference. Propensity score weighting can be applied to make the weighted sample at each exposure level have the same standardized propensity score distribution. That standardized distribution could be the distribution for any one of the exposure levels or for the full population. The latter (standardized population) was used in an examination of the effect of the Youth Anti-Drug Media Campaign based on a National Survey of Parents and Youth (Orwin et al., 2006). The propensity score weighting was applied after the survey weighting, and it was reflected in the survey sampling variance estimation procedures used for testing the effects of different levels of exposure to the media campaign. Propensity scoring can similarly be applied in the NCS to examine the effects of environmental, socioeconomic, or other exposures on health and other outcomes, controlling for many potential confounders.

10.4.2.3 Exposure/Outcome Analysis

Overview

The NCS will collect data on many types of environmental exposures. In some cases, different exposures can have related human effects; in other cases, the same type of environmental exposure can produce multiple effects, each of which can be measured at multiple times during the course of the NCS. Thus, the analysis of relationships between exposures and health and other outcomes is necessarily complex.

For example, neurotoxins, such as lead, mercury, and persistent pesticides, and nonpersistent pesticides have similar health effects (Weiss, 2000). Ozone, allergens, endotoxins, indoor air contaminants, and mold affect asthma in similar ways (Gold, 2000; Sunyer, 2001). Potential endocrine disruptors, which appear to cause reproductive problems in birds and fish, can include insecticides, herbicides, industrial chemicals, and heavy metals (Landrigan, Garg, & Droller, 2003).

Some outcomes are accentuated by the interaction between exposures over and above the additive affects of the individual exposures. Across the large number of study participants and geographic locations in the NCS, many different combinations of exposures will be observed. The relative importance of each of the analytes and their interactions can be determined by including all the different analytes in a multivariate analysis. Pathways relating different exposures to each other, to mediators, and to an outcome can be analyzed using structural equation modeling. This method is especially useful for

creating a more comprehensive model of mediating mechanisms based on the results of univariate exposure/outcome analyses in the cohort. In this instance, structural equation modeling serves as a confirmatory method, assessing the fit or appropriateness of a proffered causal model rather than as a method used to develop a causal model.

The determination of the measure or measures of exposure to use in an exposure/outcome analyses will often be far from straightforward. Since exposure may derive from different sources at any point in time, it may be measured in several different ways and vary over time. Many of the exposures being measured in the NCS will arise in multiple media. For example, pesticides can be found in air, dust, soil, water, and food, and hormonally active agents can be found in drinking water, indoor air, food, soil, dust, and commercial products. Each of these exposures and media will be measured at multiple times during the course of the study. Exposure data can be collected with such measurement instruments as interviews, medical records, diaries, chemical environmental samples, biomarkers from blood or urine, and community level assessments. Measurement error in the various exposure measures also must be taken into account (see Section 10.3.3).

The sections that follow discuss, in turn, the analysis of exposure/outcome relationships that involve one exposure in multiple media with one outcome; multiple exposures with one outcome; and one exposure with multiple outcomes.

One outcome with multiple sources of exposure

In many cases, the NCS will collect data on exposures to contaminants that exist in multiple media or sources. The example given above is of pesticides, which can occur in air, dust, soil, water, and food. Since adverse outcomes may be accentuated by the interaction between multiple sources, it is important to include all the various sources in statistical models. Statistical models for exposure interaction will be similar to those discussed in the context of gene by environment interactions (Section 10.5.2), though environmental exposures are more likely to be continuously measured. For such models define:

E1 = exposure measure from source 1 or exposure type 1;

E2 = exposure measure from source 2 or exposure type 2;

Then the association with the outcome of interest may be modeled as,

$$\text{Logit}[\Pr(\text{outcome})] = b_0 + b_1 E1 + b_2 E2 + b_3 (E1 * E2).$$

As an example, consider "exposure" to phthalate esters. Phthalates have been shown to produce male reproductive tract malformations, including cryptorchidism or undescended testes (UDT), in rats when administered during sexual differentiation (Wilson et al., 2004). The quantification of phthalate levels in the human environment is crucial to determine whether exposures are sufficient to produce UDT or other "outcomes" in humans (Fisher, 2004). Phthalates are of particular concern because exposure is ongoing and ubiquitous (CDC, 2003; Silva, Barr, et al., 2004). They are widely used as softeners of plastics, solvents in perfumes, and additives to hairsprays, lubricants, and insect repellents. These exposures can be measured using air samples. Phthalates and its metabolized forms can be measured in biological samples such as maternal blood/urine, cord blood and infant urine, meconium, and amniotic fluid (Silva, Slakman, et al., 2004).

Swan et al. (2005) developed a global score for the assessment of the phthalates as a group that took into consideration exposures from multiple sources and diverse parent compounds. They constructed their global score by categorizing individual metabolite concentrations into low (below the 25th percentile), intermediate (between the 25th and 75th percentiles) and high (above the 75th percentile) groups, assigning a score to each group and then summing these scores across the metabolites measured in each urine sample. The use of a global score allows for assessment of the phthalates as a group and takes into consideration exposures from multiple sources and diverse parent compounds.

Once a subset of exposure summaries is constructed (e.g., cumulative exposure over time from different sources or peak exposure), correlations among the various summaries can be evaluated. This information along with estimates of the individual exposure associations with outcomes of interest can then be used to develop additional models. Latent variable models (Jöreskog & Sörbom, 1996) and generalizations of such models (Sammel & Ryan, 1996; Sammel, Ryan, & Legler, 1997; Muthén & Muthén, 2004) can be used to assess and validate proposed models of exposure and outcome relationships. (Latent variable models are discussed in more detail in Section 10.4.3.) These models should be used in a confirmatory setting for inferences regarding structure to be meaningful.

As an example, consider Figure 10-1, which illustrates how a latent variable for organophosphate (OP) exposure is derived from three observed sources of information. In this structural equations framework, the underlying, unobserved true OP exposure (represented by an oval) gives rise to the observed measured exposures (rectangles) from the various sources: two body compartment measurements and one indirect personal air monitor measurement. Each λ represents the contribution of a particular exposure type to a composite OP measure. The impact of "true" OP exposure on birth weight is estimated by θ. Statistical tests for θ are a type of global test for the impact of all the observed types of OP exposures on birth weight.

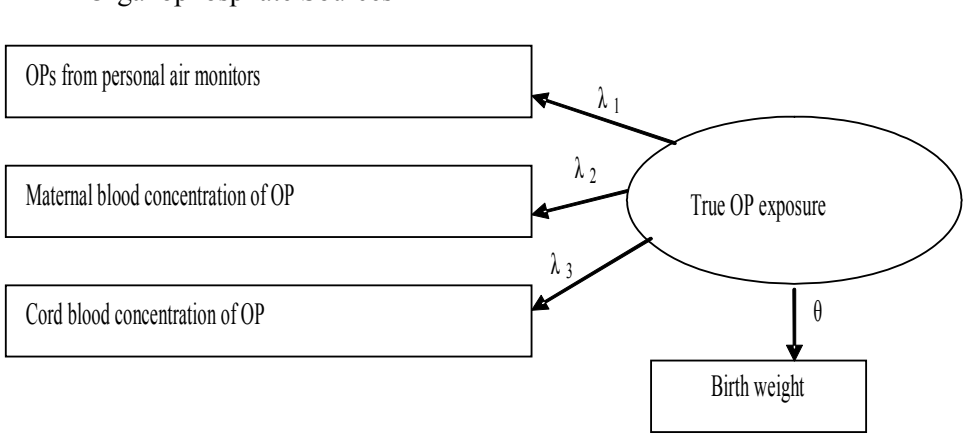


Figure 10-1. Path Diagram for Multiple Exposures

One outcome with multiple exposures

Another type of exposure/outcome relationship occurs when one outcome is associated with multiple exposures, which may act independently or in combination to influence the outcome. The issue of timing further complicates evaluating the effect of an exposure, since the time of exposure may significantly modify its influence on an outcome. Logistic regression can be used to estimate both the

independent and interactive effects of multiple exposures on the overall risk of a specific dichotomous outcome as well as the impact of timing of exposures.

Consider the example of preterm birth, which will be assessed in the NCS. Preterm birth is influenced by environmental, psychological, social, physical, and genetic factors. Two important mediators of preterm birth are inflammation and intrauterine growth restriction (Steer, 2006). The inflammatory response of the human body as it relates to preterm birth can result from bacterial vaginosis (Hartville, Hatch, & Zeng, 2005) and stress (Ruiz, Fullerton, & Dudley, 2003). In addition, stress can be the result of several factors, such as socioeconomic status (Misra, O'Campo, & Strobino, 2001) or lack of social support (Sheehan, 1998). Stress response itself is also mediated by endocrine function and corticotrophin-releasing hormone, which are related to the risk of preterm birth (Gennaro & Hennessey, 2003). The use of logistic regression modeling techniques will allow investigators to use NCS data to analyze how the interaction of these exposures affects the overall risk of preterm birth.

The impact of multiple interactive exposures on preterm births can be analyzed using a structural equation modeling framework (See Section 10.4.3). For example, Sheehan (1998) used structural equation models to show how economic stress, family stress, and lack of social support influence low birth weight.

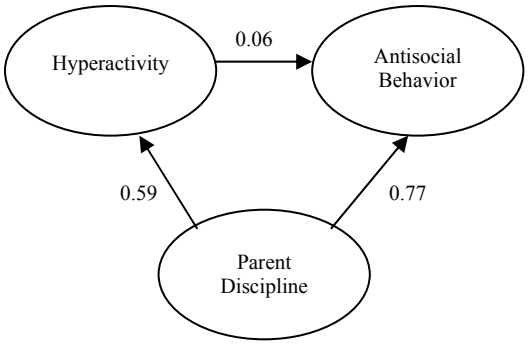
Multiple outcomes with a single exposure

Although some exposures lead predictably to a single identifiable outcome, both theory and empirical evidence suggest that a given exposure can lead to various, sometimes alternative, outcomes (Rutter, 1989). Elements of the environment, individual characteristics, and genetic predispositions can modify the trajectory such that the same exposure leads to a diversity of outcomes across, or even within, individuals. Both the various outcomes associated with an exposure and the moderating conditions that produce the different outcomes can be modeled using multivariate statistical techniques.

A set of trajectories with a unified starting point but multiple endpoints is described in the psychological and biological literature as multifinality (Cicchetti & Rogosch, 1996). Within the NCS, multifinality will arise with regard to processes of resilience. Resilience occurs when a child has an exposure that would logically lead to a negative outcome but does not due to a moderating influence. These individuals have outcomes substantially better than their history of exposure would predict, thus leading to a multifinality analysis. The resulting multiple endpoints consist of different and sometimes unrelated outcomes not simply different levels within the same outcome. For example, some children who experience physical abuse become highly aggressive toward others; some become vulnerable and open to subsequent exploitation; and others demonstrate remarkable resilience and exhibit high levels of behavioral and psychological competence.

Multifinality can be analyzed using one of several multivariate techniques that permit modeling of multiple, simultaneous dependent variables. One such technique is multivariate analysis of variance (MANOVA), which permits the prediction of multiple interval or ratio-level dependent variables from a common set of categorical independent variables. In the initial stages of analysis, MANOVA yields a multivariate *F*-statistic that indicates the significant effect of the independent variable across a multivariate response vector. Subsequent univariate tests are used to uncover the specific relations of the independent variable and any tested interactions with each of the dependent variables. The technique permits direct comparisons of strength of prediction to the multiple outcomes and the differential role of moderators across outcomes.

Multifinality can also be analyzed using structural equation models and path modeling where a single exposure can be modeled as resulting simultaneously in more than one outcome, and the relations between the exposure and each outcome can be compared. As an example, using structural equation modeling, Patterson, DeGarmo, and Knutson (2000) found that poor parental discipline predicted both child hyperactivity and child antisocial behavior in boys although the two outcomes were not significantly associated with each other (see Figure 10-2). The numbers on the arrows in the figure below are the estimated standardized coefficients for the indicated paths. Based on a separate analysis of the data, Patterson and colleagues suggested that parental antisocial behavior, a construct with a strong heritable component, might be the moderating factor that leads to this divergence in outcomes from parental discipline. The NCS would be well suited to test such multivariate models of multifinality.



Source: Patterson et al., 2000.

Figure 10-2. Model of Multifinality of Parent Discipline

10.4.3 Identifying Causal Pathways

The Children's Health Act of 2000, which authorized the planning and implementation of the NCS, also directed the Study to "investigate basic mechanisms of developmental disorders and environmental factors, both risk and protective, that influence health and developmental processes." This means that, in addition to establishing cause/effect relationships, the NCS has been directed to investigate the mechanisms mediating these associations. As an example, a number of factors have been shown to be associated with preterm birth: infection/inflammation, environmental toxins, and behavioral/psychosocial factors. What are the mechanisms through which these factors influence prematurity? Do they involve separate, independent pathways? Do they cumulatively influence the same pathway, such as prostaglandin synthesis, or do they interact in some other way? The objective of the NCS is not only to establish which risk or protective factors are associated with which outcomes but also to increase our understanding of how this occurs so that preventive efforts can be more specifically focused.

Structural equation modeling (SEM) can be used to address such issues. This method allows significant pairwise associations found between a set of single factors to be placed in a larger, theoretically derived contextual model with other significant associations to examine the interrelationships.

Thus, SEM can be used to examine relationships between exposures, mediators, and outcomes in a single overall model. The technique combines multiple regression, path analysis, and factor analysis to assist in causal inference. SEM extends the general linear model since it estimates simultaneous relationships between multiple covariates and responses (outcomes), possibly including unknown latent variables.

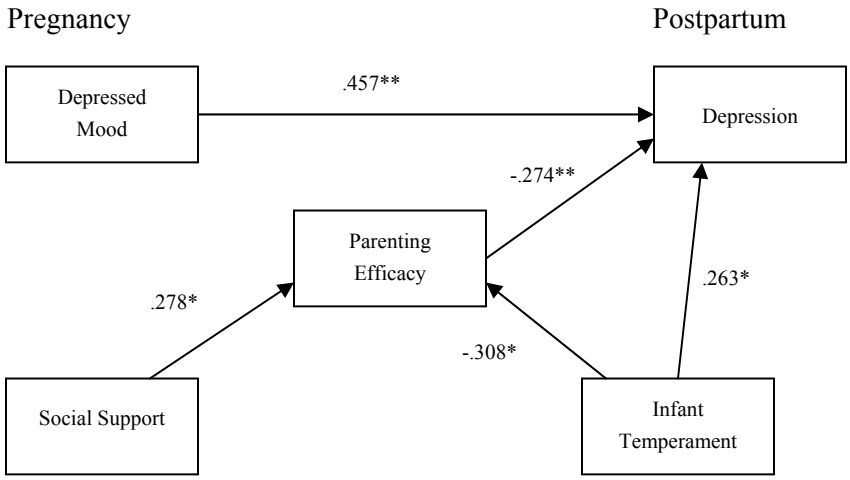
A major strength of SEM is its ability to also model constructs as latent variables simultaneously by means of separate regression equations (Bollen, 1989). Latent variables are unobserved variables or constructs (e.g., IQ) estimated indirectly in the model using measured variables (indicators) that affect them. They can be used as either independent or dependent variables.

SEM/LISREL[1] modeling focuses on two steps (Jöreskog & Sörbom, 1996): validating the measurement model and fitting the structural model. The measurement model specifies how latent variables/hypothetical constructs depend upon the observed variables, and how the association between observed variables is mediated through other observed variables. The structural model specifies the causal relationships between latent and/or observed variables, describes the causal effects, and assigns the explained and unexplained variance. At the outset, one specifies a model based on the underlying theory.

Some of the analyses in the NCS will involve SEM models that do not include latent variables. SEM also works very well in these cases (Bollen, 1989) where the objective is often to understand mechanisms that mediate the association between multiple exposures and a single outcome. As noted earlier, many of the conditions (e.g., premature birth) that the NCS plans to investigate will show associations with multiple psychosocial and physiological factors, and the aim will be to explain the causal mechanisms. For example, if infection and inflammation are shown to explain a significant amount of the variance in premature births and this is also the case with environmental toxins and stress, what are the causal pathways and how are they mediated?

A study of maternal postpartum depression by Cutrona and Troutman (1986) provides an illustration of this type of analysis. The study sought to identify maternal and child qualities associated with changes in depression from pregnancy to the postpartum period. The model was based on theoretically predicted relations from previous research on bivariate relations and significant associations resulting from subsets of analyses where social support, infant difficult temperament, and parenting efficacy were related. Thus, the model was built on theory and preliminary data analyses. Combining these multiple variables into a single model demonstrates, for example, that while having a temperamentally difficult infant predicts greater increases in depression directly, part of this effect is also mediated through feelings of efficacy about parenting (see Figure 10-3 below). Such analytic models inform both more in-depth research questions and process avenues for intervention.

[1] The terms SEM and Linear Structural relationships (LISREL) will be used interchangeably from here onwards.

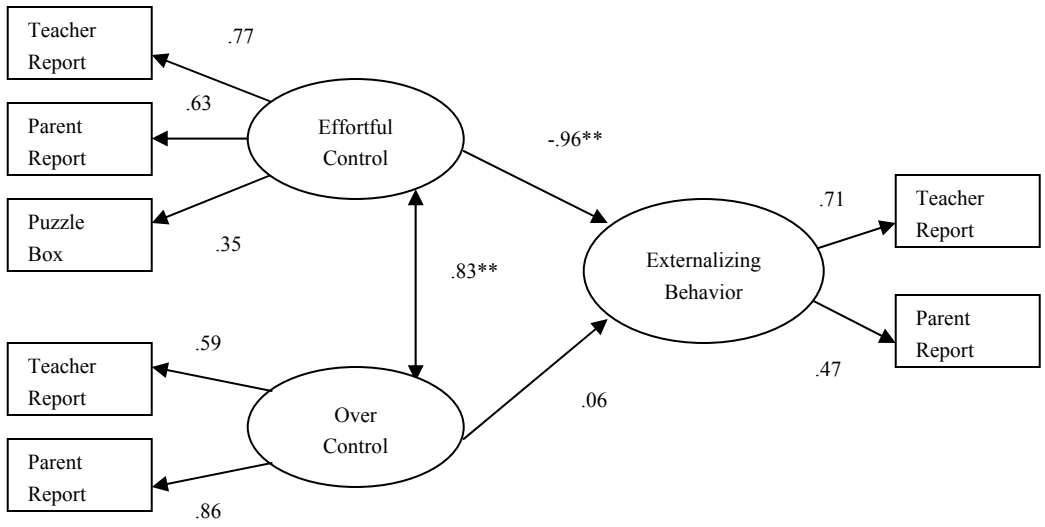


* significant path, p < .05
** significant path, p < .01
Source: Cutrona & Troutman, 1986.

Figure 10-3. Social Support, Infant Temperament, and Parenting Self-Efficacy as Predictors of Postpartum Depression

Other analyses in the NCS will involve multiple observed indicators of latent constructs, and, consequently, latent variable models will be applicable. The latent variable model of child temperamental self-control and aggressive behavior problems developed by Valiente et al. (2003) provides an example (see Figure 10-4). The latent independent variables in their model are temperamental effortful control and temperamental over-control (ellipses in the figure below). The observed variables (rectangles) associated with effortful control are parent, teacher, and task observation ratings of relevant child attention skills and persistent behavior. The observed variables (rectangles) associated with over-control are parent and teacher reports of relevant over -controlled behavior. The latent dependent variable is behavior problems (ellipse). The observed indicator variables are parent and teacher reports of child aggressive behavior. The arrows for the latent variables point toward the observed variables associated with them. The numbers on these arrows are the estimated standardized regression coefficients for the structural equation and measurement models.

This model indicates a direct relation between effortful control and child externalizing behavior. No significant direct relation is found between over-control and externalizing behavior with effortful control simultaneously accounted for in the model. The use of latent variables in this model permits more robust measurement of the latent constructs than would an analysis including only one observed assessment of each construct from a single reporter.



** significant path, p < .01
Source: Valiente et al., 2003.

Figure 10-4. The Relation Between Child Self-Control and Aggressive Behavior Problems

10.4.4 Analysis of Neighborhood Effects

The design of the NCS involves clustered sampling with clustering at the county level, and, within the county, at segment level. This type of design has the analytic benefit of providing a structured set of geographically defined neighborhoods for evaluating the effects of exposures that occur at the neighborhood level. Neighborhoods may be defined in a number of ways depending on the type of exposure and hypothesis being tested. For example, school districts might define a neighborhood for school performance assessments while geographic or administrative boundaries might define a neighborhood for examination of environmental exposures related to the water supply.

Data arising from neighborhoods or clustered structures have a hierarchical form since individual-level data can be grouped within a higher category. Since hierarchical data are nested within a higher structure, there is generally some degree of correlation between observations. For example, two individuals within one community generally are slightly more similar with regard to such factors as religiosity, socioeconomic status, education, and environmental exposures than two individuals sampled from different communities. Multilevel modeling (MLM) is the primary analytic method for analyzing the effects of the hierarchical structure.

MLM is also known as the random coefficient model (Rosenberg, 1973) and as the hierarchical linear model (HLM) (see Bryk & Raudenbush, 1992). MLM is based on the mixed-effects model with both fixed and random components. The regression coefficients are treated as random variables that can vary depending on the higher level unit (e.g., neighborhood). Consequently, MLM can be used to develop regression models with intercepts and regression weights across higher level units as outcomes and other higher-level variables as covariates.

Hierarchical models play an increasingly important role in epidemiology. For example, Juhn et al. (2005) assess the influence of neighborhood and individual-level factors on the incidence of childhood asthma among children born in Rochester, MN, between 1976 and 1979. The neighborhood-level variables considered in this study include collective efficacy, social cohesion, neighborhood socioeconomic status, and whether the Census tract contains major highways or railroads.

An important application of hierarchical modeling with NCS data will be to examine neighborhood effects on various health and developmental outcomes. The collection of standardized information on neighborhoods and counties will permit analysis of effects of both neighborhood-level and individual-level factors.

A hierarchical model can be fitted using either Monte Carlo methods (Gelfand & Smith, 1990) or some approximate methods such as penalized likelihood, penalized quasi-likelihood (Breslow & Clayton, 1993), and restricted iterative generalized least squares (Goldstein, 1995). Interaction terms can be added in the same way as in linear and nonlinear regression models. There are several software programs available for fitting multilevel models, including MLwiN (www.cmm.bristol.ac.uk/), HLM (www.ssicentral.com/), and WinBUGS (http://www.mrc-bsu.cam.ac.uk/bugs/).

10.4.5 Evaluating Temporal Effects

10.4.5.1 Overview

The longitudinal design of the NCS has the important advantage of permitting the evaluation of temporal effects. There are several statistical tools available for evaluating temporal effects depending on the research question being asked and the type of data being collected. This section discusses longitudinal data analysis, structural equation modeling with longitudinal data, and growth curve models.

10.4.5.2 Longitudinal Data Analysis

Longitudinal data analysis most often refers to repeated measurements on individuals. In the NCS, data will be collected from individuals at regular time points throughout the period of study participation. Specifically, physical, cognitive, and intellectual growth and functioning will be measured over many years. Because these measurements are taken from the same individuals over time, they are correlated and, thus, require special tools for data analysis.

The primary methods used in longitudinal data analysis are generalized estimating equations (GEE) and mixed effects models (see, for example, Fitzmaurice, Laird, & Ware 2004). Since repeated measures may be considered to be "nested" within individuals, multilevel models can be used to reflect the dependent structure of the observations (see Section 10.4.4).

In mixed effects models, individual effects are modeled explicitly. In essence, a separate regression equation is estimated for each individual based on the values of the dependent variable and the independent variables measured at the different time points. The repeated measurements are assumed to be independent within a given subject after the individual intercept and slope for the subject is taken into account. For example, in modeling dental growth, an intercept and slope might be fit for each individual; variations in projected growth about the regression line for an individual are assumed to be independent. In this example, a mixed model would include random effects for growth variations in each individual and fixed effects for factors such as gender that affect overall growth rates.

GEE is associated with marginal models, so-called because they are based on data that are averaged or accumulated for each time point. That is, the basic model is $E(\mathbf{Y}_i) = X_i'\beta$, where $\mathbf{Y}_i$ is a response vector at time i, X_i is an independent variable measured at time i, and β is a fixed regression coefficient. For example, Diggle, Liang, and Zeger (1994) show how marginal models using GEE can be used to compare respiratory infection rates between children with and without vitamin A deficiency using examination data from six medical visits, adjusting for the effects of seasonality and age**.** While marginal models and mixed effect models give similar results for continuous outcomes, GEE is more suited for binary outcomes.

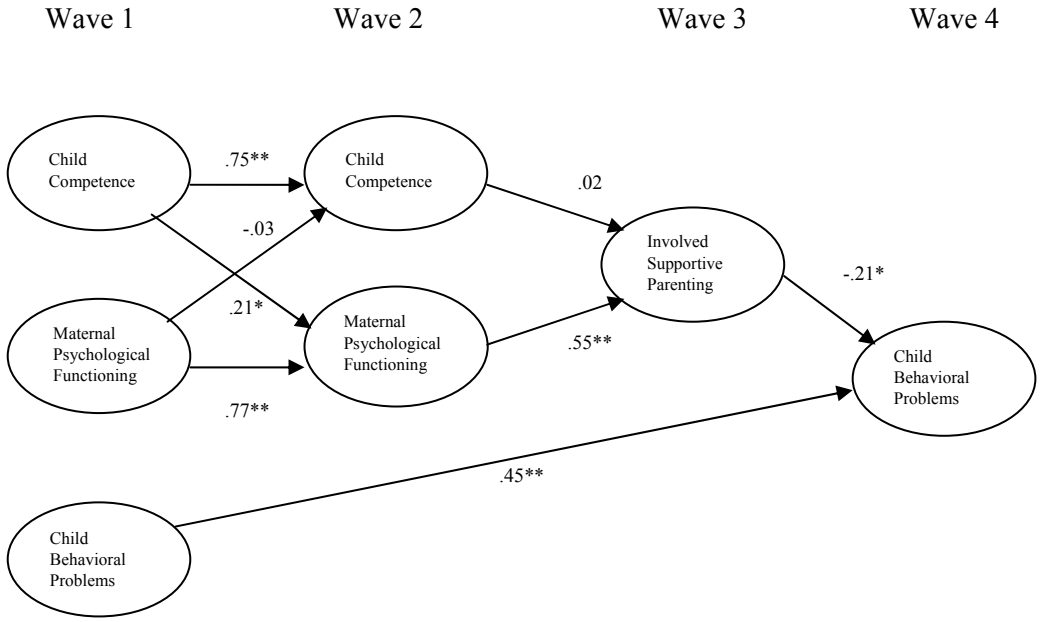
10.4.5.3 Longitudinal Structural Equation Modeling

Structural equation modeling, described in Section 10.4.3, has particular utility for modeling longitudinal data. Data sets such as the NCS that include multiple repeated measures of constructs or indicators are well suited for this analytic strategy. SEM permits modeling of latent variables from multiple indicators and the simultaneous testing of the interrelations among latent variables or individual observed variables. Longitudinal SEM extends this to make multivariate modeling of change over time possible.

When repeated measures over time are represented in SEM models, it is possible to examine predictions of later values of a latent construct while simultaneously accounting for stability of the construct from a previous assessment. Consequently, the predicted outcome represents change in that variable from the previous time point rather than being a static assessment. Because it permits complex multivariate analyses through robust model estimation techniques, SEM provides one of the most statistically elegant methods for investigating stability and change in longitudinal research.

Longitudinal SEM models also permit highly complex, temporally accurate testing of latent variable mediation models, such that the exposures precede the mediator and the outcome follows the mediator temporally, reducing the possibility of reverse-mediation. When previous values of the variables are also accounted for in the model, longitudinal SEM mediation models provide greater support for the hypothesized causal pathways than do models without these multifaceted longitudinal components.

An example of SEM with four waves of data concerning maternal and child adjustment is presented by Brody, Kim, Murry, and Brown (2004). Within these four waves were latent variables at two time points each for maternal psychological functioning, child competence, and child behavioral problems as well as a latent variable for involved supportive parenting (see Figure 10-5 below).



* significant path, p < .05
** significant path, p < .01
Source: Brody et al., 2004.

Figure 10-5. Longitudinal SEM Model of Child and Maternal Functioning

The longitudinal nature of the data permits complex inferences about the interrelations of these constructs over time. As can be seen from the figure, Wave 1 child competence predicts changes in maternal psychological functioning between Waves 1 and 2, which subsequently predicts involved supportive parenting at Wave 3. The figure suggests that child competence has effects on later parenting behavior only through its effects on intervening maternal psychological functioning. Involved Supportive Parenting also predicts changes in child behavior problems between Waves 1 and 4. Longitudinal SEM effectively models the complex bidirectionality of influence that parents and children have on each other's functioning over time. The NCS data will permit modeling such as this over the course of many waves of data and for multiple sources of influence.

10.4.5.4 Growth Curve Analysis

The primary goal of growth curve analysis is to describe patterns of change over time and identify predictors that affect these patterns. Growth curves may be modeled using exponential or logistic functions when early growth is rapid. Polynomial growth curves may also be useful. As a familiar example, height has a well understood developmental trajectory where a logistic growth curve fits the first three years of life, followed by a linear growth trend until adolescence when growth again follows a logistic curve (Bock et al., 1973).

Another example of growth curve analysis is given by Cherlin et al. (1998), who describe the effects of parental divorce on the subsequent mental health of their children at ages 7 through 33. This study, which was based on data from the National Child Development Study (Chase-Lansdale et al., 1995), assigned scale scores to emotional problems at a range of ages. Growth in these scales was then modeled as a function of age at time of divorce, gender, economic status, class background, and school

achievement. All variables except class background were statistically significant. The significant age at time of divorce variable resulted from higher scores on emotional problems scales for later ages at time of divorce up to age 22.

The latent growth curve model (LGM) is a special case of structural equation modeling and multilevel modeling. LGM treats repeated measures of individual behavior as a function of chronological development. LGM is a type of multilevel model that extends the hierarchical structure to panel data in which individuals are observed across time.

10.4.6 Case-Control Studies

10.4.6.1 Introduction

In case-control studies, a small number of cases (usually persons with a particular disease or other outcome of interest) are compared with a sample of controls, persons without the disease or the outcome being studied. There are several potential situations where this method would be relevant to the NCS. One example would be where blood or other specimens have been stored for many NCS participants but, due to laboratory processing expenses, only a relatively small number of individuals (with a given condition) would actually be selected to have their specimens analyzed. In such a situation, the specimens of an appropriate set of controls (persons without the condition) would also be analyzed and the two sets of data would then be subjected to a case-control analysis. A similar situation might arise if a new hypothesis called for additional measurements to be taken.

A second situation, which is the more traditional application, would arise when individuals with a given outcome are rare. In such a situation, all persons sampled in the NCS with the condition might be included in a comparison with a selected sample of controls to evaluate risk factors, for example.

10.4.6.2 Selection of Controls

The selection of controls is critical to the validity of case-control studies. Quite often, some form of matching is used in this process to control for confounding variables known to influence disease incidence or outcome. The two basic methods of matching are set matching, where each case is matched individually to one or more controls, and frequency matching, where cases and controls are matched in categories (e.g., a group of 15 cases who are male and aged 30 to 39 might be frequency matched to 45 controls who are male and aged 30 to 39). While set matching is a more traditional approach, frequency matching has a number of advantages both operationally and analytically, particularly in large studies.

Because case-control studies typically begin with disease cases that have already occurred, they are subject to significant sources of bias. A key step in the process of ensuring a bias-free case-control study is that cases be representative of all those who develop the disease under investigation. One threat to this process is that cases are often identified as they are diagnosed in a clinical setting, and mild cases or those that result in early mortality will not be diagnosed and are thus missed as cases. This type of bias is called incidence-prevalence bias or survival bias. Case-control studies can also give biased results if the controls are not representative of the population at risk for developing the disease under investigation. To avoid these types of bias, it will be of paramount importance for the NCS to select appropriate and representative cases and controls.

10.4.6.3 Case-Control Studies in the NCS

Nested case-control studies are those where cases are identified within a well-defined cohort, such as the NCS, where controls are selected from within the same cohort. Nested case-control studies combine some of the advantages of both cohort and case-control designs. Depending on how control subjects are selected, a few sources of bias inherent to the case-control design (e.g., recall of events and chronological differences between case and control identification) can be avoided using a nested case-control investigation. Extensive data representing the time period prior to the disease diagnosis will be available for cases, and corresponding data will be available for controls. Another weakness inherent to case-control studies that will be prevented by selecting cases and controls from the NCS cohort is the lack of extensive records before disease diagnosis. Data on many key exposures not typically available in case-control studies, such as dietary patterns, medication use, and environmental exposures, will be available to NCS researchers to determine pre-morbid risk factors more accurately. The NCS is also expected to utilize the matched case-control study design, where individual cases are matched to one or more controls based on similar demographic characteristics suspected of confounding the relationship between the exposure and outcome association under investigation.

10.4.6.4 Analysis of Nested Case-Control Studies

When analyzing nested case-control studies, the use of standard survey weights will generally lead to unacceptably large variability in estimates. By design, controls are matched to cases in terms of key confounding variables, and the distribution of these variables in the cases is usually very different from the distribution in the general population. This feature gives rise to large variation in sampling weights, which, in turn, leads to large standard errors. There are several ways to avoid this problem, two of which we describe below.

One approach is to perform an unweighted analysis. This type of analysis of case-control studies provides estimates of relative risk but not absolute risk. Thus, for example, estimates of regression coefficients would be useful but not of the intercept. Alternatively, if weights are to be used, a possible approach is to weight the sample to the case distribution, thus compensating for any disproportionate sampling of cases or nonresponse. See Scott (2006) for a discussion of these issues.

In case-control studies that used set matching, the multivariate analysis technique most frequently used is conditional logistic regression. This estimation takes into account the pairing or matching of cases and controls with respect to the variables that determined the matching. The interpretation of the coefficients in conditional logistic regression is the same as in ordinary logistic regression except that these coefficients are to be considered “adjusted” not only for the variables included in the model but also for the matching variables.

For the statistical analysis of case-control studies using frequency matching, a more efficient strategy is to use ordinary logistic regression and include the matching variables in the model. Similarly, nested case-control studies can be analyzed in the same way as matched case-control studies, where cases and controls are matched by length of follow-up. As a result, the multivariate analysis technique most often employed is conditional logistic regression, in which the conditional variable is length of follow-up. This type of conditional logistic regression model is similar to the Cox proportional hazards regression model.

10.5 Analysis of Genomic Data

The combination of a longitudinal follow-up of the NCS cohort, its large size, and its comprehensive collection of environmental exposures will provide a rich source of data with which to investigate the contribution of genetic variation to complex diseases such as autism, obesity, and asthma as well as the impact of gene and environment interactions on neurodevelopment, health, and behavior outcomes. For either genome-wide analysis (GWA) or candidate-gene approaches, the outcome can be discrete (e.g., having autism or not), continuous (e.g., quantitative measurements of depression) or censored survival data (e.g., time to onset of type 1 diabetes).

With respect to complex phenotypes that will be studied in the NCS, it is expected that multiple genes as well as gene-environment and gene-gene interactions play a key role. The large sample sizes available will greatly facilitate not only the identification of individual genes, but also the ability to identify gene-gene and gene-environment interactions. It is nevertheless clear that the ultimate success of such GWAs will depend largely on the development of highly complex and innovative analytic strategies. Methods that can efficiently account for the genome-wide linkage disequilibrium patterns and control for genome-wide error rates using false discovery rate procedures are required. Novel statistical methods that can identify gene-gene and gene-environment interactions and methods that can incorporate known biological knowledge, such as networks and pathways, in searching for complex disease genes are also greatly needed. The analysis of genomic data is a field of much active research. Analysis of genotype effects and multilocus genotype-by-genotype interactions (e.g., epistasis) as well as gene-environment interactions can be cast in a regression framework for different types of outcomes where the predictor variables include SNPs, environmental exposures, interactions among SNPs, and SNP-by-environment interactions. Due to the problem of high-dimensionality, standard regression analysis methods cannot be applied directly when many genes are involved because they produce highly variable estimates. Methods developed for analyzing high-dimensional data, such as microarray gene expression, massively parallel signature sequencing (MPSS), and evolutionary trees of haplotypes, may also be utilized. New analytic methods can be expected to emerge in the future, and researchers analyzing the genomic data in the NCS will need to apply the best methods available in every phase of the process. Some specifics of these methods are described below.

10.5.1 Haplotype Analysis

Recent advances in high-throughput technologies and the decrease in genotyping costs have made genome-wide association analysis a feasible tool in the search for genetic contributors of complex traits, including many complex diseases. As the NCS evolves and technologies mature, it is possible that genome-wide genetic profiling for each participant of the study will be available to enable possible genome-wide searches for genetic variants as well as the interactions among genes and between genes and environmental risk factors. One challenge of such data is their very high dimensionality. One solution to this problem is to fully utilize the information from tagging SNPs, haplotypes, and haplotype blocks derived from the HapMap project. Haplotypes are a set of closely linked genetic markers present on one chromosome which tend to be inherited together and which can be utilized as the unit of analyses in order to examine their effects and interactions with the environmental exposures. Haplotype analyses are potentially more powerful in identifying genes predisposing to certain health outcomes. For example, a test for association between the common haplotypes in haplotype blocks and specific outcomes can be conducted. Such association analysis can be performed using sliding windows of a small number of overlapping SNPs. Alternatively, newly developed methods such as Logic regression, FlexTree, and threshold gradient descent procedures can also be applied for considering haplotypes in multiple regions and for identifying haplotype-by-haplotype interactions and haplotype and environmental exposure interactions.

One difficulty with haplotype analysis is that haplotypes are often not observed but must be estimated from the genotyping data. This can be accomplished in a regression analysis and missing data context. The expectation-maximization (EM) algorithm can be developed for estimating the model parameters (Lin, 2004). Such EM-based estimation as well as inference procedures have been developed for binary outcomes in case-control designs and for survival outcomes in prospective cohort designs. Following the nonparametric maximum likelihood approach in Scheike and Juul (2004) for estimation of the Cox model under nested case-control sampling, methods for analyzing age of onset data and for estimating the haplotype effects could be developed in a framework of censored data regression and missing data under the case-control and nested case-cohort samplings. Specifically, we can treat the haplotype phases and the haplotypes of those who are not genotyped as missing data and use the EM algorithm and the nonparametric maximum likelihood approach to estimate the haplotype relative risk and the baseline hazard function (Chen & Li, 2005, in preparation).

10.5.2 Population Stratification

Population stratification is an important issue to consider when studying gene-trait associations using unrelated subjects, since the observed association could be spurious without appropriate adjustment for underlying population strata (Cardon & Palmer, 2003). In our analyses, we will often consider African Americans separately from Caucasians. However, population substrata could still confound gene-trait associations within African Americans and Caucasians, particularly in African Americans (Cardon & Palmer, 2003). Therefore, it will be important to adjust for population stratification in genetic association analyses. If candidate gene studies are conducted, we will select ancestry informative markers in both ethnic groups and use the STRUCTURE (Pritchard & Rosenberg 1999) approach to infer the degree of population stratification as represented by the proportion of ancestry of each individual in the study. We will test the hypothesis of two or more strata (i.e., ethnic subpopulations) within each ethnic group, and the STRUCTURE program will attempt to classify individuals as belonging to one population or another. If there is evidence of population stratification, then the multivariate analyses described previously will be repeated with adjustment for population stratum membership (e.g., using stratified logistic regression analysis). For genome-wide association studies, a recent publication by Price et al. (2006) proposed a method that enables detection and correction of population stratification on a genome-wide scale using the idea of principal components analysis. The resulting correction is specific to a candidate marker's variation in frequency across ancestral populations. We will use this method and the EIGENSTRAT software provided by the authors for genome-wide association analysis.

10.5.3 Gene-By-Gene/Gene-By-Environment Interactions

Gene-environment interactions are measured by the effects of clinical/environmental exposures on the disease risk among individuals with different genotypes. Gene classification schemes can be added to the final model for clinical risk factors, and then systematic tests for interactions between gene classification and risk factors can be conducted. Logistic regression models can be utilized to explore three types of interactions. For notation, we define:

R = clinical risk factor(s);

E = exposure(s);

G1 = genotype/haplotype/single nucleotide polymorphism (SNP) 1;

G2 = genotype/haplotype /SNP 2.

Then,

Model 1: Clinical risk factor and gene interaction

$$\text{Logit}[\Pr(\text{outcome})] = b_0 + b_1 R + b_2 G1 + b_3 (R * G1)$$

Model 2: Environmental exposure and gene interaction

$$\text{Logit}[\Pr(\text{outcome})] = b_0 + b_1 E + b_2 G1 + b_3 (E * G1)$$

Model 3: Gene–Gene interaction

$$\text{Logit}[\Pr(\text{outcome})] = b_0 + b_1 G1 + b_2 G2 + b_3 (G1 * G2)$$

In the first model, the outcome variable, say a diagnosis of undescended testes (UDT) would be an indicator of status (0 = absent or 1 = present). A previously selected exposure of interest (e.g., phthalates) would be categorized as absent or present (E = 0 or 1 depending on whether the subject was exposed during gestation) or as a continuous variable (quantitative measure of phthalate exposure from biologic specimens or from an estimated latent variable), and a candidate genotype/haplotype (e.g., HOXA9) would be characterized as nonsusceptible/susceptible (G1 or G2 = 0, 1).

Estimation of the odds ratio for clinical risk factors among the susceptible genotype (G1 = 1) group is then $\exp(b_1 + b_3)$ and for the nonsusceptible group (G1 = 0) is $\exp(b_1)$. A measure of the strength of the interaction can be evaluated by the odds ratio (OR) for susceptible and nonsusceptible, which is expressed in this model as $\exp(b_3)$. A score test for the statistical significance of the interaction OR = 1 is then a test of $b_3 = 0$. The same approach would be taken to model phthalate exposure and gene interactions (model 2), and gene-gene interactions (model 3) (Hwang et al., 1995; Yang & Khoury, 1997; Andrieu & Goldstein, 1998) such as INSL3 and GREAT, both thought to control development of the gubernaculum.

Completion of the statistical analyses described here would allow not only for the assessment of associations between allelic variants in candidate genes but also for interactions between clinical factors and in utero environmental factors such as exposure to phthalates on the risk of the outcome of interest (e.g., nonsyndromic UDT).

Another area of study is the relationship between the environmental exposures and the patterns of somatic mutations in genes. To account for potential dependency of the mutation patterns along the genome, the generalized estimating equation approach for analyzing correlated binary data can be applied to identify how environmental exposures can potentially induce somatic mutations in cells. Clustering analysis methods can also be applied to cluster the mutation patterns based on multivariate binary data and to relate the mutation clusters to environmental exposures.

10.5.4 Regression Tree Approaches to the Analysis of Interactions Between Genes

Because interactions play a key role in the analysis of genomic data, including data involving SNPs, there are multiple approaches to this type of analysis. Since the number of nucleotides

involved in susceptibility for a complex traits and conditions is often quite large, special methods are required for analyzing the even larger number of possible interactions of SNPs within and between genes. As an example, the risk of type 1 diabetes can be related to the interaction of multiple SNPs rather than to single variation sites. Analytic approaches such as the adaptive spline and tree-based methods such as MARS and CART (Friedman, 1991; Breiman et al., 1984) can be used to generate interpretable interaction rules among the SNPs. Realizing the limitations of these methods—for example, MARS is efficient on data that has interactions in at most a few variables, and CART only generates rules in disjunctive normal form—the recently developed adaptive regression method, logic regression, may be applied in order to construct predictors as Boolean combinations of the SNPs using simulated annealing.

Such Boolean combinations of the SNPs may not be detected by a standard regression tree as implemented in CART. In order to study and assess gene-by-gene interaction and gene-by-exposure interactions on the risk of developing certain outcomes, the recently developed tree-based method FlexTree (Huang et al., 2004) may be employed. FlexTree is an extension of the binary tree-structured approach such as CART and is particularly applicable to study gene-by-gene and gene-by-environment interactions. The methods work well for both the model where many genes are involved in the predisposition of certain outcomes and the model where only a small list of aberrant genotypes is predisposing.

The Bayesian variable selection approach introduces a latent binary vector to index all possible subsets of variables (George & McCulloch, 1993). A prior distribution is specified for this latent vector and the variable selection is performed based on the posterior model probabilities. When the number p of covariates is large, deriving the posterior probabilities of all 2^p possible models is computationally prohibitive. This can be handled via Markov Chain Monte Carlo (MCMC) stochastic search techniques, which are used to explore the space of variable subsets and search for promising models. At each MCMC iteration, a new candidate model is visited and retained based on its posterior probability relative to the previously visited model. This method is well suited for the analysis of high-dimensional data where the sample size is substantially smaller than the number of covariates $(n << p)$. Another advantage of the Bayesian approach is that it allows the uncertainty inherent in the model selection process to be incorporated in the inference mechanism. This is accomplished via model averaging where the estimation of parameters and the prediction of future outcomes are computed by averaging over a range of likely models.

10.5.4.1 Multifactor Dimensionality Reduction for the Analysis of Interactions Between Genes and Between Genes and the Environment

Multifactor dimensionality reduction is a method designed specifically for investigating multiple gene-gene and gene-environment interactions. In studies using related family members, the programs are currently only applicable for gene-gene but not gene-environment interactions. However, most of the children in the NCS will be unrelated and therefore this method will also be ideal for the investigation of gene-environment interactions. In logistic regression models, the number of possible interaction terms grows exponentially as each additional main effect is added (Ritchie et al., 2003). To reduce the dimensionality in data where interactions are the primary focus, one divides the data into training and test sets and then forms all possible permutations of the chosen interaction terms. The interactions become, in a sense, the "main effects" under investigation (Ritchie et al, 2003). An example would be two genes, each with a dominant and recessive homozygote and a heterozygote, making nine possible combinations. Each cell class is labeled as high or low risk according to a predefined threshold. The MDR model that has the fewest misclassified individuals is selected, and the process is then repeated using 10-fold cross-validation to evaluate predictive ability and reduce spurious significances. Since

multiple gene-gene and gene-environment interactions are the rule rather than the exception in many complex health conditions and behaviors being studied in the NCS, this process will allow for more realistic modeling of multiple interactions.

10.5.4.2 Complementary Modeling Approaches

Interactions confirmed with any of the above-mentioned approaches can also be incorporated with other causative factors into path analytic models to identify multiple cross-sectional and/or longitudinal causal pathways (see more descriptive discussions in Section 10.4). An example would be the NCS hypothesis that gene-environment interactions between ozone exposure and polymorphisims of TLR-4 and/or TNF-α play a causal role in asthma onset. This could be tested with one of the methods described above and the significant interactions inserted into a path analysis model with other factors related to the outcome but not to these interactions (e.g., prenatal factors such as low birth rate).

10.5.5 Gene Expression Data-Microarray Analysis

In addition to these established methods, others developed for analyzing high-dimensional data such as microarray gene expression data are worth exploring. Gene microarrays are a method employed for examining the expression of as many as hundreds or thousands of genes in a single tissue (Jarvis, 2006). Although the NCS will not have tissue specimens, it will have whole blood. As the Study evolves, it may be possible to collect the gene expression profiles in whole blood samples over time in order to examine how those gene expression changes detectable in blood are related to development of various health outcomes or to identify potential biomarkers for diseases and investigate how gene expression is affected by environmental exposures. Such data make it possible to learn how expression of different genes and, hence, their coded proteins, interact to provide insight into biochemical pathways and causal mechanisms on a genomic level.

Of particular interest with respect to analysis of these data are the threshold gradient methods (Friedman & Popescu, 2004; Gui & Li, 2005) and the Bayesian variable selection methods (Sha et al., 2004) for identifying important SNPs, environmental exposures, and their interactions for the risk of developing certain health-related outcomes. Bayesian variable selection methods have been developed and used successfully for the analysis of DNA microarray data in the context of multigroup classification (Sha et al., 2004) and clustering (Tadesse, Sha, & Vannucci, 2005). In the former case, the goal is to identify subsets of genes that characterize the different classes and to predict the outcomes for future samples based on their expression profiles. In the latter, the groups from which the observations arose are not known and the goal is to uncover the cluster structure of the observations and identify the discriminating variables. However, methods for analyzing longitudinal gene expression data are still relatively limited (Guo et al., 2003; Tai & Speed, 2006). We propose that the NCS develop new methods in the framework of functional data analysis and empirical Bayes analysis and treat the gene expression profiles over time as curves or functional data. Preliminary analysis of methods based on functional data analysis indicate such methods result in more sensitive procedures for identifying genes that show different expression patterns over time (Hong & Li, 2006; Leng & Mueller, 2006). We propose that the NCS generalize many commonly used multivariate analysis methods such as canonical correlation and correspondence analysis to the functional data for exploratory analysis and for graphically displaying the data. We also propose that the NCS develop regression analysis methods with functional data as predictors to account for gene expression dynamics over time. Functional data analysis provides a natural framework for accounting for gene expression levels measured over time and can potentially consider the dynamic nature of gene expression over time. These methods will be developed and made available to interested researchers on the NCS website.

10.5.6 Family Data

For population-based genetic association studies of complex traits, one of the potential areas of confounding involves the latent population substructures in the study population, which can result in the observation of spurious associations if such substructures exist and are not appropriately accounted for. Family-based study designs such as parent-child trios provide an alternative design for genetic association analysis of complex traits (Spielman & Ewen, 1996). For population-based case-control or cohort designs, genomic controls by typing a set of ancestry informative markers can be employed for adjusting for such population substructures in regression analysis (see more on this above).

Although deviations from the Hardy-Weinberg equilibrium (e.g., existence of migration) can identify systematic errors in genotyping, it is important to carry out other checks. For case-control studies of genetic association, under particular models for genotyping error there is no increase in type I errors of tests for genotype-disease associations (Gordon et al., 2002). (See Section 10.2.3 for a discussion of type I and II errors.) However, if general tests which ignore genotyping error are invalid, one solution is to integrate a realistic error model into association analysis for SNPs. A number of models for measurement error have been proposed and are described by Gordon et al. (2002) along with descriptions of how to appropriately test for association. Similarly, measurement errors in environmental exposures will be accounted for in the context of regression analysis with measurement errors (Ruppert et al., 2003, Chapter 15). Details of dealing with measurement error are provided in Section 10.3.

Genomic imprinting can be loosely defined as the gamete-of-origin dependent modification of phenotype. In other words, the phenotype elicited from a locus is differentially modified by the sex of the parent contributing that particular allele. This process results in a reversible gamete-of-origin specific marking of the genome that ultimately produces a functional difference between the genetic information contributed by each parent. In humans, the term genomic imprinting is usually described as mono-allelic gene expression or the inactivation of either the maternal or paternal allele of a particular locus. One important issue in the context of the NCS is to differentiate between fetal and maternal genotypic effects, which can be tested using the transmission test for linkage disequilibrium (Mitchell, 1997). While both parent-child triads and grandparent-grandchild designs can be used for testing maternal or parent of origin effect, the grandparent-grandchild design may in some situations provide higher power than the parent-child design (Weinberg et al., 1998; Wilcox et al., 1998). However, given the size of the NCS cohort, power should not be a problem (see Section 10.2.3, Power for Subgroups). In the framework of the log linear models as developed in Weinberg et al. (1998), one can also incorporate the environmental covariates into analysis of parents of origin and maternal-mediated genetic effects.

The parents-affected child trio design provides an alternative family-based design for studying associations between candidate genes and the risk of developing diseases or for studying gene-by-environment interactions. Designs based on the genotyping of affected individuals and their parents allow the detection of markers in linkage disequilibrium with disease genes (Spielman & Ewen, 1996). The main advantage of such a design as compared to population non-family cohort design is that it is free from the issue of spurious association caused by potential underlying population substructures. Such designs can be used for confirming the associations found in the standard population-based designs (non-family). Environmental covariates and gene-exposure interactions can be easily taken into account in the analysis (Li & Fan, 2000; Shih & Whittemore, 2002).

10.5.7 Multifactor Dimensionality Reduction and Issues of Multiple Comparisons

One important consideration with respect to genome-wide association studies is the issue of multiple comparisons. This issue usually arises in the context of hypothesis testing; however, even in exploratory studies without a formal hypothesis, there is generally an implicit hypothesis that a given discovered effect is zero.

When conducting multiple hypothesis tests, the type I error rate of 0.05 will hold for each individual test, but the overall probability of making at least one type I error is greatly increased. The most common procedure for protecting against this is to require a stringent "family-wise" error rate adjusted for the number of tests being conducted.

Although the family-wise error rate procedure is popular and performs well in genome-wide linkage analysis, it is too stringent for evaluating multiple loci, which may result in very low power. The false discovery rate (FDR) introduced by Benjamini and Hochberg (1995) provides a new notion of global error for multiple testing procedures. The idea of FDR is to use the expected proportion of false rejections of the null hypothesis among the total number of rejections as the measure of global error. Such a procedure leads to a global cutoff value that is adaptive to the data set (Sabatti et al., 2003). The FDR procedure will identify a lower cutoff level than the universal Bonferroni cutoff if a higher percentage of the null hypotheses tested are truly false. Such a procedure is most effective for the identification of loci with secondary effects. On the other hand, if all the null hypotheses are true (none of the analyzed markers is associated with the disease), controlling FDR is equivalent to controlling family-wise error rates. Although the original procedure by Benjamini and Hochberg was developed for independent tests and *p*-values, recent studies and extensions have indicated the procedure also works well when the tests are not independent, as might be expected in genome-wide association tests (Sabatti et al., 2003; Fernando et al., 2004).

Because health effects are commonly measured by multiple outcomes, the main tools we propose to apply or develop for the data to be collected are the regression models for multiple outcomes (Sammel et al., 1997; Sammel et al., 1999; Geys et al., 1999). We also propose to apply state-of-the-art models such as errors-in-variable models, missing-data methods, smoothing and methods for correlated data, such as longitudinal and spatial data analysis, to assess the health effects of dose, concentration, and duration of exposure. Semiparametric regression and generalized estimation equation methods can be developed for modeling the data and estimating the parameters in order to make fewer assumptions on the underlying models.

Another important application of multifactor dimensionality reduction is the investigation of gene-by-gene interactions. In logistic regression models, the number of possible interaction terms grows exponentially as each additional main effect is added (Ritchie et al., 2003). To reduce the dimensionality in data where interactions are the primary focus, one divides the data into training and test sets and then forms all possible permutations of the interaction terms. So for two genes, each with a dominant and recessive homozygote and a heterozygote, there are nine possible combinations. These combinations or interactions are treated as the "main effects" (Ritchie et al., 2003). Each cell class is labeled as high or low risk according to a predefined threshold. The MDR model that has the fewest misclassified individuals is selected and the process is repeated using 10-fold cross-validation to evaluate predictive ability and reduce spurious significances.

10.5.8 Twin Studies

The NCS cohort is estimated to eventually contain 3,000 twins. These twins will provide a unique opportunity to examine gene-by-environment interactions, especially with respect to complex diseases. Whereas monozygotic twins have identical genotypes, dizygotic twins have the same genetic variation as non-twin siblings. When monozygotic twins have one affected and one nonaffected twin, the ability to investigate gene-by-environment interactions with respect to specific SNPs, haplotypes and environmental factors will greatly facilitate the understanding of causal pathways.

11. DATA USE AND CONFIDENTIALITY PROTECTIONS

The National Children's Study Publications Subcommittee of the NCS Steering Committee will oversee the orderly and timely presentation of pertinent findings and data from NCS to the scientific and medical communities as well as to the public. This will include scientific papers, abstracts, and presentations. The subcommittee will also assure fair and equitable participation in the analysis of the data set and in the presentation of the study results by all NCS investigators.

Press releases and media interviews; and presentations to lay and community groups are the responsibility of the Program Office and Study Centers.

11.1 Disclosure Controls

The NCS Publications Subcommittee and the NCS Steering Committee will both have central roles in ensuring that study participants' data are appropriately protected. Methods to be used include a broad suite of disclosure control tools, which balance minimizing risk to participants with the potential for societal benefit. The ultimate goal is to protect individuals while still making data accessible to those who might make valuable contributions based on those data. There are several ways in which the NCS will strive to ensure such protection. First, the NCS will employ secure treatment of identifying data through limiting the appearance of personal identifiers on distributed data sets. Second, the NCS will control access to sensitive information by identifying different levels of access to the data and customizing data access plans across levels to ensure adequate protections on all releases of data. Third, the NCS will utilize statistical disclosure control procedures to reduce the appearance of unique personal information in the data that could result in re-identification of a participant.

11.2 Public Use Data Sets

Public use data sets for a given outcome and life stage will be developed for data sharing and made accessible to both the scientific/research community and the general public as soon as feasible, but no longer than within two years of the availability of a usable data set, and in accordance with NIH data sharing policy.[1] These types of public use data sets can be thought of as two levels of data.

11.2.1 Data Sets for the Scientific/Research Community

Data sets for professional researchers including academics, government workers, and others will be made available in compliance with the National Institutes of Health (NIH) data sharing policy:

> "Data-use sharing agreements will put some limitations on who can use the data and how they are to be used. Such agreements will contain requirements, including those to protect the privacy of subjects and the confidentiality of the data. These agreements will incorporate confidentiality standards to ensure data security at the recipient site and prohibit manipulation of data for the purposes

[1] Relevant NIH policy and guidance on data sharing can be found at the following websites: NIH Data Sharing Policy http://grants2.nih.gov/grants/policy/data_sharing/; NIH Data Sharing Policy and Implementation Guidance (Updated: March 5, 2003) http://grants2.nih.gov/grants/policy/data_sharing/data_sharing_guidance.htm

of identifying subjects. They will stipulate that the recipient not transfer the data to other users, that the data are only to be used for research purposes, that the proposed research using the data will be reviewed by an IRB [institutional review board], and the like."

Data made available to the scientific and research community will be available in de-identified data sets. Data will be released only after a full and detailed analysis of risk of data disclosure is performed. All users will sign appropriate confidentiality agreements.

11.2.2 Public Use Data Sets

Because there are no signed agreements or restrictions with regard to individual public data users, public use data files demand a very thorough initial review of the data for risk of disclosure. It is likely that a number of disclosure control techniques, jointly called perturbation, will be used on public data to ensure participants are fully protected from snoopers or inadvertent disclosures by those who are outside the research community.

11.3 Publication Policy

Study-wide publications from data that are not yet released to the general public or to the broad scientific/research community will emanate from a de-identified, validated data set issued by the Coordinating Center to NCS investigators. The data set will be available for analysis by NCS investigators after the completion of a life stage (e.g., completion of the 1-year visit) using data from either the entire cohort or from a random replicate of the entire cohort (wave of data collection). The NCS Community of Investigators consists of investigators in the Program Office, the Interagency Coordinating Committee, the Steering Committee, the Coordinating Center, and all Study Center principal investigators (and their site investigators).

A series of derived variables based on raw data, validated by the NCS Coordinating Center and approved by the Steering Committee (e.g., standardized or normed growth measurements), will be included in the database. Both core publications and non-core publications are anticipated.

11.3.1 Core Publications

Core publications are study-wide publications that address study methodology, baseline cohort descriptions, and the priority exposures and outcomes of the NCS (as identified in the 28 core hypotheses and updated over time). The scope of the core publications will be specified by the Steering Committee in collaboration with the Program Office. The NCS Publications Subcommittee will announce pending availability of data for each core hypothesis, and all interested members of the NCS Community of Investigators will be invited to submit proposals for analyses. Once formed, writing groups will be assigned a Coordinating Center statistician and may begin analysis after receiving approval from the Program Office to expend funds on the effort.

11.3.2 Non-core Publications

Non-core publications are study-wide publications not directly related to the Study's core hypotheses. Proposals for these publications will be generated by the NCS Community of Investigators as well as other government scientists (from lead agencies or otherwise). The Steering Committee and NCS Program Office may wish to advise or participate in the publication of non-core publications to ensure the maximal use of NCS data. Non-core analysis may be with or without collaboration with the Coordinating Center, resources allowing. The data access and publication proposal review process will be described in detail in the NCS Publications Subcommittee Policy Manual.

11.3.3 Approval Process

All members of the NCS Community of Investigators may request permission to publish from the Publication Subcommittee. If the proposal is rejected, the decision may be appealed. The approval process will be outlined in detail in the NCS Publications Policy Manual.

12. HUMAN SUBJECTS PROTECTIONS

The National Children's Study is primarily observational in nature and will have both a low level of subject risk and a reasonable subject burden. However, the longitudinal nature of the research, the size and scope of the Study, and the diversity of the participants, make the human subjects protection issues significant. The NCS' commitment to collecting biologic, environmental, social, and behavioral measures and creating enduring data as well as biologic and environmental sample repositories with the potential for future studies not yet conceived, make the human subjects protections somewhat complex.

12.1 Study Population

The NCS will employ a national probability sample (see Chapter 6) with no exclusions based on gender, race, or ethnicity. Women, children, and men of all of the racial and ethnic groups and economic strata represented in the United States will be subjects. The rationale for this approach is to accrue and follow a population of children that captures the range and diversity of exposures and outcomes experienced by children in the United States.

Because a primary focus of the Study includes assessing the impact of exposures that occur early in pregnancy, three groups will be enrolled and followed: pregnant women of any age and their husbands/partners; adult women planning pregnancy; and adult women not planning pregnancy but with some likelihood of becoming pregnant. All births to mothers who meet the eligibility criteria will be included.

Women who are cognitively impaired or mentally ill are not eligible if they are not able to understand fully the Study's requirements and to grant informed consent. Only women with the capacity to consent will be enrolled.

At the time when a pregnant woman is enrolled in the Study, the biological father will also be invited to participate. If an enrolled woman does not want to identify or does not want the Study to contact the biological father, the Study will not contact the father. In these instances, the pregnant woman and her child would still be eligible for participation. The father does not need to live in the same home as the mother for initial inclusion in the Study, however, there are no plans to follow biological fathers or biological mothers who have no contact with the child.

Families that move will be followed to minimize the number of participants who are lost to follow-up. Because all births to mothers who meet the eligibility criteria are eligible for the Study, there will be children in the Study born to surrogate mothers, children who will be adopted, children who will be assigned to foster homes, and children whose mothers are on active duty in the military. In addition to children whose families move, foster children, adopted children, military children, and children whose parents divorce, may change households after birth. Because the children are the primary participants, they will be followed if they move or otherwise change households. The Study will use information collected from participants, as well as publicly available data, to track and locate families and children in the Study who change households.

12.1.1 Strategies/Procedures for Recruitment

Strategies for recruitment are outlined in detail in Chapter 6. The primary approach involves screening and recruitment from households located in neighborhoods targeted for inclusion in the Study

and through providers of prenatal care. A variety of materials and strategies, including, but not limited to, media outreach and distribution of brochures and newsletters, will be utilized to increase public awareness of the Study and aid with recruitment of Study subjects.

12.1.2 Special Classes of Research Participants

12.1.2.1 Pregnant Women and Fetuses

The NCS will recruit and follow women prior to and during pregnancy. The NCS fulfills the requirements for research involving pregnant women and fetuses as described in section §45 CFR 46.204 of the Code of Federal Regulations, subpart B. The purpose of the NCS is to develop important biomedical and psychosocial knowledge about the impact of biologic, environmental, social, and behavioral exposures prior to and around the time of conception, during pregnancy, and as the child ages, on the future health and development of children. This information cannot be obtained by other means. Risks to the women and fetuses are not greater than minimal, and the research will in no way affect medical decisions about pregnancy management and outcome. Provisions in this section of the regulations also state that consent from the father of the fetus is not necessary when the research imposes only minimal risks to the fetus.

12.1.2.2 Pregnant Adolescents

The NCS will enroll pregnant adolescents who are identified during the household screening or through sites of prenatal care, and who are otherwise eligible for participation in the Study (e.g., first trimester of pregnancy). Women younger than age 18 will not be eligible for inclusion in the preconception cohort. Laws regarding the legal status of pregnant adolescents vary by state. In some jurisdictions, pregnant adolescents are considered "emancipated" from their families and can be treated as adults for the purposes of obtaining informed consent for this research project. Additionally, in many jurisdictions pregnant adolescents may legally seek medical care for pregnancy without involving their parents. In these jurisdictions, Institutional Review Boards (IRBs) may permit pregnant teens to consent to participation in research in studies such as the NCS without parental involvement. Finally, even in jurisdictions where pregnant adolescents are not considered emancipated or able to consent for their medical treatment, IRBs may waive involvement of parents in the informed consent process under certain conditions [Section §45 CFR 46.408(c)]. Local centers, in consultation with their IRBs, will determine whether parental permission is required in addition to the consent of the pregnant adolescent under the age of majority.

12.1.2.3 Children and Adolescents

Investigating the effects of environmental exposures and gene-environment interactions on the outcome of pregnancy and on the growth and development of children is the primary aim of the NCS. Thus, children from newborn to adulthood will be the subjects of this longitudinal Study. Each child's parent or guardian will be asked to grant permission for participation in the Study. It is the expectation that children, as young as toddlers and continuing through adolescence, will be informed about the Study and its goals in developmentally appropriate language, using creative methods such as newsletters, comic books, Web sites and DVDs. IRBs will receive all informational materials for review and approval prior to implementation. Issues related to consent and assent are described below.

12.1.2.4 Economically or Educationally Disadvantaged Individuals

It is anticipated that some of the participant families in the NCS will be economically or educationally disadvantaged. Section §45 CFR 46.111(b) of the federal regulations requires the IRB to assure additional safeguards are provided in a study when some or all of the subjects are likely to be vulnerable to coercion or undue influence because of economic or educational disadvantage. The NCS will design additional safeguards into the recruitment and retention activities for all participants to encourage informed participation of all eligible subjects. Each Study Center will be required to develop meaningful and enduring partnerships with the communities from which participants will be recruited. These activities along with the informed consent process described below will result in no coercion or undue influence on potential participants.

12.1.2.5 Foster Children and Wards of the State

Because of the subject matter of interest to the NCS and the probability-based sample design, it is important that every eligible child be enrolled and retained in the Study. Some children eligible for enrollment in the Study may be in foster care, may be wards of the state, or may transition into these arrangements at some time after enrollment in the Study. Permission for continued participation of the child in the Study will be sought from whatever administrative agency or institution is responsible for the care of the child and, in addition, from the foster parent. (See Section 12.6.6 for details on consent for participation of foster children and wards of the state).

12.2 Benefits

Although it is possible individuals may benefit from participation, the Study does not claim that participants will have the "prospect of direct benefit" from the Study. There are likely to be collateral benefits of participation, including information about individual examinations and tests performed during the course of the Study, health education, increased awareness of medical and social services available in the communities studied, and serendipitous findings of clinical relevance or of predictive value to participants and their families.

The potential for NCS to benefit society and children in general is extraordinary. The hypotheses being addressed and the data being amassed for future analyses are likely to impact the health and development of children for decades to come.

12.3 Potential Risks

Each of the procedures, measurements, and assessments in NCS is designed to fulfill the definition of "minimal risk" in the federal regulations [§45 CFR 46.102(i)] and to be reviewed by IRBs under §45 CFR 46.404 "Research not involving greater than minimal risk." Minimal risk as defined in the federal regulations means "that the probability and magnitude of harm or discomfort anticipated in the research are not greater, in and of themselves, than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests." In addition, the NCS staff is committed to minimizing risks even when the risks are minimal. Well-trained and competent individuals who have experience with pregnant women and children of the appropriate age will perform each procedure that might include discomfort or pain, such as a blood stick. Settings in which tests will be performed or information will be obtained from women and children will be woman- or child-friendly and respectful of the participants' needs and privacy. Questionnaires will be structured to avoid creating

discomfort for the women or children; and participants will be reminded at each data collection encounter that their participation is voluntary, they have the right to withdraw from the Study at any time, and they may refuse to answer or may skip any question.

There are additional issues associated with the Study related to testing and storage of biologic specimens and environmental samples; reporting concerns regarding possible child abuse or neglect; possible breaches of confidentiality; and informing participants of Study findings (which potentially could result in psychological effects, such as anxiety, or could have a financial impact, such as costs for additional testing). Each of these has been considered by the Study, and plans are in place to protect the welfare of participants and families involved in the Study.

The NCS staff is cognizant that while research staff is in and around the homes of participants, they may observe or learn about environmental hazards or behaviors that place a child in imminent danger, and investigators may be legally required to report such observations or information to specific authorities in some jurisdictions. The NCS staff feels morally obligated to respond to protect the interests of children when they are found to be in serious imminent danger, even if there are no reporting laws. Thus, the informed consent process will inform participants that if a data collector observes a child is in imminent danger of serious harm or the subject of child abuse, the information will be reported to the proper authorities to obtain help for the child. Study procedure manuals and interviewer training will describe the process that will be invoked to report such observations to the principal investigator or his/her designee at each site. Primary data gatherers will be trained to note such dangers to participants and inform their supervisors immediately for evaluation as to the proper course of action. It will not be the sole responsibility of the data gatherers to report the observations to authorities; rather it will be the responsibility of the professional staff under the supervision of the principal investigator to assure reporting is performed in an appropriate and timely manner. Study Centers will each have knowledge of local resources including social service providers for referral purposes. Each Study Center will develop a local mechanism for this reporting and referral process.

Primary data gatherers will also be trained to respond to observations of adults in danger, such as domestic violence between adults or suicidal tendencies. The NCS staff has a moral responsibility to assist adults in dangerous situations, but these situations will be dealt with differently. The adult victims will be involved in the process, and no reports will be filed with any authorities without the involvement and approval of the adult victims, unless required by law. Names of social service referral agencies will be provided upon request to adult victims of domestic violence.

12.4 Adjunct Studies

It is anticipated that in addition to the core protocol for the NCS, there will be adjunct studies proposed and conducted by investigators associated with the NCS. Such studies will involve a subset of the NCS cohort, at one or more Study Centers, on all or a portion of the local participants or their data. To protect the quality and integrity of the NCS, adjunct studies will be reviewed and approved through a defined process involving formal review and approval (see Chapter 16 for details).

Since Study participants may be asked to participate in these adjunct studies, the Study consent process will include a statement that participants may be contacted for other studies connected with NCS as a result of their participation in NCS, but they are not obligated to participate in any of these adjunct studies. All adjunct studies that involve additional interaction with human subjects will require IRB review and additional informed consent.

12.5 Incentives and Compensation

Recruitment and retention for the NCS will be a significant challenge in light of the respondent burden and the long-term commitment required of participants. It is expected that reasonable incentives will be part of the strategy for recruitment and retention of participants.

Compensation for participation will include reimbursement for expenses incurred in research participation such as travel to and from the research centers, parking, etc., and reasonable payment for time spent in participation in the research (approximately $25 to $50 per visit or exam, depending on the amount of time and effort involved). Adult participants and older children will be compensated for time spent completing questionnaires, for providing biologic specimens, and for other Study activities.

Small "gifts of appreciation" for continued participation will be provided to participants periodically. These may include token items such as T-shirts, tote bags, toiletries, books, and CDs. Gifts will not have sufficient monetary value as to unduly affect the voluntariness of consent to participate or of continued participation in the Study.

12.6 Consent and Assent Processes

The informed consent process will begin when potential participants are first notified about the Study and will vary depending upon the ages and types of participants and the pregnancy status of women. The first step in the process will involve advance mailings of Study material to potential participants. These materials will include a letter describing the Study. The next step will be enumerating household members. Then, pregnancy screening will be performed with all eligible females. Pregnancy screening will involve a script and a computer-assisted self-administered interview and will only include what is needed to determine Study eligibility. The Study will ask IRBs to accept oral consent for this process, because the eligibility screening will only involve questions about criteria used to determine the eligibility status of potential participants and about age in order to determine which consent process to administer. If a participant is found to be eligible and is willing to participate, only then will the full informed consent process commence.

The informed consent plan for the Study takes into account the types of participants and is tailored to address specific issues pertaining to each type. Women will provide informed consent during pregnancy for themselves and their child. There will not be a new consent process specifically for the baby at the time of birth.

The types of participants providing informed consent, or in the case of young children, assent will include:

- Adult women at risk of becoming pregnant (preconception women)
- Pregnant women (adult and adolescent)
- Biological fathers
- Other caregivers
- Children (through the phases: young children, adolescents, young adults)

The consent plan recognizes there will be transitions for some participants between types, and these transitions will affect the consent process. For example, preconception women might become pregnant and will need to provide additional consent for their own full participation in the Study and for the participation of their children. The assent/consent process for children will also change as the children grow from young children, to adolescents, to the age of majority.

The informed consent materials anticipate low literacy, they are culturally sensitive, and they reflect the diversity of potential participants. As part of the informed consent process, there is a method, described below, to ascertain if the participant understands key elements of the Study and what is involved in participation.

All consent materials will be available in English and Spanish, and other translations will be available as needed. Interpreters will be available for additional languages. A copy of the informed consent document will be made available to the participants electronically and as a paper copy.

12.6.1 Electronic Audio/Video Consent Tool Pilot

A video approach to informed consent is being developed to address some of the challenges with the traditional method for obtaining informed consent, as well as to provide a means for assuring consistency in the informed consent procedures across multiple sites of implementation. The primary goal of the tools is to enhance prospective participants' understanding of the purpose of the Study and all of the essential elements of informed consent. The videos take into account the diversity of potential participants and the reality that some eligible participants may have low literacy. They also accommodate the hearing impaired through closed captioning. There are separate versions of the tool for each of the different types of participants (preconception [nonpregnant] women, pregnant women, and biological fathers). During the Study's pilot phase, a computer-based interactive video informed consent tool will be compared to traditional written informed consent. The two methods of obtaining informed consent will be compared both in terms of understanding of the Study requirements (content of the consent) and Study enrollment.

This audio-visual presentation will be shown on the data collector's laptop or tablet computer. Study staff will be present during the entire informed consent process to assist with the computer presentation and answer participants' questions. The presentation includes embedded questions that assess the participant's understanding of what they have seen and heard to help ensure they understand the key elements of the Study and what their participation will involve. If the participant does not answer a question correctly, the presentation provides additional information and chances until the participant selects the correct answer. In that way, participants will not be excluded if they fail to answer some of the questions correctly. To consent, they will be required to keep trying until they understand which answer is correct, and the presentation will explain why the answer is correct to reiterate the information. The participant's written signature will be obtained electronically at the end of the presentation. A written copy of the material described in the informed consent video will be left with each participant.

12.6.2 Women Age 18 and Older

The NCS will initially recruit women ages 18 and older prior to and during pregnancy. Potential participants will be told that they can share the consent materials and discuss participation with family, friends, and, if they choose, their physician before deciding whether to enroll. Local research staff will be available in person to answer any questions and clarify any aspects of the NCS.

12.6.3 Women Less Than 18 Years of Age

Women younger than 18 who are pregnant will be eligible for the pregnancy portions of the Study. Special procedures will be used for women younger than 18 to ensure that encouragement to participate will not be undue or interpreted as pressure. There will be age-related differences in monitoring women for pregnancy. The Study will not enroll those younger than 18 who are not pregnant, and these young women will not be asked whether they are planning to get pregnant.

The consent process for pregnant women under the age of majority will be consistent with the laws of the local jurisdiction. Generally, federal regulations permit pregnant women of any age to consent for minimal risk research for themselves and their children. If the pregnant young woman is between 15 and 18, she will be encouraged to consult with her family prior to providing informed consent for herself and her child. If the pregnant young woman is younger than 15, then the Study protocol will require the consent of her parent or legal guardian. The young women will be asked to enroll and to provide informed consent for themselves and for their children.

12.6.4 Assent of Children

Consistent with §45 CFR 46.408(a), it is the intention of the NCS to obtain assent for participation in the Study from children beginning at approximately age 7 if they are developmentally and cognitively able. Each child who is developmentally and cognitively able to assent to continued participation in the Study will be approached. The process for obtaining and documenting "child assent" will be presented to each participating IRB at least one year before the first subjects of the Study will become 7 years old. The description of this process will include the methods that will be used to determine if a child is developmentally and cognitively competent to be approached for assent.

All children enrolled in the Study will receive continual updates on the progress of the Study through developmentally appropriate newsletters, Web sites, and other communications. They will be encouraged to continue to participate in the Study, answer questionnaires, and attend scheduled follow-up visits.

An "adolescent assent" process will be developed to obtain the affirmative agreement of each teen to continue participation in the Study. This process will be initiated at approximately age 14. A description of this process and the methods to obtain and document assent from developmentally and cognitively capable teens will be provided to each participating IRB at least one year before any of the subjects turn 14. The description of this process will include the methods that will be used to determine if an individual adolescent is developmentally and cognitively competent to be approached for assent.

12.6.5 Consent of Adolescents (When Child Participants Reach Age of Majority)

As adolescent participants in the NCS reach the legal age of majority in each jurisdiction (generally age 18), a fully informed consent will be obtained from each participant for continued participation in the Study and for continued use of stored samples for analysis. A consent process for these adult subjects will be developed and submitted to the IRB at least one year before participants turn 18.

12.6.6 Foster Children

Foster children who are wards of the state are permitted to participate in research without any additional procedural safeguards when study risks are minimal (§45 CFR 46.409). Only when the research involves greater than minimal risk and no prospect of direct benefit is there a requirement for the IRB to provide additional procedural safeguards through the appointment of an advocate. Because the NCS is primarily an observational study with a minimal level of risk, no such procedural safeguards should be required. Because knowledge about the child's living environment is essential to the Study, in addition to obtaining consent from the agency responsible for the child, a foster parent will be approached to give permission for their participation and the child's continued participation in the Study. The foster parent will be fully informed about the purposes and procedures involved in the Study, and informed consent will be obtained for their participation (as caregiver), and for continued participation of the child.

12.7 Revealing Findings to Participants, Families, and Communities

Revealing some of the Study data findings to individual participants is seen as an ethical obligation but may also be an important recruitment and retention strategy. Revealing local aggregate findings to the communities is seen as an important strategy to maintain community engagement.

12.7.1 Revealing Individual Findings to Participants and Families

Some routine physical and laboratory test results will be revealed periodically as an incentive to participation. For example, results of routine physical measurements (e.g., height, weight, and blood pressure) and routine laboratory tests performed on biologic samples (e.g., hematocrit) will be provided to participants on a regular and recurring basis. These results will be presented in a context that allows the participant to compare their individual results with normative data when appropriate (e.g., growth curves, normal range of hematocrit for age).

Unless clinically relevant and actionable, NCS generally will not provide genetic information and other medical information to participants or family members. Much of the data collected in the NCS will be of uncertain relevance to the health or well-being of individual participants, and relevant for research purposes only. Participants will be informed of this during the consent process.

If clinically relevant and actionable medical information that may impact the health of the participants is found, they will be advised of that information. Participants may opt out of any measurement, test, biological specimen collection, or environmental sample collection. However, if a test, measurement, or collection is performed, and the results indicate a known health effect or risk to the participant that is clinically relevant and actionable, the Study is obligated to reveal the finding to the participant.

If clinically relevant and actionable genetic information is found in the future, participants will be informed that such information exists and may be obtained upon request. If participants request the information, NCS staff will explain to the participants the consequences of learning such information, and if the participants still desire the information, NCS staff will inform the participants in a sensitive and knowledgeable manner.

Results of environmental sample analysis will only be revealed to participants if there is a known and generally accepted risk relation between the exposure and a significant negative health outcome. This includes the following situations:

- There are state requirements to disclose (e.g., elevated blood lead or mercury concentration).
- Federal or state standards or guidelines exist.
- Appropriate risk assessment that has been conducted and published is applicable to the community in which the samples were collected (e.g., lead levels in dust or soil).

Environmental sample results provided to participants will be accompanied by an explanation and context for the result, basic information about the sources and risks of the chemical/agent, and guidance on where to find more information.

12.7.2 Revealing Aggregate Findings to Participants and Communities

The NCS is also committed to informing participants about aggregate data on a periodic basis as Study findings unfold. Because environmental findings may reveal local problems that could impact property values, etc., there may be potential risks to individuals, (participants and nonparticipants) and to the entire community, of revealing information found in the Study. Therefore, revealing information to communities must be done thoughtfully and with some level of preparation. The NCS will always inform individual participants living in a community of any personal findings of concern before informing communities of the findings.

To help keep participants engaged in the Study, all participants enrolled in the Study, adult and child, will receive periodic national updates on the progress of the Study through newsletters, Web sites, and other media. Web sites will be developed for the adults and for children and adolescents of various ages. This continual process will include updates on the progress of the Study, health information appropriate for all participants, some insights into how large studies such as NCS analyze findings to make inferences about how an exposure might be related to an outcome, and serially, information about the Study's findings.

Each site will also integrate a local process into this national process to reveal some of the aggregate findings to the local community, to maintain contact with participants, to give site-specific information to communities and participants, and to help maintain community engagement.

12.8 Biobanking and Environmental Sample Banking

Biologic specimens will be collected from women during the preconception period, during pregnancy, and after birth. Specimens will also be collected from biological fathers (during the pregnancy period) and from the child serially after birth. At the time of birth, collection of cord blood and placental material is planned. HIV testing is not currently planned for NCS.

The NCS plans to obtain biologic specimens from participants including blood, urine, saliva, breast milk, and small samples of hair. These specimens will be used to measure various physiologic parameters (e.g., hematocrit, iron stores) and environmental exposures (e.g., lead, chemicals), and to provide genetic information about each participant. Sample volumes will be kept minimal and all child

blood samples will be less than 5 milliliters per kilogram body weight. Specimens will be analyzed and/or stored in one or more repositories for future studies.

Periodically, the NCS will also collect environmental samples of air, dust, water, and soil from the homes of participants and other places where the child spends more than 30 hours per week. These samples will be analyzed to determine and measure environmental exposures and/or will be stored in one or more repositories for future studies.

Effects of environmental exposures on gene expression are among the most important interests of the NCS. Therefore, biologic specimens for DNA analysis will be obtained from participants. The NCS is cognizant that human genomic data are private, intimate, sensitive, and create special concerns about the potential for discrimination, stigmatization, and impact on future employment or insurance. The informed consent process will include reference to the reasons and importance of obtaining genetic information on each participant.

To protect the confidentiality of participants, only unique identification numbers without personal identifiers will be used for all biologic specimens collected and all information derived from those specimens. Data that can be used to link the specimens to personal identifiers and to other data obtained from individual subjects during this longitudinal study will be maintained separately, securely, and confidentially. To further protect participant confidentiality, the NCS will obtain a federal Certificate of Confidentiality through the National Institute of Child Health and Human Development from the U.S. Department of Health and Human Services. The Certificate of Confidentiality will protect the data from forced release through a court subpoena.

PART III: STUDY MANAGEMENT AND SUPPORT

13. INFORMATION MANAGEMENT SYSTEM (IMS)

13.1 Introduction

The Information Management System (IMS) is integral to the National Children's Study. The IMS houses all NCS-related information and serves investigators and the public throughout the Study lifecycle. At the earliest stages of the Study (study design, recruitment, and enrollment of expectant mothers and the Study launch), the IMS records and tracks enrollment, personal information, and informed consent. Through pregnancy, birth, childhood, and adolescence, it supports the tracking of participants, collection of data, report of findings, and incentive management. As Study Centers and data collectors collect biological data and samples, physical measures, environmental data and samples, and questionnaire and assessment data, and as laboratories analyze those specimens, the IMS records, transforms, analyzes, reports on, and protects the information. The IMS also maintains information regarding the location and disposition of physical samples and the results of the sample analysis. To facilitate data gathering, the IMS assists in scheduling visits, including generation of visit reminders to participants and schedules of upcoming data activities for data collectors. Prior to going into the field, the data collectors upload all data needed to conduct interviews and assessments, including participant and schedule information.

Critical features of the IMS include its ability to collect, to store, and to report on the data during the Study as well as to store and report on the data after the Study is complete. To reduce the risks associated with data collection, storage, and reporting, data storage for the Study will be centralized in the IMS at the Coordinating Center. Data are gathered through multiple means (such as laptop-based survey instruments, Web-based interfaces, and measurement devices) and are electronically sent to or entered into the IMS. Backup and protection of all data are guaranteed by the centralized storage. The IMS supports centralized, uniform, high quality data collection and analysis activities for the Study. The IMS also supports uniform and consistent participant de-identification and strong controls over re-identification, as well as producing investigator-specific data sets.

Since critical Study activities are supported centrally, the IMS maintains continuous "24x7" Study operations. It incorporates state-of-the-art redundancy, fault tolerance, and disaster recovery mechanisms to ensure that operations can continue if hardware, software, or communications fail. The majority of IMS functionality is accessible to the Study Centers through an Internet browser over a secure network. Other functionality is accessible through disconnected data collection devices (e.g., laptop computers used by field data collectors).

Data collection in the home or at other field locations utilizes laptop computers and, occasionally, environmental sampling devices. This collected information is synchronized with the central database when the data collector is able to log into the Coordinating Center (either remotely or through a direct connection in a Study facility).

Clinical event data collection regarding such data as diagnoses, interventions, etc., which occur over time, constitute important outcomes and exposures to incorporate into the participant data base. Methodologies are being studied to facilitate obtaining these data during the Study from disparate sources such as primary care physicians, specialty consultants, hospitals, emergency rooms, and public health clinics.

13.2 Security and Privacy

Security and privacy are factored into every aspect of the IMS design. Security includes protection of sensitive data from corruption, theft, tampering, or unauthorized use, as well as protection from loss or corruption due to internal problems (e.g., a hardware or software failure) or external forces (e.g., a natural disaster). Privacy restricts access of sensitive information to only those individuals who are authorized to use or to view such data. De-identification of data—separating potentially identifying personal information from the actual participant data—is one aspect of privacy.

The IMS is hosted on a number of dedicated servers in a secure facility where physical access to the servers is restricted only to authorized personnel. The Study data are stored and managed in a secure network environment that is protected by continuously updated firewall, anti-virus, and anti-intrusion hardware and software. Systems are actively monitored to detect and block any attempt at intrusion or "hacking." Secure network connections are established between the Coordinating Center and external entities (e.g. Study Centers, labs, and repositories) to ensure data are not compromised during transmission. All data are encrypted during transmission and upon storage.

The IMS complies with various policies and regulations, such as the Health Insurance Portability and Accountability Act (HIPAA), to protect the privacy of the participants. Even Study staff, such as the Coordinating Center data managers and analysts, are not able to associate Study data with actual participant identities except under strictly defined conditions. To fulfill this mandate, the IMS employs a second layer of security specially designed to segregate participants' personal identifying data (PID) from the rest of the data. PID is stored in specially encrypted databases on servers that are physically separate from the main database servers. User IDs, passwords, and "digital certificates" allow access to PID only by authorized individuals and from authorized access points. The password/digital certificate system may also be augmented by a biometric identification technology such as thumbprint scanning to guarantee that any request for PID is genuine and coming from an authorized user.

13.3 Architecture/Framework

Since the Study will last more than 20 years, the IMS is designed with the ability to grow with the Study and to adapt with the evolution of technology. The IMS framework allows reusing existing applications and systems while accommodating future technology expansion. The framework accomplishes this by focusing on interoperability and component-based architecture.

Interoperability is defined as the ability of different types of computers, networks, operating systems, and applications to work together effectively to exchange information in a useful and meaningful manner. Interoperability requires not only the ability to transfer data, but a common understanding of what those data mean. The IMS supports interoperability with other systems (e.g., external databases with relevant data) by including multiple methods of transferring data between systems and by the use of industry standards to define not only the syntax but also the meaning of the data. Leveraging standards for integration enables the IMS to be flexible when future technology changes are implemented.

The IMS is a component-based architecture in which "components" (e.g. system building blocks) are responsible for specified functions. These components have well-defined interfaces. This approach supports later replacement of a component with newer or alternate versions that enhance functionality or incorporate new technology (in a "plug and play" manner). The result is a scalable IMS adaptable to the Study's long-term goals.

14. ADVERSE EVENT REPORTING AND DATA MONITORING

14.1 Monitoring Subjects and Criteria for Withdrawal from the Study

The National Children's Study is relatively noninvasive, and the research protocol has no interventions. The Study and all procedures are also of no more than minimal risk. Thus, there are no conditions envisioned, either due to Study procedures or unrelated to Study procedures, which would preclude continuation in the Study. The only situation in which we would discontinue follow-up with the family is if there is a pregnancy loss or an enrolled child dies, and the occurrence is beyond the enrollment period (such that it is beyond the point when subsequent pregnancies would be eligible for enrollment in the Study). If Study subjects develop a condition that renders them incapable of providing the continuing informed consent required of the Study, continuing consent will be sought from the legally appropriate party.

Any participant may withdraw from participation in the NCS at any time. Declining participation or withdrawing from the Study will in no way affect their relationship with the local research sites or associated medical institutions. In the event a participant withdraws from the Study or the Study is unable to locate a participant (lost to follow-up), data and samples obtained to that point will be maintained for use in future analyses unless the participant requests the samples be discarded and not used in any future analyses. Participants will also be allowed to stop participation in the Study for brief periods and then rejoin in the future. If a participant dies, all data will be maintained in the data sets for all subsequent analyses. Participants will be informed of these policies.

14.2 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) consisting of 5 to 10 individuals not associated with the NCS will be created to review data periodically. The DSMB will have expertise in biostatistics, epidemiology, environmental toxicology, pediatrics, genetics, psychology, social determinants of health, ethics, and other appropriate disciplines. The DSMB will report to the Study Director and review standard process data such as accrual rates and adverse events and possibly other appropriate aspects of study data as determined by the Study Director and the Steering Committee. The DSMB will alert the Steering Committee if data become available that might require participants to be informed about the finding. An Ethics Advisory Committee (Subcommittee of the Federal Advisory Committee to the Study) will be established to review relevant situations at the request of the NCS Study Director or the NCS Steering Committee.

14.3 Ethics Advisory Committee

During the course of the NCS, environmental findings may reveal information that could be relevant not only to participants but also to members of the community from which participants have been recruited. The Ethics Advisory Committee of the NCS will assist in considering which information is of this type and will be available to assist the regional sites, in partnership with their local community advisors, to develop a strategy for dissemination of this information in an appropriate manner.

15. QUALITY ASSURANCE AND QUALITY CONTROL (QA/QC)

15.1 General Approach

Because the National Children's Study is a multi-site, multi-year study involving the collection of complex data as well as physical/medical measures, environmental samples, and biological specimens, quality assurance and quality control (QA/QC) are vital to ensure the data, measures, samples, and specimens are collected correctly and consistently across all sites and throughout all years. All NCS partners have long-standing reputations for conducting high-quality scientific research. However, as with all large multi-center studies, standard QA/QC mechanisms and procedures must be developed and utilized to assure data quality, integrity, completeness, and comparability throughout the Study. To accomplish this, a Quality Management Plan (QMP) and a Coordinating Center QA/QC plan will be developed, applied, maintained, and updated as needed throughout the Study. The QA/QC plan will specify QA/QC procedures and policies that will apply to Coordinating Center operations and also QA/QC procedures and policies that the Study will require of the four types of collaborators: Study Centers, laboratories, clinical testing facilities, and central repositories.

An individual will be assigned by the NCS Program Office to serve as the NCS Program Office Quality Manager. This individual and, as the study grows, his or her staff, will provide independent high-level oversight of QA/QC activities conducted by the Coordinating Center, Study Centers, Information Management System (IMS) contractor, laboratories, clinical testing facilities, and repositories. Examples of the types of activities the Quality Manager will perform include conducting independent audits/assessments of the NCS components, reviewing audit reports, and making recommendations to the Study Director and Project Officers on corrective actions.

The major tool for Study and data management, and thus Study QA/QC, will be the IMS. The IMS will not only provide an array of QA/QC monitoring functions, it will also track the QA/QC activities. There are three aspects of QA/QC related to the IMS: (1) the IMS will capture all Study data allowing for Study oversight of data completeness and production; (2) the IMS will capture all Study QA/QC data allowing for Study oversight of data quality, integrity, and comparability; and (3) specific QA/QC checks will oversee the IMS.

15.2 QA/QC Activities for IMS

The IMS is the computerized heart of the Study. It must support the collection, management, and storage of the study data and manage highly complex study activities involving thousands of staff and participants across the country. The electronic edits built into the IMS will be the key to ensuring data quality, and the monitoring and tracking systems it includes will be critical to ensure proper Study management. Since the NCS will be underway for such a long period of time, it is critical that the IMS be able to accept upgrades and new technologies without going out of service. These challenges require constant attention to quality.

15.2.1 IMS System Quality

The IMS will not be able to ensure the quality of NCS data unless it is itself a quality system that performs reliably, accurately, and according to specification. System quality begins with the team that builds and integrates it. A fundamental component of the quality system for the IMS is a contractual requirement that the IMS contractor must maintain ISO 9001:2000 certification and demonstrate CMM

Level 3 compliance on an ongoing basis. During the planning phase, the IMS contractor developed a CMM plan which defined internal roles and responsibilities, record-keeping requirements, and other quality-affecting parameters. The CMM plan ensures the organization's software development processes include visibility, oversight, and checkpoints throughout the software development life cycle.

It is an axiom in software development that the earlier an error or problem is found, the easier and cheaper it is to correct. Thus, the IMS contractor will perform early reviews of requirements and design specifications, working closely with Program Office and Coordinating Center staff, who are the subject matter experts. This process ensures that when software is built (or purchased commercially) and integrated, it will meet requirements. Source code reviews during actual development will further ensure that errors are caught as early as possible.

A key step in ensuring system quality is testing. The IMS will be tested in four stages. In the first stage, the developers and integrators will test each hardware and software component to ensure it functions according to specifications, a process called "unit testing." In the second stage, and in a separate process, an independent test team will examine the IMS requirements documents and prepare a detailed test plan for each IMS system. After initial training by the development team, the independent test team will execute the test plan against each of the systems, identifying problems and placing them into a formal defect tracking system. Each problem will be prioritized and tracked to resolution, and the systems will be retested until they pass. The third stage of testing will be performed by Coordinating Center and Study Center staff who will test each of the IMS systems using real-world study scenarios to determine if the systems perform their functions properly. This process is known as "acceptance testing," and systems cannot be fielded until they pass. The final stage of testing is ongoing. Whenever a system is enhanced, upgraded, or a defect is found and corrected, not only must the new or changed elements be tested, but also a "regression test" must be performed by the independent test team to ensure the changes do not adversely affect other functionality.

Throughout the Study, the Coordinating Center will capture, track, and report IMS infrastructure outages as well as software defect reports, IMS help desk calls, and application error logs to compute an ongoing reliability factor that will be reported monthly and yearly. The IMS will include application event logs that will capture application failures along with reporting capability. In the unlikely event of a system outage, the Coordinating Center will document the outage with an incident report that describes the cause of the outage, the measures taken to resolve it, and the processes and procedures that can be implemented to prevent a similar future occurrence. Prior to implementation, the Coordinating Center will confirm the computation of the reliability statistics with the NCS Program Office.

15.2.2 IMS Data Quality

The IMS will be designed to maintain and ensure the quality of the NCS data throughout its life cycle from collection through analysis, storage and eventual archive. Quality, in this context, is defined in four broad dimensions. A data element must be:

- collected accurately;
- protected from tampering or inadvertent alteration or corruption;
- traceable and attributable to its original source; and
- associated with audit trails and decision logs that document all changes to it as well as the source and reasoning behind each change.

To support accurate data collection, the IMS will maintain calibration and test records for data collection devices and instruments, including questionnaire instruments. The IMS will also maintain records documenting data collectors' training and certification. To support data security, the IMS security features will include many technical and procedural checks and guards to protect data from tampering or corruption, including encryption, network firewalls, multi-factor authentication of users, and role-based access controls. To ensure that data are always traceable and attributable to source, each data element will carry associated metadata to document its history and context. IMS data management systems will use audit trails, include timestamps, and will identify the source as well as the nature of data changes. Decision logs will document the reasons behind any changes made to data post-collection as well as larger study-level decisions that may cause wholesale instrument or methodology changes.

The IMS will not only maintain data quality, it will provide the information needed to make improvements in data collection instruments, methods, and techniques. Edit reports produced by the IMS will document edit failures and their resolution. The IMS will use the edit failures to identify possible data collection or manipulation or metadata errors that will be used to compute an overall accuracy statistic for data collected by the IMS.

In addition to system reliability and accuracy statistics, reports and audits will be used to assess the quality of the IMS products at any given time in the project life cycle. Reporting on defects and change requests provides some useful quality indicators, such as team productivity and bottlenecks, evaluation of workload distribution, the need to insert more or less flexibility into processes, and overall schedule progress. Audits provide verification that processes are being followed and that traceability exists between coded software and requirements or change requests.

15.3 Training Data Collectors

Comprehensive training of Study Center data collection staff will be an important aspect of the QA/QC plan. Highly experienced Coordinating Center staff will develop and implement a carefully designed and thorough training program, including training manuals, training exercises, role-play scenarios, audio/visual tools, and certification procedures. The Coordinating Center staff will conduct initial training of Study Center staff using a "train-the-trainer" method to prepare the Study Center staff who will subsequently conduct the training and retraining for data collection at their site. The training sessions and materials will be structured around specific competency-based objectives using a variety of teaching strategies to maintain the active involvement of the trainees. The techniques used during the training will follow the fundamental concepts of effective adult learning theory and require extensive active participation of the trainees. A basic and important requirement of the training will be to give every member of the staff the tools he or she needs to gain respondent cooperation at every level of participation and to acquire the skills needed to combat nonresponse and promote continued response.

As part of the QA/QC plan, a training roster will be developed for each Study Center that will include the type of competency assessment or certification required for each Study Center staff person. Almost all of the training modules will require a competency assessment at the end of training before the trainee can begin data collection. For example, staff members who collect anthropometry data will be tested against the "gold standard" expert in a series of competency sessions at the end of training. Additionally some staff (e.g., phlebotomists and ultrasonographers) must have up-to-date certifications before they can begin data collection. The team responsible for the training will determine the competency criteria. As training and certifications are completed, the training roster will be updated to indicate the training and certifications received. The roster will be maintained through the IMS.

The QA/QC plan will include periodic staff retraining. Refresher training may be necessary to introduce new Study procedures and forms and to sharpen data collector skills. This will be done throughout the Study as a standardized means of delivering new information. The Study may identify a Study Center whose study staff members, when audited, are not passing standards or whose data do not correlate with standard examiners, and may decide to conduct refresher training at that Study Center. As the Study progresses, some attrition among Study Center staff is expected. This will make it necessary to train new staff. There may be a need for special training during the course of the Study, for example, to teach techniques for improving response rates among special populations (e.g., minorities, very young mothers, or single mothers), or to elicit feedback from interviewers on the effectiveness of outreach materials and the need for new items to target specific groups. Remedial training may be necessary when data collectors do not meet acceptable performance standards as identified using QC measures.

15.4 QA/QC for Data Collection Activities

QA/QC procedures will be developed and applied to all Study data collection and management activities including interviewing, taking physical and medical measurements, collecting and handling environmental samples and biological specimens, and processing the collected data. QA/QC procedures regarding maintenance and calibration will be developed and applied to the measurement equipment used in the Study. There will also be QA/QC procedures developed and applied to the environmental and clinical laboratories and testing facilities utilized.

All Study data will be carefully and thoroughly reviewed and edited for consistency and range checks. Inconsistencies, anomalies, and outliers will be identified, examined, and verified when necessary. For example, participant demographic characteristics will be checked against reported health conditions and medical events for logical consistencies, and blood pressure measurements will be checked for end-digit preferences. All data collectors will be directly observed, indirectly monitored, and evaluated for quality issues such as protocol adherence and inter-rater reliability measures.

Study staff will observe Study Center data collection staff to evaluate procedures and protocols during participant identification activities, while completing the interviews, while collecting specimens and samples, and while taking physical measurements, during the field pilot tests and dress rehearsals. After this initial period, Study staff will conduct at least one in-person audit in the field per data collector per year to monitor interviewing techniques and all other data collection activities. Data collectors will be observed while conducting the home visits as well as the clinic visits. Study staff will develop a standardized electronic form for use by auditors in evaluating performance during these observations.

The Study will assign senior staff, trainers, or trained designees to conduct the field audits. The field auditors will record the results of each audit item on the form and will use the completed forms as the basis for providing rapid feedback. Individual and/or group feedback may be provided. Completed observation forms will be kept for the duration of the Study and will be used to assist in identifying topics for review during refresher trainings.

The procedures described above will be applied to all data collection activities, but there will be additional QA/QC procedures developed and applied to each of the specific types of data collection activities. Sections 15.5 through 15.9 summarize these additional QA/QC procedures.

15.5 QA/QC for Interviewing

Re-interviews, or "verifications," will also be used to monitor interviewer work. The verification will confirm that the interview was conducted and verify a few selected responses. Verification QC will be conducted by telephone by Coordinating Center staff. Cases to be verified will be selected through the IMS as work is completed. All of an interviewer's work will be eligible for verification regardless of the final disposition. Typically, 10-15 percent of each interviewer's cases will be selected for verification. Only a certain number of highly objective questions will be selected for verification, both to reduce respondent burden and to protect against discrepancies due to legitimate response changes. Interviewers will be told their work will be verified but will not know the number of cases or the procedure for selecting cases. If at any time verification indicates the possibility of falsification, the Coordinating Center will begin a 100 percent verification immediately of the interviewers' work. The Coordinating Center will report verification rates and results through monthly progress reports.

Falsification will be further substantiated through the use of digital time stamp reports and tracking GPS coordinates. A systematic review of digitally entered time stamps for work done by each interviewer will be an important indicator of potential problems in the field. These time stamps will generate several reports that will be routinely reviewed by Study staff. Any unusual or suspicious pattern in the digital entry trail must be explained and will trigger a higher validation rate for the interviewer.

Study Center interviewers will be required to edit all work before finalizing the data collection case. After completing each case, the computer will display any outstanding data collection activities and exams which the Study Center staff would review and finalize. If the data are collected at a home or birth visit or some other facility, it will be further reviewed at the Study Centers before uploading to the IMS. If necessary, the interviewers will receive immediate feedback to rectify any problems. After this edit, the completed work will be uploaded to the IMS. There will be built-in editing procedures in the IMS that will support a further review of the data. For example, all text entries in the questionnaires, as well as other critical data items, will be reviewed. Whenever possible, the Study Center coordinator or an assistant will re-edit 10 percent of each interviewer's work.

15.6 QA/QC for Collecting and Handling Samples and Specimens

All procedures for the collection of environmental samples and biological specimens will have data collection forms specific to each specimen or sample to be collected. These forms will be developed to allow the monitoring of data quality across the Study. All procedures and corresponding forms will be evaluated regularly for effectiveness, and, if a modification is required, changes will be implemented seamlessly and the modification will be documented. On a periodic basis, all parties affected by the procedure and data collection forms will be solicited for any needed modification or update.

Observing or auditing the work of sample collectors will be done to evaluate procedures and protocols as described in Section 15.4. In addition, Study staff will observe sample labeling at the collection site and processing, storage, packaging, and shipment of biospecimens and environmental samples to ensure these activities are conducted according to Study procedures.

In addition to conducting visits to observe field procedures, the Study will establish a schedule for regular reviews of biospecimen and environmental sample data, problem logs, equipment logs, maintenance records, and calibration results for all field work. Review of biospecimen and environmental sample data can be considered an indirect observation, a variation on the method of direct observation that may be suitable for some collection tasks, either as a substitute for or supplement to the

field audits. During an indirect observation, field staff performance will be monitored after the activity is complete, for example, by review of data from completed collection forms and comparison of the collection data to the laboratory results of analyzed specimens. The resulting data can be used as a measure of the quality of data collection or specimen collection.

All Study Centers will be required to keep logs of reported problems with specimen and sample labeling, processing, transfer, and shipment. These logs will be maintained on the IMS, as would similar logs from the biorepositories and analytic laboratories. The Study will track these logs on the IMS to identify, investigate, and resolve these types of problems with the Study Centers, laboratories, and repositories and to make recommendations for modifying procedures as necessary.

All biospecimen and environmental sample measurement equipment used in the field will be required to have regularly scheduled maintenance and logs of the maintenance, operating status, and all calibration results. The written procedures will describe, in detail, calibration procedures for all biomedical and environmental measurement equipment. If a particular instrument is required to be calibrated prior to each use, the Study will specify these calibration tests as well. Study procedures will include instructions on how to handle situations where equipment does not meet the specified calibration criteria. Study Center staff will be trained to calibrate and maintain all instruments and equipment in accordance with the approved procedures, including equipment that may need to exceed manufacturer recommendations because of extensive use.

The results of equipment maintenance and calibration activities will be automatically tracked in the IMS. If missing logs, failed calibrations, drifting, or other problems are found, the Coordinating Center will contact the affected party to discuss and correct the problem. If needed, a site visit will be made to observe the questionable equipment and procedures.

The Coordinating Center will work with the NCS Program Office to develop procedures designed to address the need for resampling and duplicate or repeat collection of samples. These procedures could apply to collection of most biospecimens or environmental samples (e.g., more blood, urine, or breast milk, or another dust, air, or soil sample). The Study will identify quality control samples to be used, including specifications as to their content, number of samples to be obtained, possible sources, and assurance of the quality of the samples and specimens.

15.7 QA/QC for Environmental/Clinical Labs, Repositories, and Testing Facilities

The Study will require all laboratories to submit the standard operating procedures, which will be used for the NCS. These documents will be logged and evaluated to ensure the standard operating procedures are written in accordance with current guidelines and other regulatory requirements, as well as Study procedures. Revisions will be requested as needed. All current and past standard operating procedures will be submitted and maintained electronically in the IMS, which will be easily accessible and searchable by the NCS Program Office.

The Coordinating Center will work with the NCS Program Office to define and implement procedures for monitoring the performance of the laboratories, testing facilities, and repositories. The monitoring will continue throughout their performance. The performance monitoring will include implementing external QC through use of split duplicate and other QC samples and review of those results. Reports will be developed to ensure production standards are met; to identify inconsistencies and inaccuracies in specimen type or labeling; to identify results that fall outside of expected parameters; to identify any trends in analysis over time; and to review internal standardization and proficiency sample analysis conducted as part of accreditation or certification programs. On-site observation will be done to

verify that procedures adhere to the NCS procedures and to verify equipment calibration procedures and internal QC. The Study will institute a methodology for regularly receiving data from the laboratories (monthly or semimonthly) to ensure quality and production standards are maintained. The Coordinating Center will perform this task by verifying the laboratory QC data and production levels are within acceptable parameters set forth by the NCS Program Office. The Coordinating Center will provide the results of the verification process to the NCS Program Office on a monthly basis.

The Coordinating Center will generate data collection forms for all audits and data collection mechanisms. Based on the data elements collected, the Coordinating Center will generate reports for the NCS Program Office. The Coordinating Center will request the input from the NCS Program Office into what data elements would be needed for reports of varying types. Based on these requests, the Coordinating Center will ensure all data are collected in a timely manner and any discrepancies will be reconciled.

The Coordinating Center will submit the audit procedures for each type of facility to the NCS Program Office to approve prior to any site audit. The Coordinating Center will arrange for and oversee audits of all laboratories and repositories before samples are sent, and every six months thereafter. All laboratory audit inspectors will have initial training and refresher training for current guidelines and will maintain training levels throughout the Study.

The audit staff will conduct six-month on-site laboratory reviews. Prior to the audit visit, the Coordinating Center will work with the NCS Program Office to address any particular concerns for the specific site to be audited by developing a site-specific audit plan. During the audit visit, the audit staff will operate from the approved standard operating procedures as well as from the site-specific audit plan of all Study-related equipment calibration documentation, internal assay QC specimen or sample results, and environmental control logs. The audit team will verify all components of the Study-related procedures conducted at the site, including staff training, procedures, security, environmental monitoring. The team will document any deviations or violations uncovered, as well as the corrective actions the site implemented to rectify them. The team will also work with the sites to obtain copies of all necessary documents and maintain these documents as a tracking mechanism for site performance. The Coordinating Center will document all findings and report to the NCS Program Office within one month of the site visit.

The Study will develop and implement procedures to work with laboratories to improve performance on an as-needed basis. The Coordinating Center will submit the procedures to the NCS Program Office for approval prior to implementation. The Coordinating Center will draft a procedure that addresses distributing QC specimens or samples. The procedure will address methods for distribution from the source to the Coordinating Center, the Study Centers, and the central repository, including sample handling and storage, as appropriate. For stable samples and analytes, many QC specimens may be ordered and sent out at one time; however, for unstable samples, there may need to be a steady stream of QC samples shipped out to the various entities.

The IMS will also specify the frequency with which each Study Center will insert the QC specimens and samples into the sample stream. This will primarily be by affixing a bar-coded label to each QC specimen or sample as if it were an actual Study specimen or sample during collection of actual specimens or samples. No sample type identifying information will be provided to the laboratories, (e.g., for environmental samples), and the surface area or volumes will not be provided. This will help prevent the laboratory from knowing which specimens or samples are QC checks.

The Coordinating Center will ensure that all QC specimens and samples are tracked in the IMS regardless of the source of the material. Ideally, the sources would enter the specimen/sample

information directly into the IMS. The IMS will have a specimen and sample tracking system (STS) component to track the shipment, handling, and results for all specimens and samples. The STS should have a set of specimen/sample ID numbers for QC specimens and samples that look like actual sample numbers.

15.8 QA/QC for Physical Measures Data Collection

QA/QC measures will include periodically reviewing equipment logs, maintenance schedules, and calibration results. Study staff will conduct any duplicate data collection specified in the Study documents, will ensure that all data collection forms are completed accurately in the field, and that all data such as ultrasound images and digital photographs are collected, labeled, and transmitted in accordance with specified study procedures. Study staff will document data maintenance efforts in the form of log files, summary operating procedures, and logs of changes to data.

Additional on-site observation audits of testing may include duplicate or repeat tests. Duplicate data will be collected on participants as part of the data collection protocol when the data are recognized as difficult to obtain. For example, in a typical ultrasound exam, each of the images will be taken twice to have at least two measurements of each type. Blood pressure measurements will be measured three to five times following a specific resting period. All measurements will be captured to allow an average reading to be computed based on an algorithm determined by the data analyst.

Gold standard examinations may also be used to measure the agreement between a recognized expert and an examiner by conducting examinations on the same participant during a single examination session. This type of QA is particularly relevant to some of the clinical examinations such as anthropometric measurements. The number of gold standard examinations required to assess the level of agreement, as well as acceptable levels of agreement, will be specified. The IMS will have the capability to generate a report that displays a side-by-side comparison of results from the primary and gold standard examinations. The gold standard examiner would be able to print and use this report to provide immediate feedback to the primary examiner. There should also be a program in the IMS that Study Centers run to produce inter-rater reliability statistics. If statistically significant differences between the gold standard and the primary examiner are identified, these will be addressed through retraining.

Replicate examinations, in which a second examination is performed on a participant by the same examiner as the primary examination, may be used to measure intra-examiner reliability on some clinical exams. Although replicate exams provide a good measure of reliability, they are burdensome for the participant and time consuming for the Study Center. Replicate examinations require that the participant return to the examination center (or the examiner return to the home) at a later date for a second-day examination. Not all exam procedures will be slated for inclusion in the second-day examination, and only those components that require this level of QA will be included.

15.9 QA/QC Activities for Data Management

The general approach to QA/QC for data management will be to rely on the approved procedures for ongoing structure to the program. Each procedure will include steps to facilitate identification of issues as they arise, support tracing problems to the source, and determine whether changes to procedures and/or the IMS will mitigate such problems in the future. QA/QC steps will be an integral part of each procedure and will be reviewed on an established basis but no less than annually.

The Coordinating Center will work closely with the NCS Program Office to ensure that NCS data are handled, processed, and managed with the highest level of quality. For data coding, the Coordinating Center will ensure metadata entry and maintenance, data review, and other manual data preparation procedures are performed properly. The Coordinating Center will ensure staff are trained and certified prior to beginning work and data management processes are designed specifically to identify errors resulting from data collection instrumentation or data processing activities.

A random sample of coders and data entry staff work will be reviewed by a second, more senior data management staff member or supervisor. Should consistent issues be identified, the staff member will be given additional guidance and training. If this guidance and retraining is not effective, the staff member will be removed from the project.

Automated edit software included in the IMS will be employed to detect item value, range, and inter-item logical inconsistencies, and checks will be implemented against historical data collected for the case. Results and resolutions of these edits will be maintained in automated form in the IMS. Any updates to NCS IMS databases as a result of data review will be accompanied by an annotation that includes the reason for the change, prior value, date of change, and authorization for the change, if required. Data management procedures will be fully documented and the documentation maintained online, accessible to data management staff. A log of all exceptional data management events will be maintained automatically within the IMS to support historical data questions.

Digital images will be used in several aspects of data collection such as radiology, pathology, or photography. Management of the digital images will include procedures for obtaining, transmitting, and storing images at the Study Centers and ultimately in image libraries in the IMS overseen by the Coordinating Center. A QA/QC protocol for management of digital images will be developed.

Approval guidelines for digital imaging equipment used in the Study will be developed. Study-approved protocols for technical parameters and measurements, calibration procedures and certifications, routine QC testing, and maintenance checks will all be addressed. Study Centers will be encouraged to participate in QA programs and certification programs for diagnostic imaging used in health care settings and will be expected to provide evidence of certification.

Procedures that ensure the capture of acceptable images for use in the NCS and the monitoring of images will be developed. Image transmission QC procedures that monitor the flow of complete image sets within the Study Centers and after transfer to the libraries will be addressed. Confidentiality and accuracy procedures for de-identification and anonymization of digital images, as well as ensuring accuracy in cataloging images, will be detailed in the QA/QC protocol. Evidence of robust backup, archival, and disaster recovery procedures will be required. Image files will be expected to be made available and accessible on secure Web sites.

16. SUB-STUDIES, OUTSIDE ADDITIONS TO THE CORE PROTOCOL, AND ADJUNCT STUDIES

As the National Children's Study proceeds, it will serve as a platform on which to build additional scientific research. Aspects of the NCS will yield ideas for sub-studies within the core protocol planning process and opportunities for adjunct studies, as well.

16.1 Sub-Studies Within the Core Protocol

The core protocol was developed through the NCS Program Office protocol planning process and paid for with NCS funds. This core protocol is to be performed on the entire cohort. Additionally, there will be some "sub-studies" of the core protocol planned by the NCS protocol planning team but performed on just a portion of the cohort. Sub-studies are generally funded by NCS and planned and approved through the same process as the core protocol. The Study Centers will carry out a sub-study as an integral portion of the core protocol.

16.2 Outside Initiated and Funded Studies That Pertain to the Entire Cohort

Outside initiated and funded proposals for the entire cohort will be considered as proposed modifications of the core protocol, ultimately decided upon and incorporated into the protocol by the same process that is used for the core protocol planning. The review process for such proposals will be a combination of the adjunct study review process and the core protocol planning process, as appropriate for the specific proposal.

16.3 Adjunct Studies

An adjunct study is performed on a portion of the cohort at one or more Study Centers, on all or a portion of their Center participants and/or their biospecimens or environmental samples. Adjunct studies are derived from or initiated and planned outside the NCS Program Office protocol planning process (e.g., can be initiated by a Study Center, government agency, independent investigator, industry, etc.), and are funded by such mechanisms as government grants applied for by the initiator, public-private partnerships, etc. Adjunct studies are reviewed and approved through a defined process and are implemented with the concurrence of the specific involved Study Centers. Individual Centers have the option of not participating in any particular ("outside initiated") adjunct study. Adjunct studies, therefore, are considered optional for the Study Centers.

While adjunct studies are generally neither planned by the NCS protocol planning team nor funded by NCS, in very specific circumstances the NCS may require or authorize and fund specific adjunct studies (for a portion of the cohort) to be planned "outside" the regular protocol planning process yet paid for with NCS funds (e.g. specific studies at specific Study Centers). These are referred to as "Internal Adjunct Studies," to reflect their internal (NCS) direction and funding despite "external" (i.e., other than NCS protocol planning team) scientific development.

16.4 Review Process

Adjunct studies and other additions to the protocol will undergo a formal review to assure maintenance of the quality and integrity of the Study and to address scientific merit; scientific "fit" with the NCS; burden to the participant and the Study; risk and other human subjects' issues; etc. A brief preliminary application has been developed as well as an in-depth full application to assure attention to the quality of the proposal and also to facilitate the submission, review, and approval of such proposals. Both are electronic and allow for relevant sections of government grant applications to be "cut and pasted" into the form.

17. THE HEALTH INSURANCE PORTABILITY AND ACCOUNTABILITY ACT

Requirements for the release of identifiable health information by covered entities (e.g., certain health providers, health plans, and health clearinghouses) were set forth by the Health Insurance Portability and Accountability Act (HIPAA) of 1996, which became effective on April 14, 2003.

The Coordinating Center will work with the Study Centers to ensure that authorizations to release identifiable health information meet the HIPAA requirements. These authorizations must include a description of the information that will be used or disclosed; who may use or disclose the information; who may receive the information; the purpose of the use or disclosure; the expiration date (if there is no expiration date, it must be explicitly stated as such); notice that the authorization may be revoked; notice that the information may be disclosed to others not subject to the Privacy Rule (redisclosures may not be protected); notice that an individual may refuse to sign the authorization (if any treatment or payments are conditional upon the individual's signing the authorization, the individual must be informed of this); and the individual's signature and date.

These requirements for authorizations may be combined in a consent form or may be a separate document. A Privacy Board (or IRB serving as a Privacy Board) may authorize the release of identifiable health information if additional risks are not created for Study subjects.

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19. REVISIONS, ERRATA AND ADDENDA

This chapter documents revisions, changes, and additions to The National Children's Study Research Plan and Appendices.

19.1 Revisions From Original to Version 1.1

The following revisions and additions were made to Volume 1 effective on June 20, 2007:

- Revised title of Chapter 6.
- Deleted bullet point four on page 6-10, subsection "Summary of preconception visits for women with a high probability of pregnancy," Chapter 6.
- Revised Table of Contents to reflect title change to Chapter 6 and addition of Chapter 19.
- Added "Version 1.1" to title and changed date to "June 20, 2007."

19.2 Revisions From Version 1.1 to Version 1.2

The following revisions and additions were made to Version 1.1 effective on August 17, 2007:

- Consolidated Volumes 1 and 2 into a single volume.
- Added "Preface."
- Corrected label in column 2 of table on page A2-7, subsection "7. Power and Sample Size," Appendix A2.
- Revised Table of Contents to reflect addition of "Preface" and consolidation of Volumes 1 and 2.
- Changed version number to "1.2" and changed date to "August 17, 2007."

19.3 Revisions From Version 1.2 to Version 1.3

The following revisions and additions were made to Version 1.2 effective on September 17, 2007:

- Revised number of hypotheses to 28 on pages 4-1, 7-1, 7-2, and 11-2.
- Revised Table 4.1 on page 4-3 of Chapter 4 to include titles of two additional hypotheses.
- Revised Table 7.1 to include two additional hypotheses.

- Revised Appendix A-1 to include two additional hypotheses.
- Added pages 149-164 in Appendix A-2, subsection Child Health and Development, and pages 239-250 in subsection Asthma.
- Added Appendix K, Statistical Analysis Plan for Birth Defects From Impaired Glucose Metabolism.
- Revised Table of Contents to reflect addition of Appendix K.
- Changed version number to "1.3" and changed date to "September 17, 2007."

National Children's Study Hypotheses

Pregnancy Outcomes		
Birth defects from impaired glucose metabolism	Among women without diabetes before pregnancy, impaired glucose metabolism during pregnancy is associated with risk of major congenital malformations of the heart, central nervous system, musculoskeletal system, and all birth defects combined.	Page A2-1
Increased risk of preterm birth from intrauterine exposure to mediators of inflammation	Intrauterine exposure to mediators of inflammation due to infection of either vaginal, cervical, or uterine sites or of more distal sites (e.g., periodontal disease) is associated with an increased risk of preterm birth.	Page A2-13
Increased risk of fetal growth restriction, preterm birth, birth defects and developmental disabilities in children born through assisted reproductive technologies	Children whose conceptions were aided by assisted reproductive technology (ART) are at increased risk of fetal growth restriction, birth defects, and developmental disabilities in comparison to children who were conceived without ART.	Page A2-25
Maternal subclinical hypothyroidism and neurodevelopmental disabilities/adverse pregnancy outcomes	Maternal subclinical hypothyroidism is associated with adverse pregnancy outcomes and neurodevelopmental disabilities.	Page A2-39

National Children's Study Hypotheses (continued)		
Neurodevelopment and Behavior		
Non-persistent pesticides and poor neurobehavioral and cognitive skills	Repeated, low-level exposure to nonpersistent pesticides, including carbamates, organophosphates, and pyrethroids in utero or postnatally increases risk of poor performance on neurobehavioral and cognitive examinations during infancy and later in childhood.	Page A2-49
Prenatal infection and neurodevelopmental disabilities	Prenatal infection and mediators of inflammation are risk factors for neurodevelopmental disabilities such as cerebral palsy and autism.	Page A2-61
Gene-environment interactions and behavior	Exposures to adverse psychosocial, chemical, and physical environments and other stressors during vulnerable periods of pregnancy and early childhood can interact with genotype to cause or modulate behavioral problems in childhood.	Page A2-71
Prenatal and perinatal infection and schizophrenia	Prenatal infection and mediators of inflammation during pregnancy and the perinatal period are associated with increased risk of schizophrenia.	Page A2-87

National Children's Study Hypotheses (continued)		
Child Health and Development		
Family influences on child health and development	Family resources and processes shape the structure and quality of children's home, childcare, and school experiences and economic opportunities. These resources and processes affect children's developmental and health trajectories and mediate and/or moderate other environmental influences on children's outcomes.	Page A2-101
Impact of neighborhood and communities on child health	Geographic area of residence is associated with exposure to social, physical, psychological, and environmental factors that increase the risk of developing health problems and decrease access to protective resources.	Page A2-113
Impact of media exposure on child health and development	Exposure to media from stationary and mobile sources can have both positive and negative short- and long-term effects on children. Home- or school-based media include television, video, and interactive media such as electronic games and the Internet. Multimedia mobile devices including cellular phones, portable digital music players, and portable computers integrate traditional radio, television, print media and film. The amount, type, content, and context of media exposure from infancy through adolescence influence brain and neurological development; cognitive and social development; and risk-behavior factors related to aggression, injury, substance use, sexual health, obesity, and other aspects of physical development. Exposure to specific media content will lead to developmental trajectories along a continuum of prosocial to antisocial behavior.	Page A2-125
Social institutions and child health and development	Interactions between children and families and the formal child care, school, and religious institutions in their communities influence children's cognitive, social, and emotional development.	Page A2-137
Influences on healthy development	Positive influences and protective factors in children's development, including family processes and parenting, biologically based child characteristics, and access to and use of high quality community services, have direct and indirect positive effects on development. These positive influences promote competence and buffer the negative effects of social, environmental, and biological risk (e.g., poverty, stress, birth weight/gestational age, integrity of cognitive, sensory, and motor systems, genetic polymorphisms) on development, leading to healthy cognitive, social, and physical child outcomes.	PageA2-149

National Children's Study Hypotheses (continued)

Asthma		
The role of prenatal maternal stress and genetics in childhood asthma	Prenatal, maternal stress increases the risk of childhood asthma. Genetic and environmental factors that influence immune development and lung growth/airway inflammation in early life modify the association between maternal psychological stress and the development of asthma.	Page A2-165
Exposure to Indoor and outdoor air pollution, aeroallergens and asthma risk	Exposures to indoor and outdoor air pollution. aeroallergens, and other environmental agents are associated with increased risk of asthma onset and progression in children, and is modified by genotype and other risk factors.	Page A2-177
Dietary antioxidants and asthma risk	Intake of antioxidants in diet affects the risk of asthma.	Page A2-193
Social environmental influences on asthma disparities	Disparities in the prevalence, severity, and effective management of asthma by race and socioeconomic status are explained, in part, by social environmental factors and processes that influence exposure to physical environmental risk factors, psychosocial stress, and health-related behaviors.	Page A2-209
Early exposure to structural components and products of microorganisms decreases the risk of asthma	Early exposure to heterologous structural components and products of biologics (microorganisms, e.g., viruses, bacteria, fungi, and parasites, and common indoor aeroallergens) significantly decreases or increases the risk of asthma and other atopic diseases (e.g., eczema, allergic rhinoconjunctivitis), and/or this will be mediated by genetic and other risk factors.	Page A2-223
Environmental exposures interact with genes to increase the risk of asthma and wheezing in children	There will be a significant association with gene-environment, gene-gene, and genotype-phenotype relationships that contribute to wheezing and asthma in children.	Page A2-239

National Children's Study Hypotheses (continued)

Obesity and Growth		
Obesity and insulin resistance from impaired maternal glucose metabolism	Impaired maternal glucose metabolism during pregnancy is directly related to risk of obesity and insulin resistance in offspring.	Page A2-251
Obesity and insulin resistance from intrauterine growth restriction	Intrauterine growth restriction (IUGR) is associated with subsequent risk of central-body obesity and insulin resistance in offspring, independent of subsequent body mass index.	Page A2-259
Breastfeeding associated with lower rates of obesity and lower risk of insulin resistance	Breast milk feeding compared with infant formula feeding is associated with lower rates of obesity and lower risk of insulin resistance.	Page A2-275
Fiber, whole grains, high glycemic index and obesity and insulin resistance	Consumption of a high glycemic load diet, during childhood, is associated with obesity and subsequent insulin resistance in childhood.	Page A2-287
Genetics, environmental exposures, and Type I Diabetes	The development of beta cell autoantibodies and subsequent type 1 diabetes is causally associated with the interaction between genetic susceptibility, early exposure to viral infections, and early exposure to cow's milk protein or other dietary components.	Page A2-295

National Children's Study Hypotheses (continued)		
Injury		
Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Repeated mild traumatic brain injury has a cumulative adverse effect on neurocognitive development.	Page A2-303
Behavioral exposures, genetics, and childhood or adolescent onset aggression	Biological, physical, and psychosocial components of the environment and their interactions with specific genetic variations are associated with and determine patterns of increased onset and maintenance of antisocial physical aggression.	Page A2-313
Antecedents and resiliency to traumatic life events in childhood	Antecedent factors such as genetic risk, family structure, neighborhood and community factors, interact with traumatic life events to predict the risk of anxiety disorders.	Page A2-325

National Children's Study Hypotheses (continued)		
Reproductive Development		
Hormonally active environmental agents and reproductive development	Prenatal and postnatal (including peripubertal) exposure to hormonally-active environmental agents can alter development of the reproductive system resulting in multiple types of outcomes that can occur at various stages of development and may result in cumulative effects over time.	Page A2-343

BIRTH DEFECTS FROM IMPAIRED GLUCOSE METABOLISM

1. Meta Hypothesis

Among women without diabetes before pregnancy, impaired glucose metabolism during pregnancy is associated with risk of major congenital malformations of the heart, central nervous system, musculoskeletal system, and all birth defects combined.

2. Specific Hypotheses

1. Elevated maternal HgbA1C during the first trimester of gestation is associated with increased risk of birth defects.

2. Children of mothers with gestational diabetes have an increased risk for birth defects.

3. Mothers with impaired glucose metabolism who supplement with anti-oxidant vitamins or folic acid reduce the risk of having children born with birth defects.

3. Background and Justification

Among women who have type 1 or type 2 diabetes before pregnancy, the risk of congenital anomalies in offspring is increased, and animal models confirm the teratogenicity of impaired glucose metabolism. Whether women first diagnosed with diabetes during pregnancy (gestational diabetes) or those with lesser degrees of impaired glucose metabolism during pregnancy have offspring with increased frequency of birth defects has not been determined, though limited data suggest an association (Farrell, Neale, & Cundy, 2002; Schaefer et al., 1997; Anderson et al., 2005; Kanwar et al., 2005).

3.1 Public Health Importance

Prevalence/incidence

Impairments in glucose metabolism during pregnancy are associated with an increased risk of birth defects; thus, infants of women with type 1, type 2, and gestational diabetes are at greater risk of having a birth defect (Casson et al., 1997; Schaefer-Graf et al., 2000). The risk of birth defects among these infants is estimated at three to five times higher than for nondiabetic mothers and increases as glycemic control worsens (Kucera, 1971). Major congenital malformations of the heart, central nervous system, and musculoskeletal system, among the most common defects seen in the offspring of diabetic women, are induced before the seventh week of gestation (Mills, Baker, & Goldman, 1979). The prevalence in the general population of targeted birth defects is:

- 0.6 percent of births with major congenital malformations of the heart (Hoffman & Kaplan, 2002),
- 0.3 percent of births with central nervous system defects (Branum et al., 2003),
- 0.2 percent of births with musculoskeletal birth defects (Feuchtbaum, 1999), and
- 3-4 percent of births with major birth defects combined (Leppig, Werler, Cann, Cook, & Holmes, 1987; Lynberg & Edmonds, 1994).

The total prevalence of diabetes in women ages 20-39 is 1.1 percent and 4.4 percent among those ages 40-49 (Harris et al., 1998 as cited in Beckles & Thompson-Reid, 2001). Among reproductive-aged women with diabetes, 0.6 percent younger than 40 and about 1.6 percent of those 40 or older did not know they had the disease (Harris et al., 1998 as cited in Beckles & Thompson-Reid, 2001). The combined prevalence of diagnosed diabetes, undiagnosed diabetes, and impaired fasting glucose is 2.9 percent and 10.3 percent among white women ages 20-39 and ages 40-49, respectively. Among Mexican-American women ages 20-39 and ages 40-49, 5.6 percent and 22 percent, respectively, had diagnosed diabetes, undiagnosed diabetes, or impaired fasting glucose. Combined prevalence of the same for African-American women ages 20-39 and 40-49 were 5.8 percent and 17.2 percent, respectively. About 4-7 percent of pregnancies are complicated by gestational diabetes, possibly even higher numbers in various ethnic groups (Kjos & Buchanan, 1999).

There are two types of impaired glucose tolerance: impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). Depending on the test used to diagnose impaired glucose tolerance, prevalence estimates differ. In a cross-section of U.S. adults ages 40-74 tested from 1988 to 1994, 33.8 percent had IFG, 15.4 percent had IGT, and 40.1 percent had pre-diabetes (IGT or IFG or both). When these percentages of IGT and IFG are applied to the 2000 U.S. population, approximately 35 million adults ages 40-74 would have IFG, 16 million would have IGT, and 41 million would have pre-diabetes (National Diabetes Information Clearinghouse, 2004).

Based on data from the 1999-2000 National Health and Nutrition Examination Survey, approximately 2 percent of U.S. adults between 20 and 39 years old are affected by impaired fasting glucose, a type of impaired glucose metabolism (Centers for Disease Control and Prevention [CDC], 2003).

Economic and/or social burden

The estimated lifetime cost of birth defects among children born during one year in the United States is $2 billion for heart defects, central nervous defects, and musculoskeletal defects combined, and $8 billion (1992 dollars) for all birth defects combined (CDC, 1995).

3.2 Justification for a Large Prospective Longitudinal Study

The study of the broad hypotheses proposed in this document within a longitudinal study is justified for several reasons: (1) birth defects associated with poor glycemic control can be lethal or cause morbidity resulting in significant expenditures of health care resources; (2) preliminary studies suggest the effects of impaired glucose metabolism can be lessened by readily accessible therapies, providing a base for possible preventive public health recommendations; (3) the prevalence of obesity, impaired glucose metabolism, type 2, and gestational diabetes in women of childbearing age is increasing steadily; and (4) the longitudinal nature and potential scope of the National Children's Study (NCS) provide the most robust, and perhaps the only adequate, study design to understand the relationship between impaired glucose metabolism and birth defects.

3.3 Scientific Merit

The NCS can provide new information on the relationship between impaired glucose metabolism and birth defects. Researchers will be able to identify overt diabetes and occult forms of abnormal glucose metabolism, ideally before conception, and evaluate their effects on the developing fetus. Such comprehensive assessment is important because latent disorders of glucose metabolism are more prevalent than overt diabetes, therefore, many birth defect cases could be attributed to small increases in associated teratogenic risk. Because the effects of these metabolic abnormalities are poorly

understood relative to those associated with overt insulin-dependent diabetes, the longitudinal study will expand the knowledge base of preventive clinical and public health relevance.

3.4 Potential for Innovative Research

The NCS can study glucose metabolism prospectively using objective measurements. This is an improvement over the many retrospective (e.g., case-control) studies in which the ascertainment of disease is based solely on maternal self-reports. Where possible, measurements will begin before conception because glucose metabolism can change during pregnancy. Abnormalities of glucose metabolism might have multiple effects upon the fetus that manifest themselves during pregnancy (in fetal death), at birth (as a birth defect), or later (as childhood obesity). The longitudinal component of the study provides an opportunity to discover and assess the potential multiple effects of altered glucose metabolism during gestation on children over time.

3.5 Feasibility

The current literature concerning impaired glucose metabolism and birth defects suggests a number of well-established biomarkers as well as potential confounders and effect measure modifiers can be collected within the NCS. Some procedures may represent a slight burden to participants, but most samples and data should be easily obtainable.

Ascertainment of birth defects in the NCS will follow the approach used by existing birth-defects surveillance programs. There is extensive experience in the ascertainment of birth defects and collection of clinical information for classification purposes by population-based birth defects surveillance registries in various states around the country. Some of these registries have served as the basis for a number of large population-based case-control studies of birth defects such as the National Birth Defects Prevention Study (NBDPS) in which clinical information was reviewed by clinical geneticists for classification purposes. The Metropolitan Atlanta Congenital Defects Program has been successful in achieving high levels of case ascertainment with relatively high quality of the clinical information gathered (Correa et al., 2007).

Proper classification of cases will be somewhat challenging within a nationwide study such as the NCS, and experience gained by investigators during the NBDPS could be useful. The NCS conducted a workshop that included clinical geneticists, pediatric cardiologists, and birth defects experts from around the country to review and prioritize possible strategies and pilot studies for ascertainment and classification of birth defects in the NCS.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Maternal reports of pregestational and gestational diabetes
- Blood glucose and HgbA1C
- Serum lipid profiles

- Serum insulin or related samples (e.g., C-peptide)
- Serum inositol
- Genetic samples (for exploration of potentially relevant genes such as variable number tandem repeats [VNTR] insulin, glucokinase)

Primary/child

- Serum glucose
- Serum insulin or related samples (e.g., C-peptide)
- Genetic samples (for exploration of potentially relevant genes such as VNTR insulin, glucokinase)

4.2 Methods

Primary/maternal

- Blood samples
- Medical record reviews

Primary/child

- Blood samples
- Medical record reviews
- Physical exams

4.3 Life Stage

Primary/maternal

- Preconception, prenatal, postnatal (follow-up of maternal carbohydrate metabolism would be a valuable ancillary study)

Primary/child

- Birth

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

Assessment for birth defects:

- Standard physical examinations (done routinely on all newborns and infants)
- Review of medical records from birth through age 21
- Photographs of the face in infancy

5.2 Methods

Primary

- Direct observations by a medical professional, photographs of the face, or via medical record reviews
- Clinical information reviewed by experts for classification of birth defects

5.3 Life Stage

Primary

- Birth through age 21

6. Important Confounders, Mediators, and Effect Modifiers

- **Previous history of birth defects:** Mothers whose first child had a birth defect are 2.4 times more likely than other women to have a second infant with a birth defect. Most of the risk is accounted for by the same defect (Lie, Wilcox, & Skjaerven, 1994).
- **HgbA1C:** Low HgbA1C is associated with the reduced incidence of congenital abnormalities (Haire-Joshu, 1996).
- **Maternal obesity (body mass index):** Maternal obesity is associated with an increased risk of birth defects and with impaired glucose metabolism (Anderson et al., 2005).
- **Lipid profile:** Maternal fat-modified diets result in lower total and HDL cholesterol in infants and could be a suitable way to prevent cardiovascular disease among infants from the beginning of life (Fard, Mehrabian, Sarraf-Zadegan, & Sajadi, 2004).
- **Maternal serum inositol:** The mechanisms by which hyperglycemia leads to birth defects are not clear but are probably complex and could be related to oxidative stress, low inositol levels, and other metabolic abnormalities associated with hyperglycemia.

Studies in animals suggest low levels of inositol may be associated with an increased risk of NTDs and other defects (Cockroft, Brook, & Copp, 1992; Hashimoto et al., 1990; Baker, Piddington, Goldman, Egler, & Moehring, 1990; Green & Copp, 1997; Cogram et al., 2002). A recent case-control study of spina bifida also suggests that low maternal serum levels of inositol may be associated with an increased risk for spina bifida (Groenen et al., 2003). So in evaluating the role of hyperglycemia, it is important to take into account the inositol status of women early in pregnancy. Inositol, a sugar with important structural and functional properties, is part of the diet but is also synthesized by the body. Therefore, measurements of inositol status during pregnancy will need to be based on maternal serum levels as well as on interview data on dietary intake.

- **Insulin gene VNTR:** There is some conflicting information regarding this insulin gene. Most data suggest VNTR variations do not influence early growth, but some data suggest an increased risk of childhood obesity and insulin resistance (Bazaes et al., 2003; Bennett et al., 2004; Kraemer, Ratliff, Bartholdi, Brown & Longmire, 1989).

- **Glucokinase mutation:** The intrauterine environment is associated with insulin resistance in childhood. Birth weight is reduced if one parent has a glucokinase mutation (Hattersley et al., 1998).

- **Hormone levels such as cortisol:** Fetuses exposed to glucocorticosteroids in the first trimester had a lowed median birth weight and were born at an earlier gestational age, which increased the risk for being underweight or having birth defects resulting from underdevelopment, but did not exhibit an increased teratogenic risk (Gur, Diav-Citrin, Shechtman, Arnon, & Ornoy, 2004).

- **Smoking status:** The more a pregnant woman smokes, the greater the cumulative health risk for premature birth and birth defects for her baby. Nicotine and carbon monoxide play a role in causing adverse pregnancy outcomes (U.S. Department of Health and Human Services [HHS], 2004; Law et al., 2003; American College of Obstetricians and Gynecologists [ACOG], 2000; Wang et al., 2002; Little, Cardy, & Mungar, 2004).

- **Use of medication:** Anticonvulsants can cause serious problems, including mental retardation and slow growth, in the developing fetus. Other drugs associated with birth defects include antipsychotic and antianxiety agents and certain antibiotics (Jones, 1996; Hernandez-Diaz, Werler, Walker, & Mitchell, 2000; Hernandez-Diaz, Werler, Walker, & Mitchell, 2001).

- **Use of nutritional supplements:** Supplementation with folic acid attenuates the risk for neural tube defects (Berry, 1999; Bower & Stanley, 1989; Czeizel, 1993; Czeizel & Dudas, 1992; Daly, Kirke, Molloy, Weir, & Scott, 1995; Laurence, James, Miller, Tennant, & Campbell, 1981; Milunsky, 1989; MRC Vitamin Study Research Group, 1991), cardiac defects (Botto, Khoury, Mulinare, & Erickson, 1996; Botto, Mulinare, & Erickson, 2000; Czeizel, 1996, Czeizel, Toth, & Rockenbauer, 1996; Lewis, Van Dyke, Stumbo, & Berg, 1998; Shaw et al., 2002), oral clefts (Itikala, Watkins, Mulinare, Moore, & Liu, 2001; Lewis et al., 1998; Shaw, Lammer, Wasserman, O'Malley, & Tolarova, 1995; Tolarova & Harris, 1995; Yang, Khoury, Olney, & Mulinare, 1997), and urinary tract defects (Czeizel, 1996; Czeizel et al., 1996;

Lewis et al., 1998; Werler, Hayes, Louik, Shapiro, & Mitchell, 1999; Yang et al., 1997). Supplementation with antioxidant vitamins appears to attenuate risk for cardiac defects (Correa, Botto, Liu, Mulinare, & Erickson, 2003). For markers of oxidative stress, consider collecting first voided morning urine for acrolein-lysine adducts (ELISA), 8-hydroxy-2-deoxyguanosine (8-OHdG) (ELISA), and nitric oxide metabolites (colorimetric, non-enzymatic assay). These have been used as markers for oxidative stress in children with type 1 diabetes (Hata et al., 2006).

- **Gene-nutrient interaction:** Methylenetetrahydrafolate reductase (MTHFR) polymorphisms appear to be associated with increased risk of birth defects (Botto & Yang, 2000). Reduced folate carrier and MTHFR appear to interact with folic acid supplementation to modify risk of birth defects (Shaw et al., 2002; Shaw, Rozen, Finnell, Wasserman, & Lammer, 1998).

- **Family history:** About 20 percent of birth defects are hereditary, resulting from the interaction of genes from one or both parents plus environmental influences. Defects may include cleft lip and palate, spina bifida, and heart defects (Shaw, Rozen, Finnell, Wasserman, & Lammer, 1998; Lott, 1996).

- **Other factors:** Recreational drugs have been associated with arm and leg abnormalities and central nervous system problems (Jones, 1996).

7. Power and Sample Size

For women with and without diabetes prior to pregnancy and their offspring, assuming 100,000 infants are born into the Study, with a prevalence of impaired fasting glucose early in pregnancy of 2 percent, the power to detect relative risks in the range of 1.5-2.5 for selected major defects is as follows:

Birth defect group	Rate per 1,000 births	Relative Risk			
		1.5	1.75	2.0	2.5
Heart defects	6	47.4	72.3	87.8	98.3
Central nervous system	3	29.8	48.7	65.6	87.0
All major birth defects	30	96.3	99.9	99.99	99.99

The Study has more than adequate power to assess associations of maternal impaired fasting glucose and all major defects as a group. Statistical power to analyze subgroups of defects is less than that for the main defect categories. The power to detect these associations may be also reduced when controlling for confounders and evaluating effect measure modifiers. If, however, various defects are associated with maternal hyperglycemia as suggested by the literature on diabetes and birth defects, it may be possible to group them into a larger group and, thereby, increase the power of the Study to examine smaller risks and various interactions (e.g., with folic acid or antioxidants).

8. Other Design Issues

- **Drug/supplement classification:** Data collection concerning prescription and over-the-counter medications and herbal and multivitamin supplements must contain accurate information about the specific brand, product line, and dosage to be effectively used in evaluating confounders and effect measure modifiers.

- **Ethical/burden considerations:** Identification of clinically significant abnormalities in carbohydrate metabolism in preconceptional or early pregnant women will require notification of the woman's care provider.

- **Cost/complexity of data collection:** Diabetes status prior to pregnancy may be unknown for currently pregnant women who enter the sample. Postnatal assessment of diabetes status may serve as a surrogate.

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INCREASED RISK OF PRETERM BIRTH FROM INTRAUTERINE EXPOSURE TO MEDIATORS OF INFLAMMATION

1. Meta Hypothesis

Intrauterine exposure to mediators of inflammation due to infection of either vaginal, cervical, or uterine sites or of more distal sites (e.g., periodontal disease) is associated with an increased risk of preterm birth.

2. Specific Hypotheses

1. Intrauterine infection, as indicated by histological evidence in the fetal membranes or placenta at the time of birth, is associated with increased risk of preterm birth (less than 37 weeks) and early preterm birth (less than 32 weeks).

2. Pro-inflammatory cytokines in the maternal circulation during pregnancy are biomarkers of systemic infection that places the fetus at risk for preterm birth.

3. Pro-inflammatory markers in cervical/vaginal secretions are biomarkers of local infection that increase the risk for preterm birth.

4. Genetic predisposition to an enhanced inflammatory response (e.g., increased production of tumor necrosis factor [TNF]) among women with evidence of vaginal or other infection during pregnancy is associated with an increased risk of spontaneous preterm birth.

3. Background and Justification

While the cause of most preterm births is unclear, evidence of placental or chorioamniotic infection is present in as many as 40 percent of all spontaneous preterm births and in up to 75 percent of those born before 32 weeks gestation (the infants at greatest risk of subsequent adverse outcome) (Romero, Espinoza, Chaiworapongsa, & Kalache, 2002; Andrews, Hauth, & Goldenberg, 2000). Numerous common organisms of generally low virulence (e.g., ureaplasma, mycoplasma, gardenerella) have been associated with spontaneous preterm birth, and multiple micro-organisms can be present concurrently. Chorioamniotic or vaginal markers of inflammation and infection have been associated with preterm birth in the absence of identifiable infectious agents. Most work has focused on the association between local infection in gestational tissues and the vagina and preterm birth (Goldenberg, Hauth, & Andrews, 2000). However, systemic infection or infection at sites remote from the fetus, for example, periodontal disease, have been associated with preterm birth (Offenbacher et al., 1998; Goepfert et al., 2004; Jeffcoat et al., 2001) although a recent meta-analysis reported that this association was weaker in high-quality studies than it was in studies of lower quality (Vergnes & Sixou, 2007). The latter findings suggest a systemic infection of the mother influences the fetus through the blood. At present, it cannot be ruled out that an association between periodontal disease and preterm birth is the result of confounding, for example, by socioeconomic status (Goldenberg et al., 2006).

Observation of higher prevalence of infection at birth for preterm births compared to normal gestation infants has led to suggestions that screening and treatment for infection has the potential to reduce the incidence of preterm birth. However, interventions intended to reduce risk of preterm birth by addressing infectious causes of inflammation have, to date, not provided consistent evidence of benefit, (Pararas, Skevaki, & Kafetzis, 2006). An example includes a randomized trial of antibiotic therapy during

the interpregnancy interval for mothers with a prior preterm birth in which no difference in preterm birth was found among 124 analyzable pregnancy outcomes (Andrews et al., 2006). Another trial randomized 823 women for treatment of periodontal disease (scaling and root planning) at 13-17 weeks of pregnancy but failed to find a resulting difference in proportion of preterm births (Michalowicz et al., 2006) despite promising results for this therapy in a small pilot study (Jeffcoat et al., 2003). Several explanations can be proposed for the lack of efficacy of interventions tested to date. First, the choice of an agent for antibiotic interventions or the timing of their administration (i.e., after infection became symptomatic, when it has been suggested infections that have a role in preterm delivery may have been present at conception) (Goldenberg et al., 2000) were not suitable to obtain the desired effect. Second, the presence of infection and inflammation at preterm birth may not be a case of cause-and-effect but of phenomena with a common risk factor. Certain research gaps regarding infection and inflammation in preterm birth are best addressed through observational studies: to more specifically identify the infectious agents and inflammatory processes most closely related to preterm birth; to obtain better evidence of a causal link between infection and preterm birth, including evaluating temporal sequence; to determine if infection causes preterm birth mainly among women or fetuses with variant inflammatory response genes; and to detect biomarkers for risk of preterm birth.

Observational studies are also needed to investigate genetic susceptibility to the influences of infection and inflammation on birth outcomes. A putative susceptibility allele of the proinflammatory cytokine TNF-alpha has been examined in several studies, but a pooled analysis revealed no overall association between the polymorphism and preterm birth (Menon et al., 2006). A woman with a history of preterm birth is at high risk of another preterm birth, and incidence varies by race. This implies factors intrinsic to the mother, possibly genetic, play an important role. There is continued interest in finding genetic factors that act in the pathway of infection and inflammation effects on preterm birth (Pararas et al., 2006).

The National Children's Study (NCS) is an ideal setting in which to address these issues, because it will enroll a representative sample of pregnant women. This will enable the determination of the contribution of inflammation to the burden of preterm birth among different ethnic groups. The large size of the cohort will allow for more definitive data on the presence and magnitude of an interaction between the TNF-alpha promoter mutation and the presence of bacterial vaginosis on the occurrence of preterm birth (Goldenberg et al., 2000).

In summary, it is likely inflammation and infection are intimately associated with preterm birth as well as with short- and long-term neonatal outcomes including neurologic impairment. The exact mechanism of these associations, including timing and mode of action, remain unclear. And while there are sufficient data to suggest certain populations are at increased risk (e.g., African-American women), there may be other predisposing facts such as single nucleotide polymorphisms in inflammatory genes or other genes also present in women or their infants who ultimately experience this complication. There are also likely specific pathogens and gene-environment interactions that result in preterm birth and poor neonatal outcomes.

3.1 Public Health Importance

Prevalence/incidence

Each year in the United States, approximately 12 percent of all births are preterm (less than 37 weeks gestation) and 1-2 percent are early preterm (less than 32 weeks) (Martin, Hamilton, Ventura, Menacker & Park, 2002). Two-thirds of all infant deaths in the United States occur among those born preterm; preterm birth is associated with substantial neonatal morbidity, a high risk of long-term neurodevelopmental deficits, and low academic achievement (Hack et al., 2002). In term pregnancies,

about 1-2 percent are affected by chorioamnionitis (intrauterine infection), but in pregnancies ending in preterm births, the prevalence of such infection is higher (Wu & Colford, 2000).

Economic and/or social burden

Hospital charges in 2003 for infants who were preterm or low birth weight totaled $18.1 billion in 2003, and these infants accounted for half of the hospital charges for all infants (March of Dimes, 2006). The estimate does not include the monetary value related to use of community health services, the increased educational costs, the costs of social services, nor the out-of-pocket expenses incurred by the guardians of these children (Petrou, Sach, & Davidson, 2001). This underestimates current costs because of increasing preterm birth rates and improved survival of preterm infants. Due to large socioeconomic and racial or ethnic disparities, the U.S. population does not evenly share the medical, educational, and economic costs of preterm births.

3.2-3.3 Justification and Scientific Merit for a Large Prospective Longitudinal Study

It is likely that inflammation and infection are intimately associated with preterm birth as well as with short- and long-term neonatal outcomes including neurologic impairment. The exact mechanism of these associations, including timing and mode of action, remain unclear and can only be elucidated within the context of a large prospective longitudinal study such as the NCS. The Study will enroll a representative sample of pregnant women. This will enable the determination of the contribution of inflammation to the burden of preterm births among different ethnic groups. In addition, the large size of the cohort will allow for more definitive data on the presence and magnitude of an interaction between the TNF-alpha promoter mutation and the presence of bacterial vaginosis on the occurrence of preterm births (Goldenberg et al., 2000).

3.4 Potential for Innovative Research

There is evidence for an association between preterm birth and infection/inflammation as outlined above, but important questions remain to be answered to develop appropriate screening and interventions. As a longitudinal study, the NCS can examine issues of causality (exposure preceding disease) and timing of exposure that cannot be studied in other settings. The study will have good feasibility and statistical power, as noted below, to address research questions about infection, inflammation, and preterm birth.

3.5 Feasibility

Given the present knowledge about infection and inflammation and their relationship to preterm birth, a number of specific infectious agents can by hypothesized to be risk factors and a number of proinflammatory cytokines can be hypothesized to be mediators in this pathway. Therefore, the list of potential biomarkers to be measured in a study is long and may change as knowledge advances. However, as long as suitable biological samples are obtained in appropriate quantities and stored in a manner that prevents degradation of DNA and proteins, researchers will have the opportunity to return to these samples and examine a number of specific biomarkers of exposure as the science develops.

In designing research to examine infection and preterm birth, there are questions about what tissue and what timing of sample collection will best capture the biologically relevant exposure. For example, a case-control study observed an association between symptomatic periodontal disease and preterm birth found periodontal disease was not reflected in local markers of infection (i.e., placental culture, cord plasma IL-6) (Goepfert et al., 2004). This is indicative of questions about measurement of local versus systemic infections and potential influence of each on the fetus. Table 1 summarizes the

types of samples and timing of their collection relevant for assessment of fetal exposures to infection and inflammation. The study protocol calls for collection of two of the most critical tissues for assessment of infection: the placenta (including membranes) and the umbilical cord. Amniotic fluid will not be obtained. The study protocol includes good coverage of maternal samples of interest (i.e., a blood draw and a vaginal swab at each trimester).

Periodontal disease as a risk factor for preterm birth has received attention in recent literature. Collection of dental records or physical exams under the study protocol to ascertain presence of periodontal disease in the mother will not be feasible in the NCS. It is possible several sites might propose an ancillary study of periodontal disease and preterm birth. Given that periodontal disease is found among approximately 25 percent of African-American and 15 percent of white and Mexican-American reproductive age women (Xiong, Buekens, Vastardis, & Wu, 2006), a study of approximately 6,000 women would be needed to detect a 2.4-fold increase of birth at less than 28 weeks of gestation to women with periodontal disease compared to women with normal periodontal health. Just such a relative risk was reported in a recent meta-analysis (Xiong et al., 2006). Adding periodontal examinations for this number of women may be feasible as an ancillary study. Alternatively, assessment of bacterial DNA specific to pathogens responsible for periodontal disease in maternal sera may provide a surrogate marker for periodontal disease itself. Biomarkers of infection in maternal serum are another method for the indirect assessment of periodontal disease.

Table 1. Types of biological samples useful for assessment of infection during pregnancy, and planned collection under the NCS protocol

Priority*	Type of sample	Timing of collection	Collected under current NCS protocol?	Biomarkers of interest
1	Placenta	At birth	Yes, B1	Histologic evidence of inflammation, biomarkers†
2	Membrane (amnion and chorion)	At birth	Yes, B1	Histologic evidence of inflammation, biomarkers†
3	Umbilical cord segment	At birth	Yes, B1	Histologic evidence of inflammation, biomarkers†
4	Vaginal secretions	Each trimester and at birth	Yes, vaginal swab at T1, T2/3	Biomarkers†, Gram stain
5	Maternal cervical fluid	To be decided	No	PCR for specific pathogens
6	Maternal urine	Each trimester and at birth	Yes, T1, T2, T3, B1	White cell count, PCR for specific pathogens
7	Fetal cord blood serum	At birth	Yes, (cord is collected) B1	Biomarkers†
8	Maternal serum	Each trimester and at birth	T1, T2, T3	Biomarkers†
‡	Mother and infant DNA	Any	Yes	Genetic polymorphisms

* Qualitative ranking of importance of this sample type for assessment of in utero exposure to infection.

† Biomarkers include PCR for specific pathogens, cytokine levels, specific gene expression, and glucose level. The former can feasibly be assessed retrospectively from stored samples.

‡ For evaluation of gene-environment interaction, not assessment of infection.

4. Exposure Measures

Primary/maternal

Maternal infection/inflammation:

- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers, metalloproteinases)
- Gram stain for bacterial vaginosis
- Medical history of fever and infection (medicine usage) during pregnancy
- Dental exams (potential adjunct study in subpopulation)

Primary/child

- Prenatal infection: Umbilical cord/placental histology
- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers, metalloproteinases)

4.1 Individuals Targeted for Measurement

The most efficient design would be to conduct detailed assessment of exposure for a case-cohort or nested case-control sample of participants.

4.2 Methods

Primary/maternal

- Blood samples
- Vaginal fluid swabs self-collected
- Interviews
- Medical record reviews

Primary/child

- Physical sampling at delivery
- Histologic exam of placenta, umbilical cord, and fetal membranes at birth
- Cord blood

4.3 Life Stage

Primary/maternal

- Prenatal

Primary/child

- Birth

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Preterm birth: Gestational age; birth weight

5.2 Methods

- Longitudinal data from prior study contacts, including prenatal ultrasounds
- Medical records

5.3 Life Stage

- Birth

6. Important Confounders, Mediators, and Effect Modifiers

- **Economic status:** Increased risk associated with low educational level; unmarried status (Centers for Disease Control and Prevention [CDC], 1990), and psychosocial stress (Hogue, Hoffman, & Hatch, 2001).
- **Race/ethnicity:** As a percent of live births, 17.6 percent among blacks are preterm, 11.4 percent among Hispanics, and 10.8 percent among whites (Division of Vital Statistics, National Center for Health Statistics, CDC, 2003).
- **Mother's medical history:** Increased risk associated with maternal smoking, alcohol consumption; older maternal age (indicated preterm births); younger maternal age (spontaneous preterm births); low or high parity; previous preterm birth or stillbirth (Heffner, Sherman, Speizer, & Weiss, 1993; Meis et al., 1995; Wisborg, Henriksen, Hedegaard, & Secher, 1996).
- **Others:** Increased risk; unwantedness of the pregnancy (Olsen et al., 1995; McDonald, Armstrong, & Sloan, 1992).
- **Potential effect modifiers:** Antibiotic treatment (which may decrease exposure to infectious agent-initiated processes of inflammation, especially if systemic, or may increase exposure through alteration of normal vaginal flora, allowing bacterial

vaginosis, invasion of inappropriate organisms, or through the dissemination of proinflammatory mediators after bacterial death).

- **Corticosteroids** (Goldenberg et al., 2006).

- **Genetics and gene-environment interactions:** Some (Macones et al., 2004), though not all (Menon et al., 2006), evidence suggests a specific polymorphism in the TNF gene leading to an exaggerated inflammatory response is associated with an increased risk of preterm birth among women with bacterial vaginosis. Variation in other proinflammatory genes (e.g., IL-1, IL-6) (Engel et al., 2005) may also be associated with increased risk of spontaneous preterm birth among women with infection during pregnancy.

- **Intermediate variables:** Data collection and analysis must be conducted with the awareness that certain conditions may fall in a causal pathway between infection and preterm birth (e.g., preeclampsia). Another issue is that factors other than infection can affect biomarker of inflammation. An example of this is that inflammation has been cited as possible mechanism of action of noninfectious exposures that contribute to risk of preterm birth (e.g., air pollutants) (Huynh, Woodruff, Parker, & Schoendorf, 2006).

7. Power and Sample Size

For a number of specific hypotheses, data analysis will be based on a case-cohort or case-control sample rather than the whole cohort. These designs are appropriate for exposures requiring expensive or time-consuming assays or data collection (e.g., cytokine assays, PCR for detection of microbial DNA, or retrospective chart review). Study power for specific hypothesis tests will vary depending on the outcome selected for analysis (e.g., preterm birth, defined as less than 37 weeks with an estimated prevalence of 12 percent, or early preterm birth, which may be a more specific endpoint with infection-associated inflammation but is much less frequent, estimated at 1-2 percent). The exposure of interest also affects power. Periodontal infection is very prevalent, as high as 30 percent among adults, whereas the percentage of intrauterine infections with any single specified microorganism will be quite low. Evaluation of study power should also take into account missing data. Given that missing data can be expected due to missed clinic visits, subject refusal for certain samples, laboratory assay failure, etc., it should be expected that not all subjects will have complete data for the exposure of interest and for covariates. Minimum detectable odds ratios for a range of scenarios are presented in Table 2.

Table 2. Minimum detectable odds ratios* (MDOR) for two preterm birth outcomes, by exposure prevalence

Outcome	Frequency	N cases	Percent with missing data	N cases with complete data	MDOR for each % exposure prevalence					
					0.5%	1%	2%	5%	10%	20%
Preterm birth	12%	12,000	10%	10,800	1.50	1.35	1.25	1.15	1.12	<1.10
< 37 weeks			25%	8,000	1.60	1.40	1.30	1.20	1.14	1.10
Early preterm birth	2%	2,000	10%	1,800	2.40	2.00	1.65	1.40	1.30	1.25
< 32 weeks			25%	1,500	2.60	2.10	1.70	1.45	1.35	1.25
Early preterm birth	1%	1,000	10%	900	>3.00	2.50	2.00	1.60	1.45	1.35
< 32 weeks			25%	750	>3.00	2.70	2.10	1.65	1.50	1.35

* At 80% power, alpha = 0.05, 1:1 case-control ratio; calculated using Stata software.

8. Other Design Issues

- **Changing biomarkers:** The list of potential biomarkers to be measured in a study is long and may change as knowledge advances. However, as long as suitable biological samples are obtained in appropriate quantities and stored in a manner that prevents degradation of DNA and proteins, researchers will have the opportunity to return to these samples and examine a number of specific biomarkers of exposure as the science develops.

- **Ideal tissues to collect:** In designing research to examine infection and preterm birth, there are questions about what tissue and what timing of sample collection will best capture the biologically relevant exposure. The study protocol calls for collection of two of the most critical tissues for assessment of infection: the placenta (including membranes) and the umbilical cord. Amniotic fluid will not be obtained. The study protocol includes good coverage of maternal samples of interest (i.e., a blood draw and a vaginal swab at each trimester).

- **Use of periodontal measures:** Periodontal disease as a risk factor for preterm birth has received attention in recent literature. Collection of dental records or physical exams under the study protocol to ascertain presence of periodontal disease in the mother will not be feasible in the NCS. It is possible several sites might propose an ancillary study of periodontal disease and preterm birth.

- **Reporting of findings:** Issues of reporting of findings to participants and parents of participants, dependent on what samples are collected and analyzed at what time, will require special attention.

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INCREASED RISK OF FETAL GROWTH RESTRICTION, PRETERM BIRTH, BIRTH DEFECTS, AND DEVELOPMENTAL DISABILITIES IN CHILDREN BORN THROUGH ASSISTED REPRODUCTIVE TECHNOLOGY

1. Meta Hypothesis

Children whose conceptions were aided by assisted reproductive technology (ART) are at increased risk of fetal growth restriction, birth defects, and developmental disabilities in comparison to children who were conceived without ART.

2. Specific Hypotheses

1. Singleton fetuses resulting from ART treatment will have higher rates of fetal growth restriction as compared to singleton fetuses from the general population and also as compared to singleton fetuses from couples with subfertility who did not undergo ART.

2. Women with singleton pregnancies resulting from ART treatment will have higher rates of preterm delivery as compared to women with singleton pregnancies from the general population and also as compared to women with singleton pregnancies arising from couples with subfertility who did not undergo ART.

3. Infants born from singleton pregnancies resulting from ART treatment will have higher rates of any birth defects as compared to infants born from singleton pregnancies from the general population and also as compared to infants born from singleton pregnancies arising from couples with subfertility who did not undergo ART.

4. Children born from singleton pregnancies resulting from ART treatment will have higher rates of developmental disability as compared to children born from singleton pregnancies from the general population and also as compared to children born from singleton pregnancies from couples with subfertility who did not undergo ART.

3. Background and Justification

ART is infertility treatments in which both oocytes and sperm are handled in the laboratory. These treatments include in vitro fertilization (IVF) with transcervical embryo transfer (common), IVF with intracytoplasmic sperm injection (ICSI) and transcervical embryo transfer (common), gamete and zygote intrafallopian transfer (gametes or zygotes transferred into fallopian tubes rather than uterus, which is rare), frozen embryo transfer (less common), and donor embryo transfer (less common). ART is recognized as an important contributor to the U.S. low birth weight rate because of the known association between the use of ART and multiple births (Centers for Disease Control and Prevention [CDC], 2004b; CDC, 2000) and between multiple births and low birth weight (Martin & Park, 1999). It has been suggested that concerns about adverse outcomes of ART could be mitigated by single embryo transfer, avoiding the major complications associated with multiparity (Wennerholm et al., 2000; Catt, Wood, Henman & Jansen, 2003; Davis, 2004). However, as reviewed below, there is substantial evidence suggesting increased risk for singleton pregnancies from ART. This hypothesis therefore focuses on the outcomes for singletons.

Fetal growth restriction

Many retrospective studies have found rates of low birth weight and preterm birth are increased among singleton infants conceived with ART as compared with naturally conceived singleton infants or population-based rates (MRC Working Party on Children Conceived by In Vitro Fertilisation, 1990; FIVNAT, 1995; Gissler, Malin Silverio, & Hemminki, 1995; Verlaenen, Cammu, Derde, & Amy, 1995; Bergh, Ericson, Hillensjo, Nygren & Wennerholm, 1999; Dhont, De Sutter, Ruyssinck, Martens, & Bekaert, 1999; Westergaard, Johansen, Erb, & Andersen, 1999; Schieve et al., 2002; Schieve et al., 2002; Jackson, Gibson, Wu, & Croughan, 2004; Schieve, Ferre, et al., 2004; Poikkeus, Gissler, Unkila-Kallio, Hyden-Granskog, & Tiitenen, 2007). The question remains whether the observed risks for singletons conceived with ART is a direct effect of the procedure or reflects another factor related to the underlying infertility of the couples who conceive using these procedures (Westergaard et al., 1999; Buck Louis, Schisterman, Dukic, & Schieve, 2005). Subfertile controls (i.e., trying for more than one year) who conceive spontaneously are necessary to address this question. A recent large population-based study from the Netherlands using subfertile couples conceiving naturally as controls suggests ART (or some component of it) may have independent adverse effects for low birth weight and preterm birth (Kapeiteijn et al., 2006). In addition, studies to date have been limited in their ability to evaluate low birth weight due to intrauterine growth restriction separately from low birth weight due to preterm delivery. This is an important distinction for understanding etiology and developing prevention strategies. No study has monitored fetal growth changes prospectively. Rapidly changing ART treatments (most notably, a rapid increase in the use of ICSI in the past five years) result in a need to re-examine the association with precise data about treatments (Schultz & Williams, 2002).

Birth defects

Mixed results exist regarding the association between ART and birth defects in the offspring, with some studies finding no increased risk (MRC Working Party on Children Conceived by In Vitro Fertilisation, 1990; Friedler, Mashiach, & Laufer, 1992; FIVNAT, 1990; Westergaard et al., 1999), and other studies finding an increased risk for all birth defects and/or some conditions (Bergh et al., 1999; Wennerholm et al., 2000; Ericson & Kallen, 2001; Hansen, Kurinczuk, Bower, & Webb, 2002; Bonduelle et al., 2005; Kallen, Finnstrom, Nygren, & Olausson, 2005; Klemetti et al., 2005; Merlob, Sapir, Sulkes, & Fisch, 2005; Olson et al., 2005). A recent meta-analysis concluded ART carried a 30-40 percent increase in the risk of all birth defects as compared with naturally conceived infants (Hansen, Bower, Milne, de Klerk, & Kurinczuk, 2005). Prior studies have suffered from methodological problems, most notably low statistical power, and differential case ascertainment and coding schemes for infants conceived using ART vs. infants conceived naturally (Schieve, Rasmussen, & Reefhuis, 2005). Nearly all studies have relied on retrospective registry data. Most studies of birth defects have not analyzed singleton infants separately from infants from multiple gestations, and multiple gestation is a risk factor for birth defects. Analogous to low birth weight, the question remains as to whether increased risk is due to the treatment or the underlying infertility (Schieve, Rasmussen, et al., 2004; Kallen et al., 2005; Olson et al., 2005; Schieve et al., 2005). Work in progress from Iowa suggests underlying ovulation disorders are themselves a risk factor for low birth weight, regardless of infertility treatment received (ART or other treatment) (VanVoorhis et al., 2006). A large population-based cohort from Denmark found that when compared to spontaneously conceiving subfertile controls, the incidence of all genital malformations was increased fourfold although the incidence of birth defects overall was not increased (Zhu, Basso, Obel, Bille, & Olsen, 2006). (This study is discussed further below.) Retrospective studies will substantially underestimate the incidence of birth defects because some result in spontaneous abortion or in some cases, elective abortion, so they are not identified in retrospective cohorts based on births. If such early terminations of pregnancy were more common in ART pregnancies, then retrospective studies based on births would underestimate the risk.

Developmental disabilities

The study of longer-term outcomes such as developmental disabilities among ART children has been hampered by inadequate sample sizes and follow-up. Cross-sectional studies have generally not found increased developmental problems for children conceived by ART (Olivennes et al., 2002; Ponjaert-Kristoffersen et al., 2005). A retrospective population-based study from Sweden reported an increased risk for cerebral palsy (odds ratio 2.8, 95 percent confidence interval 1.3-5.8), and a nonsignificant trend for suspected developmental delay (odds ratio 2.0, 95 percent confidence interval 0.7-5.4) among singleton children conceived with ART (Stromberg et al., 2002). The study suffered from a number of methodological drawbacks, including a reliance on registry data and a lack of standardized neurodevelopmental evaluation. There have been no longitudinal prospective studies with thorough follow-up and standardized assessment across children conceived with and without ART.

Epigenetic effects have been shown with ART techniques applied to animals (Khosla, Dean, Brown, Reik & Feil, 2001, Young et al., 2001) and this may be a possible mechanism for some adverse outcomes with ART in humans (Schultz, 2005). The rare imprinting disorders Angelman Syndrome and Beckwith-Wiedemann Syndrome have been linked to ART, though the absolute risk is small (Cox et al.., 2002; DeBaun, Niemitz, & Feinberg, 2003; Chang, Moley, Wangler, Feinberg, & Debaun, 2005; Maher, 2005; Sutcliffe et al., 2006). Nevertheless, this highlights the potential value of investigating potential genetic and epigenetic mechanisms for adverse outcomes of ART within a large cohort study (Bjornsson, Fallin & Feinberg, 2004).

3.1 Public Health Importance

Prevalence/incidence

In the United States and worldwide, the use of ART to overcome infertility is increasing rapidly. In 2004, the most recent year for which U.S. population-based data are available, more than 128,000 ART procedures were performed, resulting in more than 49,000 live-born infants (CDC, 2004b). These infants are estimated to represent slightly more than 1 percent of the total infants born in the United States in 1999. However, ART treatments represent only a fraction of the infertility treatments currently used. Results from the population-based National Survey of Family Growth suggest that in 2002, treatment with ovulation stimulation medications without ART was about 15 times more frequent than the use of ART, and artificial (or assisted) insemination was about five times more frequent (Chandra, Martinez, Mosher, Abma, & Jones, 2005). In addition, 15.1 percent of married women of reproductive age reported some type of subfertility, but only 36 percent of women with subfertility ever received any form of infertility service (of which about half received medical advice only), and only 1 percent ever received ART. This represents a large pool of subfertile women who may have spontaneous conceptions and also a large growth potential for increased use of ART and other infertility treatments as accessibility improves. In a representative sample of births based on 2004's Pregnancy Risk Assessment Monitoring System (PRAMS) in Utah, 7 percent of intended births without infertility treatment had a time to pregnancy of greater than one year (representing spontaneous conceptions in subfertile women) (Stanford, Ellis, Simonsen, & Baksh, 2007).

In the United States, the incidence of fetal growth restriction is approximately 5 percent (Peleg, Kennedy, & Hunter, 1998). The preterm birth rate has been rising and is 12.5 percent of all births as of 2004 (Committee on Understanding Premature Birth and Assuring Health Outcomes, 2006). Major birth defects occur in approximately 3 percent of children born in this country (CDC, 2005; Boyle & Cordero, 2005). The prevalence of serious developmental delay is estimated from 2-3 percent in national surveys (Simpson, Colpe, & Greenspan, 2003; Van Naarden Braun, Autry, & Boyle, 2005), while the presence of any developmental impairment, including mild impairment, is estimated to be 17 percent

(Rice, Schendel, Cunniff, & Doernberg, 2004; Boyle & Cordero, 2005). Cerebral palsy affects approximately 0.2 percent of children (Kuban & Leviton, 1994), and autism affects about 0.3 percent (Yeargin-Allsopp et al., 2003).

Economic and/or social burden

Children with birth defects and those born too small contribute disproportionately to infant and pediatric health care costs (Sandhu, Stevenson, Cooke, & Pharoah, 1986; Lynberg & Edmonds, 1994; Committee on Understanding Premature Birth and Assuring Health Outcomes, 2006). An investigation conducted in the United States in 2003 concluded that the proportion of preterm and low-birth-weight infants has been growing. Ten billion dollars was spent on care for preterm births in the form of acute care, education, and child care costs (Cuevas, Silver, Brooten, Youngblut, & Bobo, 2005). A report estimated an aggregate incremental cost of $26 billion in 2005 associated with preterm birth. That was $20 billion in direct costs for health care, early intervention, and education and $6 billion in lost productivity due to the increased risk of developmental disabilities (Institute of Medicine Report, in press). The lifetime costs for persons born in 2000 were estimated at $51.2 billion for those with mental retardation, $11.5 billion for those with cerebral palsy, $2.1 billion for those with hearing loss, and $2.5 billion for those with impaired vision (in 2003 dollars) (CDC, 2004a). More than 25 percent of pediatric hospital admissions are estimated to occur among children with birth defects (Lynberg & Edmonds, 1994). Children with developmental disabilities require special education services, medical services, and supportive care.

3.2 Justification for a Large Prospective Longitudinal Study

There is strong scientific consensus about the importance of understanding the health outcomes of ART, including longitudinal studies to identify concerns about child development (President's Council on Bioethics, 2004). Although a large body of literature suggests an association between ART and the adverse outcomes discussed above for children from singleton pregnancies, critical questions remain that can be answered only by a large prospective study that includes appropriate measures (Schieve, Rasmussen, et al., 2004; Schieve et al., 2005). These questions include parsing whether the associations are due to the ART or are due to the underlying condition of infertility itself, estimating the potential relative contribution of different types of ART treatment (most notably, ICSI), the relative contribution of ART to fetal growth restriction and preterm delivery (important for understanding underlying etiologic pathways and possible routes for prevention of low birth weight), and identifying the true magnitude of the association based on standardized prospective identification of outcomes. Prospective identification of outcomes is essential for adequate ascertainment of fetal growth restriction, birth defects, and developmental disabilities.

3.3 Scientific Merit

One previous study that has similar design characteristics and is of a similar size to the National Children's Study (NCS) is the Danish national birth cohort. This study enrolled subjects during the first or second trimester of pregnancy from 1997 to 2003 (Zhu et al., 2006). This study included 50,897 singleton births and 1,366 twin births born to normally fertile women, 5,764 singletons and 100 twins born spontaneously to subfertile couples, and 4,588 singletons and 1,690 twins born to subfertile couples with treatment. Exposure data for treatment were obtained by questionnaires, and pregnancy and child outcomes were obtained by linking to the national hospital register and medical birth register. As noted, this study found an increase in genital organ malformations among singletons conceived with the aid of infertility treatment compared to subfertile couples who conceived naturally. When broken out by treatment class, there was some evidence this might be most related to undergoing ICSI. There was also an increase of all malformations among spontaneously conceiving couples with longer times to pregnancy

(adjusted hazard ratio 1.3 for couples taking longer than 12 months to conceive compared to couples taking two months to conceive). Thus, this study found some evidence suggesting both connections to adverse outcomes both for underlying infertility and for infertility treatment.

The NCS could examine these questions:

- **Exposure:** Retrospective assessment through questionnaires of the type of infertility treatment, including different types of ART and other treatments. Prospective data from maternal reports may be available from women participating in the prepregnancy aspects of the Study.

- **Outcome:** Fetal growth trajectories assessed by ultrasound in utero. Standardized clinical examinations for malformations at birth. Capture of most early pregnancy outcomes, including early miscarriage or abortion. Standardized sequential neurodevelopmental evaluations for childhood.

- **Extended outcomes:** Longer term follow-up for developmental issues in adolescence, including pubertal transitions.

- **Mechanisms:** Genetic and epigenetic investigations of both parents and of the children.

- **Potential confounders:** Detailed data on environmental and chemical exposures, dietary exposures, as well as data on treatments received during pregnancy.

3.4 Potential for Innovative Research

As described immediately above, the NCS would be able to examine these hypotheses more rigorously than any study. Perhaps the greatest potential for innovation in relation to these hypotheses comes from the opportunity to investigate the potential genetic and epigenetic mechanisms for adverse outcomes of ART within a large cohort study (Bjornsson et al., 2004). In addition, structuring the study to test these hypotheses will result in a cohort able to answer questions about other specific child health outcomes from subfertility and ART that may arise in the future.

3.5 Feasibility

Most of the measurements necessary for this hypothesis are already incorporated into the study protocol or else can be easily added with minimal change of burden for the participants. The follow-up schedule is adequate for the study. The major alteration that would be required in the study to address this hypothesis would be to expand the sampling frame to include a targeted subsample of couples undergoing ART as described under “Power and Sample Size.” Addressing these hypotheses with the study protocol is feasible.

4. Exposure Measures

The following lists include measures relevant to a robust exploration of the hypotheses, including exploring possible mechanisms and confounders:

4.1 Individuals Targeted for Measurement

Primary/maternal

- Maternal reports of reproductive health/infertility.
- Genetic information.
- Hormones.

Primary/paternal

- Infertility.
- Genetic information.

4.2 Methods

Primary/maternal

- Questionnaire materials: Age; prior menstrual and pregnancy history; history of any infertility evaluation and treatment for index or expected pregnancy; time "at risk" of pregnancy at time of study entry and again at time of diagnosis of pregnancy; smoking; alcohol; and socioeconomic status.
- Desirable questionnaire measures: Daily diaries of bleeding, coitus, and vaginal mucus discharge for at least one of the cycles leading to the index pregnancy performed and recorded by the ART center. For women not completing daily diaries, usual frequency of sexual intercourse will be queried upon entry into the study. Prior gynecologic history, including the history of sexually transmitted infection. Family planning history (especially use of hormonal methods). History of infertility evaluation and treatment in past. Time to pregnancy question for past pregnancies. Prior history of periods of noncontracepted sexual intercourse and family planning use.
- Anthropomentric measures: Height and weight.
- Exact infertility diagnoses and details of treatments, including ART (IVF and ICSI), and other evaluations or treatments.
- Prenatal treatments (such as progesterone) for index pregnancy. (These cannot be obtained reliably from the couple by questionnaires alone).
- Basal serum FSH and estradiol levels if available from the ART Center.
- DNA of both parents.

Primary/paternal

- Essential questionnaire materials: Age; history of infertility evaluation or treatment for index pregnancy; smoking; alcohol; and socioeconomic status.

- Exact infertility diagnoses and details of treatments, including ART (IVF and ICSI), and other evaluations or treatments.
- Prior andrologic history, including sexually transmitted infection, pregnancies caused, and prior history of infertility treatments.
- Anthropomentric measures: Height and weight.
- DNA of both parents.

4.3 Life Stage

Primary/maternal

- Preconception/periconception and following birth (depending on contact availability)

Primary/paternal

- Preconception/periconception and following birth (depending on contact availability)

5. Outcome Measures

The following lists include measures relevant to a robust exploration of the hypotheses, including exploring possible mechanisms and confounders:

5.1 Outcomes Targeted for Measurement in Child

Primary

- Preterm birth.
- Congenital malformations.
- Developmental disorders.

5.2 Methods

Essential questionnaire measures

- Child developmental measures: Screening questionnaires regarding the child periodically in childhood; assessment of social environment for the child in relation to potential impacts on development.
- Standardized physical examination of all neonates and infants with attention to the ascertainment of birth defects.
- Abstraction of relevant clinical information on birth defects from medical records.

- Reviews of clinical information for classification of birth defects by clinical geneticists.
- Standardized psychoneurologic assessment for developmental milestones periodically through childhood.
- Neonatal complications; evaluations or treatments in relation to possible birth defects or developmental delay.
- Desirable measures from medical records (ideally prospectively or perhaps retrospectively): School records.
- Biologic measures: DNA of infant/child.
- Fetal biometric measurements from ultrasound.
- Radiology: Neonatal cardiac ultrasound, if available (for structural defects).

5.3 Life stage

Prenatal, birth, and childhood through early adulthood.

6. Important Confounders, Mediators and Effect Modifiers

- Maternal age at conception
- Parity
- Previous obstetric outcome
- Ethnicity
- Socioeconomic status
- Genetic carrier states of parents

7. Power and Sample Size

The study design must ensure the sample selected is appropriate to study the effects of ART on various outcomes for singleton births separately. Because a high proportion of ART births are multiple, this must be factored into sample size estimations. It may be desirable to inflate the ART sample in order to have full power to assess major subtypes of ART (e.g., IVF with and without ICSI). Based on the following table, a minimum of 3,000 and a maximum of 16,000 ART-exposed pregnancies will be needed to adequately address the hypotheses, depending on whether power is sought for specific conditions of low incidence, and whether full power is desired to analyze two subgroups of ART. By comparison, the Danish national birth cohort included 4,588 singleton infants exposed to infertility treatment of which only a subgroup of unreported size actually had ART (Zhu et al, 2006). Within the existing study sample frame, approximately 1,000 infants will be ART-exposed, meaning that targeted oversampling for participants with ART will be necessary for sufficient power for the hypotheses. Of the

approximately 85,000 non-ART pregnancies available for the nonexposed comparison group, approximately 7 percent (5,950) can be expected to be pregnancies resulting from spontaneous conceptions to subfertile couples, allowing for somewhere between a 1:1 and 10:1 ratio of ART-exposed to unexposed pregnancies resulting from spontaneous conceptions from subfertile couples.

Table 1. Sample Size Calculation

1 Outcome of Interest	2 Prevalence in general population	3 Minimum expected increase in ART singleton population	4 Sample size of singleton births needed in ART group	5 Number of ART live birth deliveries needed to obtain number of singletons in column 4	6 Number of ART pregnancies needed to obtain number of deliveries in column 5
Intrauterine growth restriction (term infants)	5%	1.5 fold (7.5%)	925	1,423	1,674
Preterm delivery	10.0%	1.5 fold (15.0%)	434	668	809
Birth defects (all)	3.0%	1.5 fold (4.5%)	1,578	2,428	2,856
Serious developmental disabilities (all)	2.0%	1.5 fold (3.0%)	2,395	3,685	4,335
Mild-serious developmental disabilities (all)	17.0%	1.5 fold (25.5%)	233	358	421
Specific birth defect or developmental disability	1.0%	2.0 fold (2.0%)	1,445	2,223	2,615
Specific birth defect or developmental disability	0.5%	2.0 fold (1.0%)	2,909	4,475	5,265
Specific birth defect or developmental disability	0.3%	2.0 fold (0.6%)	4,861	7,478	8,798

Assumptions: Column 4: At least 4:1 ratio non-ART (nonexposed) to ART infants; alpha = 0.05 (two-sided); Beta = 0.2. Column 5: 65% of deliveries will be singletons. Column 6: 15% fetal loss rate.

8. Other Design Issues

- **Ethical/burden considerations:** Couples seeking ART are already undergoing intensive and time-consuming medical testing and treatments. Preconception data collection could be structured to coincide with their regular visits to an infertility clinic. While it would be ideal to recruit all ART-exposed women preconceptionally,

it would also be possible to recruit women and their partners at the time of conception. For many couples, infertility is an emotionally charged condition, and it may turn out that willingness to participate is more likely once conception has occurred.

- **Cost/complexity of data collection:** A sampling strategy that incorporates selected recruitment at infertility treatment centers will be required. These centers should be located at or near the study sites to maximize comparability of the women recruited. Arrangements will need to be made with infertility clinics to facilitate coordination of data collection, including review of records of diagnosis and treatment.

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MATERNAL SUBCLINICAL HYPOTHYROIDISM AND NEURODEVELOPMENTAL DISABILITIES/ADVERSE PREGNANCY OUTCOMES

1. Meta Hypothesis

Maternal subclinical hypothyroidism is associated with adverse pregnancy outcomes and neurodevelopmental disabilities.

2. Specific Hypotheses

1. Maternal subclinical hypothyroidism during pregnancy results in changes in neurodevelopmental trajectories in the offspring.

2. Maternal/fetal environmental exposures during gestation result in changes in neurodevelopmental trajectories in children, at least in part, via disruption of the maternal thyroid system.

3. Maternal subclinical hypothyroidism during gestation results in adverse pregnancy outcomes, specifically preterm birth (delivery earlier than 37 weeks) and preeclampsia.

4. Maternal hypothyroidism in the first trimester of pregnancy before the fetus can produce its own thyroxine is more damaging to childhood neurodevelopment than hypothyroidism later in pregnancy (American Thyroid Association [ATA], 2004).

3. Background and Justification

Epidemiologic data show suboptimal thyroid function in pregnancy is associated with impaired intellectual development. Two studies have shown an association between low thyroid hormone concentrations in early gestation and significant IQ decrements in children at 7 years and 10 months of age, respectively (Klein et al., 2001; Pop et al., 1999) although both studies are single-center studies with small sample sizes. Further evidence for this association comes from a recent study showing an inverse correlation between the severity of maternal hypothyroidism and IQ of the offspring (Klein et al., 2001) and from a study suggesting maternal hypothyroidism in the first trimester of pregnancy before the fetus can produce its own thyroxine is more damaging to childhood neurodevelopment than hypothyroidism later in pregnancy (Pop et al., 2003).

In 2004, a statement from the ATA and the American Association of Clinical Endocrinologists workshop "Impact of Maternal Thyroid Status on Pregnancy and Fetal and Childhood Development" concluded although hypothyroidism from iodine deficiency in the United States has been addressed, "there are still additional adverse outcomes for maternal health, maintenance of pregnancy, and child development that may occur as a result of overt maternal hypothyroidism, as well as subclinical hypothyroidism (normal serum thyroxine concentration and elevated serum thyroid-stimulating hormone concentration), maternal hypothyroxinemia (depressed serum free thyroxine concentration), and the presence of thyroid autoantibodies (Pop et al., 2003)." The ATA highlighted several recent research findings, among which two are particularly relevant to this proposed hypothesis and need further data with regards to "magnitude:"

- Pregnant mothers with overt or subclinical hypothyroidism are at an increased risk for premature delivery.

- The offspring of mothers with (subclinical) thyroid hormone deficiency or thyroid-stimulating hormone elevation during pregnancy may be at risk of mild impairment in their intellectual function and motor skills (Casey, 2005).

Autoimmune disorders are considered to be the most common cause of hypothyroidism in reproductive-age women (Brucker-Davis, 1998). However, endocrine disruption as a mode of action for the toxicity of chemicals has been of increasing interest and concern during the past decade. Of particular interest are chemicals demonstrated to alter thyroid hormone function in laboratory animals and wildlife. There is much less information from studies that assess thyroid effects in humans (Howdeshell, 2002). A recent review listed 116 chemicals that could interact with thyroid hormone status (Landrigan, Garg, & Droller, 2003). Although the potential of environmental chemicals to interfere with thyroid function is well established, the role of endocrine disruption in hypothyroidism at the population level is not known.

While the literature suggests variations of endocrine parameters, such as thyroid dysfunction in utero, appear to be associated with developmental disabilities in the resulting child, the threshold for manifestation of these effects is currently undefined. There are information gaps regarding the potential effects of hormonally active agents in the environment on the developing fetus via maternal endocrine disruption. There also are potential direct interactions with the fetus for agents that can cross the placental barrier. A recent conference that focused on evaluation of endocrine disruption within the National Children's Study (NCS) investigated the issue of thyroid hormone function as well as other endocrine effects. Scientists who participated in this workshop highlighted the potential for chemical exposures to cause subclinical hypothyroidism or subclinical hypothyroxinemia and thus increase the risk for adverse health and developmental outcomes in children as an important priority for the Study (Longnecker et al., 2003; Chung, Lau, Yip, Chiu, & Lee, 2001).

Maternal depression affects fetal health (Van den Bergh, Mulder, Mennes, & Glover, 2005; Chrousos, Torpy, & Gold, 1998). Although psychological and biological explanations have been researched, hypotheses focused on hormonal exposures have received more attention. Depression is known to affect thyroid and pituitary function and is associated with hypothalamo-pituitary-adrenal (HPA) axis hyperactivity. Maternal stress, anxiety, or depression (factors regulated by peptides derived from the activated HPA axis) each affect birth outcomes (Paarlberg, Vingerhoets, Passchier, Dekker, & van Geijn, 1995; Sandman et al., 1994; Sandman, Wadhwa, Chicz-DeMet, Dunkel-Schetter, & Porto, 1997; Smith et al., 1990; Wadhwa, Dunkel-Schetter, Chicz-DeMet, Porto, & Sandman, 1996). Thus, increased HPA-axis activity, including subclinical hypothyroidism and hypothyroxinemia, through psychological influences such as depression, may directly affect maternal and fetal function, fetal growth, and ex utero growth and function.

Clinical maternal hypothyroidism also has been linked to adverse pregnancy outcomes, including infertility, preeclampsia, placental abruption, postpartum hemorrhage, and associated perinatal morbidity and mortality with a high frequency of low birth weight and fetal death (Davis, Leveno, & Cunningham, 1988; Lao, Chin, & Swaminathan, 1988). Subclinical hypothyroidism has been linked to eclampsia, preeclampsia, and gestational hypertension (Leung, Millar, Koonings, Montoro, & Mestman, 1993). Thyroid hormone status (decreased free T4 and increased thyroid-stimulation hormone) also has been found to be related to severity of preeclampsia and degree of low birth weight (Basbug et al., 1999). Preterm babies born to mothers with preeclampsia and hypothyroxinemia show lower thyroid hormone levels in utero and at birth (Belet, Imdat, Yanik, & Kucukoduk, 2003; Chan, Chiu, & Lau, 2003). Evidence supports the premise that clinical hypothyroidism is associated with preeclampsia, preterm birth, and associated adverse outcomes in infants. Subclinical hypothyroidism and hypothyroxinemia may be implicated as well, but it has not been adequately studied.

3.1 Public Health Importance

Prevalence/incidence

The prevalence of subclinical hypothyroidism during pregnancy in the United States is estimated at 2.4 percent (Casey et al., 2005). No data exist to define the proportion of these cases resulting, either in part or in total, from environmental exposures. While preeclampsia and spontaneous preterm birth remain major contributors to adverse long-term outcomes for children, it is not clear what proportion of these cases are from subclinical hypothyroidism, environmental exposures, or the interaction between environmental exposures and resultant thyroid dysfunction.

Economic and/or social burden

No studies have precisely calculated the costs associated with thyroid dysfunction during pregnancy. Although newborn screening for congenital hypothyroidism already occurs in all states, maternal screening for thyroid dysfunction during pregnancy is not yet considered an obstetric standard of care. The screening of all million pregnant women delivering live-born infants each year in the United States could lead to several billion dollars annually in screening costs alone just for detection of subclinical hypothyroidism or hypothyroxinemia. The costs of treatment would add substantial additional expenditures. All these costs should be adequately justified in a prospective fashion.

3.2 Justification for a Large Prospective Longitudinal Study

No data exist to guide clinical obstetric decision making regarding appropriate interventions for subclinical hypothyroidism or hypothyroxinemia during pregnancy. Although a randomized clinical trial of thyroxine replacement vs. placebo for such women is registered with clinicaltrials.gov (NCT 00388297), results will not be available until at least 2015, and the study is not addressing environmental influences on laboratory testing or outcomes. In addition, this randomized clinical trial will follow subjects only to age 5.

As a result, a large prospective longitudinal study that collects information both about maternal thyroid function and environmental exposures and correlates these data with truly adequate long-term outcomes is needed in order to guide future interventions and public policy.

3.3 Scientific Merit

Such a study has obvious clinical applicability and scientific merit. All of the dependent and independent variables described below are validated and accepted as appropriate clinical markers capable of answering the relevant study hypotheses. The potential clinical implications are tremendous.

3.4 Potential for Innovative Research

The evolving disciplines of genomics, proteomics, and epigenetics will offer multiple opportunities to assess the interactions between maternal and fetal thyroid genetic regulation and environmental exposure.

3.5 Feasibility

This study is likely to be accomplished within the NCS framework. The independent variable samples will be collected within the basic framework of the study (maternal and cord blood, environmental exposures, long-term function of children). No extra interventions are required beyond

those already outlined in the Study's basic framework. Information will also be available on all newborns from newborn metabolic screening.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Maternal thyroid status, including L-thyroxine (T4), free thyroxine (free T4), L-triiodothyronine (T3), free T3, thyroid stimulating hormone, thyroid gland enlargement, and serologic markers of thyroid autoimmunity (anti-thyroid peroxidase, anti-thyroglobulin, thyroid-stimulating, and TSH receptor-binding inhibitory antibodies), prepregnancy or in the first trimester, as well as in the third trimester.

- Maternal stress during pregnancy as assessed by stress hormones (e.g., cortisol) and report of stressful situations, anxiety, or depression.

Primary/child

- Neonatal thyroid status as assessed by L-thyroxine (T4), free thyroxine (free T4), L-triiodothyronine (T3), and thyroid stimulating hormones.

4.2 Methods

Primary/maternal

- Biological specimens: Blood, urine, saliva, breast milk

- Examination by a medical professional

- Interview(s)

Primary/child

- Umbilical cord blood culture/pathology

- Blood samples/newborn blood spots

4.3 Life Stage

Primary/maternal

- Repeated measures: Preconception, first through third trimesters, birth, and during nursing period

Primary/child

- Repeated measures at birth, during infancy

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

- Neurodevelopmental trajectories via neurocognitive and developmental tests including IQ, domain-specific evaluations of attention/concentration, executive function, learning, memory and motor skills
- Preterm birth: Gestational age, birth weight

Secondary

- School performance

5.2 Methods

Primary

- Study personnel will include a neurological examination and clinical and observational tests, including Bayley Scales of Infant Development III, with subtests for cognition, motor skills, and language. Future neurological exams will be age-appropriate.

Secondary

- School record examination for grades/performance

5.3 Life Stage

- Primary/child: 1, 6, 12, and 18 months; 3, 5, 7, 9, 12, 16, and 20 years
- Secondary/child: Follow-up in year 7, 9, 12, 16, and 20

6. Important Confounders, Mediators, and Effect Modifiers

- **Child's exposure to neurotoxicants (e.g., lead) during childhood:** Increased exposure to other neurotoxins would increase adverse neurodevelopment behavior (Steenland et al., 1994; Calvert et al., 1998).
- **Family history:** Twin and family studies have suggested a genetic link, which may be shown through family histories, to neurodevelopmental disorders (Muhle, Trentacoste, & Rapin, 2004).
- **Mother's medical and obstetrical histories:** Particularly in cases without a family history, various obstetric complications are often cited as possible causes for fetal neurodevelopmental disruption and consequent disorders (Nelson & Willoughby, 2000).

- **Economic status:** Increased risk of preterm birth is associated with low educational level; unmarried status (Centers for Disease Control and Prevention, 1990).

- **Race/ethnicity:** As a percent of live births, 17.6 percent among blacks are preterm, 11.4 percent among Hispanics, and 10.8 percent among whites (U.S. Department of Health and Human Services, 2003.).

- **Mother's medical history:** Increased risk of preterm birth is associated with maternal smoking, alcohol consumption; older maternal age (indicated preterm births); younger maternal age (spontaneous preterm births); low or high parity; previous stillbirth (Heffner, Sherman, Speizer, & Weiss, 1993; Meis et al., 1995; Wisborg, Henriksen, Hedegaard, & Secher, 1996).

- **Function of endocrine disruptors:** The role that endocrine disruptors play in the relationship between maternal hypothyroidism and child neurodevelopment will be investigated. Measurements of endocrine disruptors will be made available through data collected for other NCS hypotheses (in particular, Hypothesis 5) and will allow for the examination of whether endocrine disruptors have an impact on maternal thyroid status during pregnancy and on neurodevelopment of the offspring.

- **Others:** Unwantedness of the pregnancy (Olsen et al., 1995; McDonald, Armstrong, & Sloan, 1992).

7. Power and Sample Size

Assuming 100,000 infants born into the Study with an exposure prevalence of 2 percent, a type I error of 0.05, and power of 0.8, the smallest detectable relative risk would be, for cerebral palsy, 2.8, and for autism, 2.4. Adjusted odds ratios for Bayley PDI are less than 85 for infants of women with free T4 values less than the tenth percentile at 12 weeks' gestation have been reported as 5.8 (95 percent confidence interval 1.3-12.6) (Pop et al., 1999). In Maine, 15 percent of children born to women with hypothyroidism measured in the second trimester scored below 85 on WISC full-scale IQ at a mean age of 8 years, compared to 5 percent of children of women who were euthyroid (Haddow et al., 1999). These studies, which suggest three-fold or greater risks of intellectual disabilities in the offspring of mothers with hypothyroidism indicate the Study will have the power to detect similar relative risks.

8. Other Design Issues

- **Cost/complexity of data collection:** Since the Study will collect maternal serum in the first and third trimesters of pregnancy and the children will be routinely followed for their cognitive and neurological development, this hypothesis could be addressed through data and specimens already included in the study plan.

- **Cost of sample analysis:** Analyses to assay TSH in maternal serum are inexpensive and can be automated. Free T4, however, is somewhat more complex and expensive to assay.

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NONPERSISTENT PESTICIDES AND POOR NEUROBEHAVIORAL AND COGNITIVE SKILLS

1. Broad Meta Hypothesis

Repeated, low-level exposure to nonpersistent pesticides, including carbamates, organophosphates, and pyrethroids, in utero or postnatally increases risk of poor performance on neurobehavioral and cognitive examinations during infancy and childhood.

2. Specific Hypotheses

1. After adjusting for potential confounders, exposure to nonpersistent insecticides will be associated with decrements in standardized tests of development, intelligence, language, motor ability, and visual-motor integration.

2. The risk of poor performance on neurobehavioral and cognitive examinations due to exposure to nonpersistent pesticide exposure will be more pronounced among children with genetically decreased paraoxonase activity.

3. Children with certain polymorphisms affecting paraoxonase activity (i.e., $PON1_{Q129}$) will be vulnerable to pesticide exposures at all ages while children without this polymorphism will be more susceptible to pesticide exposures during the first 6 months of life than at later ages (gene-by-exposure-by-age interaction).

3. Background and Justification

3.1 Public Health Importance

National survey data show that the general adult population has widespread exposure to pesticides as reflected by levels of urinary metabolites (Hill et al., 1995). In many settings, children have a greater opportunity for exposure because they have greater dermal contact with surfaces near the floor or ground and because of their greater hand-to-mouth activity (Moya, Bearer, & Etzel, 2004). Within existing literature, investigations of children's exposures to pesticides as reflected by the levels of urinary metabolites reveal surprisingly frequent exposure even in populations without agricultural exposure. Because children may be more sensitive than adults to low levels of neurotoxic substances and because pesticide exposure is generally widespread, concern about the potential health effects is warranted. Although recent regulations have decreased the use of organophosphate pesticides (e.g., chlorpyrifos) in homes, other pesticides with similar mechanisms are still used. Organophosphate pesticides are also used extensively in agriculture, and children's exposure from food sources is substantial (Curle, Fenske, & Elgethun, 2003). Members of the pyrethroid and organophosphate classes of synthetic insecticides have been identified as toxic to developing nervous systems (Olson, Blank, & Menton, 1998; Roy, Andrews, Seidler, & Slotkin, 1998; Weiss, 2000). Data from animal models and epidemiological studies suggest that even low levels of exposure during critical periods of development could cause subtle neurological effects in humans (Dam, Garcia, Seidler, & Slotkin, 1999; Auman, Seidler, & Slotkin, 2000; Dam et al., 2000; Rice & Barone, 2000; Shafer, Meyer, & Crofton, 2005; Kofman, Berger, Massarwa, Friedman & Jaffar, 2006). Experiments on animals show that low levels of organophosphate pesticides in utero or postnatally have subtle, detrimental, and permanent effects on behavior (Eskanazi, Bradman, & Castorina, 1999). Their toxicity owes to inhibition of cholinesterase (Brimijoin & Koenigsberger, 1999), an action shared by carbamate pesticides. The lowest level of organophosphate exposure used in these experiments

(Muto, Lobelle, Bidanset, & Wurpel, 1992) resulted in exposures within an order of magnitude of what humans experience in buildings where pesticides are used (Currie, McDonald, Chung, & Higgs, 1990).

Prevalence/incidence

It is assumed that the frequency of detectable exposure may vary with the specific pesticide being measured. A recent report detected chlorpyrifos, diazinon, and propoxur in 100 percent of personal air samples of pregnant urban women correlated significantly with maternal and cord plasma concentrations (Whyatt et al., 2003). The 1999 National Health and Nutrition Examination Survey (NHANES) indicated that more than 90 percent of a sample of the U.S. population had detectible levels of at least three metabolites of organophosphate insecticides in their blood or urine. The metabolites detected in the highest number of samples, diethylphosphate and diethylthiophosphate, are nonspecific metabolites of approximately 10 regularly used organophosphate insecticides.

Estimating the prevalence of poor cognitive development in children is a more difficult task. The definition of poor cognitive development can include cases of severe mental retardation to mild cases of attention deficit in the absence of hyperactivity. While mental retardation can be easily assessed using IQ testing, the diagnosis of more subtle developmental disabilities, such as hyperactivity and some autism spectrum disorders, makes a true national estimate of developmental disabilities in the United States difficult. In addition, the standards used to identify and classify developmental disabilities continue to change as more is understood regarding childhood cognitive development. Developmental disabilities affect approximately 17 percent of children younger than 18 years old in the United States and have resulted in substantial financial and social costs for affected families and educational and health care systems (Centers for Disease Control and Prevention [CDC], 2006).

Economic and/or social burden

Applying the methodology in Grosse, Matte, Schwartz, and Jackson (2002), a one IQ point loss in 90 percent of births results in $49 billion in annual cost in 2003 dollars, and lifetime savings from an IQ point gain of 0.5 percent of at-risk live births (2003) is about $294 million.

While no studies have precisely calculated costs associated with autism, a U.K. report estimates the lifetime custodial costs of autism spectrum disorders from $3 million to $4 million per child, with societal costs likely to be triple the individual estimate (Jarbrink & Knapp, 2001). The lifetime cost of mental retardation for persons born in 2000 was estimated at $51.2 billion (in 2003 dollars) (CDC, 2006). These costs are likely to underestimate the true cost of mental retardation since they are based on prevalence estimates obtained through only one CDC monitoring program.

3.2 Justification for a Large Prospective Longitudinal Study

Well-designed prospective, longitudinal studies of the impact of prenatal and postnatal insecticide exposure on child outcomes are critical in determining actual effects. It is likely that there are interactions between genotypes at multiple loci and that gene-by-environment and gene-by-environment-by-age interactions also exist. For example, the paraoxonase-1 (PON1) enzyme plays a role in detoxification of organophosphates but its levels are lower in neonates than in older children and adults (Cole et al., 2003), suggesting neonates have a reduced ability to detoxify organophosphates. In addition, individuals with a particular allele related to PON1 (i.e., $PON1_{Q129}$) may be more vulnerable to organophosphates at all ages. While all individuals without this allele are vulnerable to organophosphates during the neonatal period, they do not demonstrate this vulnerability later in life. Detecting these interactions will require larger sample sizes than studies to date. In addition, prospective studies will

allow for measurement of both prenatal and postnatal exposures prior to the development of adverse outcomes.

3.3 Scientific Merit

This study will help discover the effects of exposure to pesticides at various points in development from prenatal to early adulthood and possibly identify critical periods at which exposure is most damaging. The large sample size will allow for detection of subtle effects of exposure and gene-by-environment-by-age-at-exposure interactions. The nationally representative sample will help describe types of exposures by setting or geographic region and further elucidate and perhaps reveal exposure effects most damaging to development.

3.4 Potential for Innovative Research

This large, longitudinal study could lead to discovering the best markers of exposure, peak periods of exposure, and various aspects of neurodevelopment and behavior affected by exposure to pesticides with the possibility of determining very specific effects (e.g., contribution to learning disabilities). The size of the study also will enable investigators to study gene-by-environment and gene-by-environment-by-age interactions, allowing for the identification of subpopulations or age groups at increased risk from pesticide exposure.

3.5 Feasibility

This hypothesis fits within the general scope of the National Children's Study (NCS). Careful consideration of information gained by repeated environmental and biological measurements will be weighed against costs and participant burden. Recent investigations have demonstrated little within-home variability in a two-week average of indoor air pesticide concentrations, indicating that reducing the frequency of obtaining such samples can reduce the burden to NCS participants without sacrificing access to valuable data (Whyatt et al., 2007). The number of molecular techniques currently available to the study, combined with the number of environmental exposures to be measured, lends to the feasibility of investigating multiple types and sources of exposure to nonpersistent pesticides and outcomes related to poor cognitive development in children. Utilizing readily accessible molecular and genetic techniques to investigate gene-by-environment interactions is not only feasible, it can be beneficial and cost efficient. It is important to stress the need to utilize multiple approaches to assess pesticide exposure and not to rely on either biomarkers or physical indoor/outdoor air samples (Bradman & Whyatt, 2005).

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Pesticide concentration: Pesticide metabolites
- Genetic markers (e.g., paraoxonase gene)

Secondary/maternal

- Predictors of maternal pesticide exposure: Pesticide usage log, occupational data, food frequency questionnaire during pregnancy, food preparation patterns (washing fruits/vegetables or special cooking techniques)

Primary/paternal

- Some paternal measures (genetic markers such as paraoxonase activity [inheritable to child], pesticide usage log, occupational data)

Primary/child

- Child pesticide concentration: Pesticide metabolites in body fluid or concentrations in environmental media
- Child paraoxonase activity: Genetic markers of susceptibility

Secondary/child

- Predictors of child pesticide exposure: Food frequency diary, food preparation patterns (washing fruits/vegetables or special cooking techniques)

4.2 Methods

Primary/maternal

- Biological specimens: Blood, urine, breast milk
- Environmental samples: Air, dust, soil, water, food (residential/occupational)

Secondary/maternal

- Predictors of maternal pesticide exposure: Pesticide usage log, occupational data, food frequency questionnaire during pregnancy, food preparation patterns (washing fruits/vegetables or special cooking techniques)

Primary/child

- Biological specimens: Blood, urine
- Environmental samples: Air, dust, soil, water, food (microenvironments)

Secondary/child

- Interview/questionnaires with parent

4.3 Life Stage

Primary/maternal

- Repeated measures from preconception through birth
- Repeated measures from birth to age 1 (for milk collection)

Secondary/maternal

- Repeated measures from preconception through birth
- Repeated measures from birth to age 1 (for milk collection)

Primary/child

- Repeated measures from birth through age 7

Secondary/child

- Repeated measures from birth through age 21

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

- Poor neurobehavioral and cognitive performance

Secondary

- Poor academic performance (under cognitive performance)

5.2 Methods

Primary

- Examination by a trained professional (neurological and psychological testing): assessments selected will include those that evaluate broad areas of abilities (e.g., neurodevelopment, intelligence, behavior) as well as more specific skills (e.g., executive function, memory, reading, language, motor skills, and attention). At birth, general neurobehavioral functions will be assessed using the Neonatal Intensive Care Unit Network Neurobehavioral Scale, which is considered to be sensitive to toxic exposures. In early childhood, poor performance on the cognitive, language, and motor subscales of the Bayley III at 12 months will be used to identify delays in general cognitive development. The MacArthur-Bates CDI will be used at 12 months to assess delayed language development from parental report. Social competence and behavioral problems will be assessed at 12 months with the Brief Infant-Toddler Social and Emotional Assessment short form. The Modified Checklist for Autism in

Toddlers will be used for autism screening. For those instruments not yet specified, the location of the study visits will be considered when selecting the instrument to be used at each visit.

Secondary

- School record reviews will be used to demonstrate poor academic performances in children. Achievement testing, possibly age-appropriate sections of the Woodcock-Johnson test, may also be administered at multiple points in time to participating children to identify poor academic performance.

5.3 Life Stage

Primary

- Repeated from birth through 21 years with age-appropriate instruments

Secondary

- Repeated throughout childhood and adolescence with age-appropriate instruments

6. Important Confounders, Mediators, and Effect Modifiers

- **Genetically decreased paraoxonase activity and stored DNA:** Genetic polymorphisms of paraoxonase may decrease paraoxonase activity and increase risk for neurotoxic effects (Berkowitz et al., 2004). It is likely that additional, yet currently nonidentified, genes influence the metabolism of nonpersistent pesticides. DNA will be stored so gene-by-environment and gene-by-environment-by-age interactions can be investigated in the future.

- **Exposure to other neurotoxins such as lead, mercury, and persistent pesticides:** Increased exposure to other neurotoxins would increase adverse neurodevelopment and behavior (Steenland et al., 1994; Calver et al., 1998).

- **Residential and daycare environment:** Home environment is one of the most prominent factors in children's intellectual and behavioral development. Rural living and size of farm is associated with decreased performance on neurobehavioral test batteries (e.g., digit span, Benton visual retention, simple reaction time) (Van Wijngaarden, 2003; Misra, Prasad, & Pandey, 1994). Although pesticide exposures could cause these associations, it is possible other factors analogous with rural lifestyle may be causing these differences.

- **Prenatal and postnatal nutrition:** Folate and vitamin D deficiency have been implicated in adverse neurodevelopmental outcomes. Caffeine and tobacco consumption during pregnancy may be associated with neuropsychological functions and behavioral problems (e.g., ADHD).

- **Socioeconomic status:** Low socioeconomic status, including low levels of parental education, is the strongest and most consistently identified risk factor associated with poor performance across all cognitive domains.

- **Prenatal and recent infection and cytokine response:** Prenatal infection and cytokine concentrations are associated with neurodevelopmental disabilities and will need to be controlled for the analysis (also see Prenatal Infection and Schizophrenia and Prenatal Infection and Neurobehavioral Disabilities hypotheses).

7. Power and Sample Size

The example given above for the relationship between the presence of the $PON1_{Q129}$ allele (occurs at a rate of 0.3 to 0.75 among the general population) (Furlong, Richter, Seidel, & Mutulsky, 1988; Costa et al., 2003) organophosphate (OP) exposure and age may be hypothesized to affect IQ in the following direction:

Table 1. Mean IQ Scores by Presence of $PON1_{Q129}$ allele, Organophosphate Exposure, and Age at Exposure

$PON1_{Q129}$	Organophosphate	Neonate	Older	Totals
Present	Exposed	↓↓↓	↓↓↓	↓↓↓
	Non-exposed	100	100	100
Absent	Exposed	↓↓↓	↓	↓↓
	Non-exposed	100	100	100

To simplify the sample size calculation, we used the presence of the $PON1_{Q129}$ gene (present/absent) and a four-level variable exposure: 1) as a neonate, 2) as an older child, 3) as a neonate and an older child, or 4) not exposed. Loss to follow-up was assumed to be 2 percent per year and assessment age 18 was used. It is estimated that approximately 70,000 children will still be in the Study at 18. The minimum difference that can be reliably detected is 0.53 IQ units with a 30 percent exposure rate. The minimum detectable difference will be smallest if 50 percent of children are exposed (0.49) and bigger if the percentage is close to 0 percent or 100 percent (minimum effect size is 0.82 if 10 percent are exposed).

For a two-way analysis of variance model with an interaction, the minimum interaction that can be reliably detected is about twice that for a main effect. The minimum interaction that could be reliably detected would be 1.11 IQ units assuming that 30 percent are exposed as neonates, 20 percent are first exposed when older, and 50 percent are not exposed.

These results indicate that if the proportion exposed is not too close to 0 percent or 100 percent, the minimum detectable difference will be between 0.5 and 1.0 IQ units. For the interaction of the allele and exposure, if the proportion of the population with the allele is not too close to 0 percent or 100 percent, an interaction of between 1.0 and 2.0 IQ units can be reliably detected.

8. Other Design Issues

- **Ethical/burden considerations:** The study will have a formal process for effectively communicating results of physiological and biochemical measures to the child's parents and a responsible health care provider; and the results of environmental monitoring to the child's parents along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings.

- **Repeated tests are potentially burdensome:** The study will have a protocol for reporting neurodevelopmental and behavioral test results to primary care providers when they suggest significant developmental delay or behavioral disorder and may indicate need for more detailed assessment or intervention services.

- **Cost/complexity of data collection:** Potentially time consuming and costly instruments to assess exposures and outcomes in later stages of the study will be selected on the basis of providing most accurate data while maximizing retention rate through minimization of subject burden.

- **Cost of sample analysis:** Some tests are costly and repeated assessments can add to the overall cost. The use of nested case-control design for some outcomes will reduce expenses.

- **Need for community involvement:** Daycare and school cooperation will be required for some of the intended measures.

- **Training on proper administration of neurobehavioral assessments:** Rigorous training will guarantee all individuals are tested properly according to the Study protocol. Provisions will need to be made for training, certification, and annual recertification of examiners. Given the magnitude of the Study, training efforts may be costly and time consuming but are imperative to ensure quality data.

- Other potential measurement issues that could be investigated:

  - Body burdens or excretion rates of nonpersistent insecticides or their metabolites in children are directly related to insecticide concentrations in environmental exposure media, and these relationships are modified by human behaviors.

  - Longitudinal insecticide exposure for individual children can be ascertained from repeated short-term sampling of noninvasive biological markers of exposure (e.g., insecticide metabolites in urine).

  - Longitudinal insecticide exposure for individual children can be estimated from repeated (e.g., annual) residential exposure media (e.g., settled dust indoors) and longitudinal questionnaire/diary information on residential pest pressure, residential insecticide use, microactivity patterns, and food consumption.

- Long-term (e.g., annual) exposure to nonpersistent insecticides varies among groups of children defined by age and other demographic parameters.

- Long-term exposure to nonpersistent insecticides for populations of children can be estimated from personal exposure information obtained from representative samples of individual children.

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PRENATAL INFECTION AND NEURODEVELOPMENTAL DISABILITIES

1. Meta Hypotheses

Prenatal infection and mediators of inflammation are risk factors for neurodevelopmental disabilities such as cerebral palsy and autism.

2. Specific Hypotheses

1. The risk of neurodevelopmental disabilities will vary depending on the type of infection (i.e., chorioamnionitis, etc.) and timing of infection during gestation.

2. Maternal changes in cytokines in response to infection will predict the risk of neurodevelopmental disabilities.

3. The risk of neurodevelopmental disabilities due to exposure to infection will be influenced by obstetric complications and genetic risk factors.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Cerebral palsy affects as many as 0.3 percent (Bhasin, Brocksen, Avchen, & Van Naarden, 2006; Kuban & Leviton, 1994) and autism affects about 0.7 percent of children (Centers for Disease Control and Prevention [CDC], 2007; Yeargin-Allsopp et al., 2003). Whether the frequency of autism is increasing is controversial. Recent estimates of higher prevalence may be due to inclusion of less severe cases, better identification, or other disorders that may now be classified as autism whereas previously they were not (e.g., intellectual disabilities).

Seizures, reduced intellectual and communication skills, and increased injuries can co-occur with cerebral palsy. Fractures are prevalent in children with cerebral palsy, and repeated fractures are common. They diminish the quality of life and add to the care requirements for these children (Henderson et al., 2002). Increased mortality from seizures, injuries, and respiratory diseases is observed among persons with autism, and, although mortality is especially increased among persons with accompanying severe intellectual disabilities, life expectancy is also reduced for persons with mild intellectual disabilities (Shavelle, Strauss, & Pickett, 2001).

Literature findings suggest prenatal exposure to infection is a risk factor for cerebral palsy, autism, and other neuropsychiatric disorders (Meyer et al., 2006; Nelson & Willoughby, 2002). Chorioamnionitis is thought to play a role in the development of cerebral palsy, and approximately 1-2 percent of term pregnancies are affected by chorioamnionitis. Pregnancies ending in preterm births have an even higher prevalence of such infection (Wu et al., 2003).

Fetal inflammatory response to chorioamnionitis (intrauterine infection) includes increased levels of fetal cytokines, which can be neurotoxic (Yoon et al., 1998; Dammann & Leviton, 1998). While few studies of chorioamnionitis and cerebral palsy among children born at term have been done (Grether & Nelson, 1997; Nelson & Ellenberg, 1985), it has been estimated that about 28 percent of cerebral palsy

in preterm infants and 12 percent of cerebral palsy in term infants may be due to chorioamnionitis (Wu & Colford, 2000). A study by Wu et al. (2003) observed a four-fold increased risk (adjusted) of cerebral palsy in subjects with diagnosed chorioamnionitis. Periodontal disease is also a source of infection during pregnancy and may be associated with adverse pregnancy outcomes due to metastatic spread of infection, metastatic injury from circulating microbial toxins, or metastatic inflammation induced by microorganisms (Li, Kolltveit, Tronstad, & Olsen, 2000).

Another recent study, which found an association between fetal herpes group B exposure and cerebral palsy, found a prevalence of neurotropic viral nucleic acids from any herpes virus (group A and B) to be 38.3 percent (95 percent confidence interval: 34.8 to 41.9) in stored neonatal blood spots from a control population (Gibson et al., 2006a). The odds ratio for the association between herpes exposure and any type of cerebral palsy was 1.52 (1.09 to 2.13).

Economic and/or social burden

Cerebral palsy and autism are serious developmental disabilities that have a dramatic effect on the lives of the affected persons and their families. The lifetime economic costs of cerebral palsy have been estimated at $11.5 billion per annual cohort (CDC, 2004). A U.K. report estimates the lifetime custodial costs of autism spectrum disorders in the range of $3 million to $4 million per child (Bradstreet, 2002; Jarbrink & Knapp, 2001). The estimated annual monetary cost of autism in the United States is $26 billion (Maltby, 2000) with lifetime incremental societal costs per capita estimated at $3.2 million. Lost productivity and adult care are the largest components of these costs (Ganz, 2007). Estimates of dollars currently spent on special education annually are from $30.9 billion to about $34.8 billion (National Center for Education Statistics, 1998). Research indicates early identification of and interventions for children with autism using behavioral modification programs would lead to significant cost savings (Jacobson, Mulick, & Green, 1998).

3.2 Justification for a Large Prospective Longitudinal Study

Investigation of the influence of prenatal infection or mediators of inflammation on the origins of neurodevelopmental disabilities such as cerebral palsy and autism requires a large prospective cohort that has been followed from early gestation through childhood.

A prospective study is required to obtain prenatal exposure data. For the exposures of interest (e.g., prenatal infection), biological specimens are essential to obtain data during early and late gestation. Although case-control studies have shown an association between cerebral palsy and either recall of prenatal viral infection or presence of viral nucleic acids at birth, there are no prospective studies that have investigated the influence of the timing of the infection, whether the infection is primary or recurrent, the trajectory of the immune response to the infection, or genetic factors that may influence the risk of adverse outcomes of the trajectory in the immune system. To conserve specimens and reduce cost, a nested case-control design will be employed when assessing viral nucleic acids.

The longitudinal design is required because it is important to trace the development of autism through childhood. Identifying and validating early predictors of autism may lead to significant improvement in outcomes.

A large cohort is required to study infrequent outcomes such as autism and the possible gene-by-environment interactions. Using a nested case-control approach, the National Children's Study (NCS) can be used to identify genes associated with autism that are conditional upon infection or inflammatory responses to infection.

3.3 Scientific Merit

Herpes viruses and enteroviruses can cross the placenta and infect the fetus. These neurotropic viruses can either contribute directly to the causation of cerebral palsy or indirectly by increasing proinflammatory cytokines that may adversely affect the developing brain (Cai, Lin, Pang, & Rhodes, 2004). An association between perinatal viral exposure, as determined by detection of specific viral nucleic acids in newborn blood samples, and the risk of developing cerebral palsy has recently been reported (Gibson et al., 2006a). Children with specific cytokine polymorphisms (i.e., tumor necrosis factor-alpha [TNF-∀] and mannose-binding lectin) are at a greater risk of developing cerebral palsy than children without the polymorphisms (Gibson et al., 2006b). These polymorphisms are associated with a greater immune response to infection.

While there are some data on the relation of viral infections in pregnancy to occurrence of autism, few causal agents have been established. One specific infection, rubella, has been commonly associated with autism spectrum disorders (Rodier & Hyman, 1998). The general relationship of prenatal infection, such as chorioamnionitis, and mediators of inflammation to risk of autism has not been well studied. Such studies are overdue because of the role immune abnormalities may play in autism and the increased knowledge of the neurotoxicity of inflammatory cytokines (Meyer et al., 2006).

The genetic basis for autism is covered in greater detail in a separate hypothesis (see "Gene-Environment Interactions"). The focused research efforts and an intense search for genes associated with autism have not identified a gene with a large effect, but linkage scans have identified some overlap of regions harboring many genes with some related to the central nervous system (Muhle, Trencoste, & Rapin, 2004). The heterogeneity of findings across genetic studies of autism may be due to unidentified gene-by-environment interactions that may be masking some of these effects.

The risk of neurodevelopmental disabilities resulting from infection is determined, in part, by the specific type of infection (i.e., viral, bacterial, etc.), the gestational age of the fetus at the time of infection, whether the maternal infection is primary or recurrent, and possibly an inherited predisposition that makes the fetus more susceptible to infection or inflammation. Prospective studies are needed to determine timing of the specific maternal infection, the gestational age at the time of infection, whether the infection is primary or recurrent, and genetic predisposition. In addition, there is increasing evidence neurodevelopmental disorders such as cerebral palsy can result from subclinical maternal infection that cannot be identified retrospectively (Schendel, 2001).

3.4 Potential for Innovative Research

The technologies used in the assessment of relevant prenatal exposures will include serologic and viral nucleic acid analyses, including measurement of inflammatory cytokines, and are common to other research hypotheses within the NCS. Each assessment during pregnancy provides a window on fetal exposure and holds the potential to improve our knowledge and understanding of the identity, timing, and mechanisms of exposures adversely influencing neurodevelopment and increasing risk for cerebral palsy and autism.

In addition to basic serologic analyses for specific infections (e.g., influenza, HSV2), high throughput methods will be used to detect and quantitate viral nucleic acids, antiviral antibodies, and cytokine response. This will permit the simultaneous measurement of multiple analytes using small amounts of sera. The use of nested case-control design will allow this to be done in a cost-effective manner. No human studies have been reported that investigated longitudinal changes or timing of prenatal infection and immune response and how these changes or timing influence the risk of neurological outcomes of the infant.

3.5 Feasibility

Due to the size and longitudinal nature of the Study, these hypotheses can be addressed. Collection of biological samples during the first, second, and third trimesters will allow investigation of longitudinal changes in infection and immune response and determine whether the gestational age at the time of exposure to infection or cytokines has a significant impact on these neurobehavioral outcomes. Obtaining family history and genetic material also will allow for the determination of genetic susceptibility to infection and inflammatory response and whether this genetic susceptibility modifies the risk of neurodevelopmental disabilities resulting from exposure to infection. The burden of sample collection at multiple time points during pregnancy is feasible, and the use of a nested case-control design is cost-effective.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers)
- Blood for viral nucleic acids. The specific type of viral infection is thought to modify the risk of neurological disorders (Gibson et al., 2006a)
- Medical history of fever and infection (medicine usage)
- Dental exams

Primary/child

- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers)
- Umbilical cord/placental (antibodies, cytokines, viral nucleic acids)

Secondary/maternal

- Retrospective medical records reviews

4.2 Methods

Primary/maternal

- Blood samples
- Vaginal/cervical cultures
- Examinations by a medical professional
- Interviews

Secondary/maternal

- Medical and obstetrical history
- Family history

Primary/child

- Umbilical cord blood culture/pathology

4.3 Life Stage

Primary/maternal

- Repeated measures at early and late gestation

Secondary/maternal

- Repeated measures at early and late gestation

Primary/child

- Birth

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

- Neurological development

Secondary

- Parental assessment and screening for child's autistic traits (e.g., modified-checklist for autism in toddlers)

5.2 Methods

Primary

- Direct observation by medical professional: Fetal ultrasound, neurological exam, autism screening and diagnostic tests (e.g., autism diagnostic observation schedule)

Secondary

- Parental and perhaps teacher screening and assessment for autistic traits in child (e.g., modified checklist for autism in toddlers, autism diagnostic interview-revised)

5.3 Life Stage

Primary

- Prenatal through year 7

Secondary

- Follow-up in year 7 and older

6. Important Confounders, Mediators, and Effect Modifiers

- **Family history of psychiatric and neurodevelopmental disorders:** Children of parents with a history of psychiatric disorders (including schizophrenia-like psychosis or affective disorders) may be at increased risk of autism (Larsson et al., 2005). Twin and family studies have suggested a genetic component to neurodevelopmental disorders, which may be determined by assessing family histories (Muhle et al., 2004).

- **Maternal and infant genetic polymorphisms:** Genetic polymorphisms have been identified that influence the concentration of circulating cytokines in response to a viral infection (Gibson et al., 2006b). Therefore, it is expected the presence of specific polymorphisms or genes may modify the risk of exposure to infectious agents.

- **Gestational age:** Studies have shown the relationship between viral type and neurological disorders varies depending upon whether the infant was born preterm or term and small for gestational age (Larsson et al., 2005).

- **Mother's medical and obstetrical histories:** Various obstetric complications are often cited as possible causes for fetal neurodevelopmental disruption and consequent disorders (Nelson & Willoughby, 2002). Increased risk for autism has been associated with specific breech presentation and low Apgar score at 5 minutes (Larsson et al., 2005).

- **Environmental exposures and drug use during pregnancy:** Maternal smoking (Hultman, Sparen, & Cnattingius, 2002) and maternal drug usage (Rodier & Hyman, 1998; Williams et al., 2001) have been implicated as risk factors for autism. Prenatal use of thalidomide early in gestation was correlated with increased risk for autism, implicating xenobiotics as a possible factor in the pathway to autism (Newschaffer, Fallin, & Lee, 2002). Prenatal use of valproic acid and other anticonvulsants has been associated with increased risk for autism in animal models (Rodier & Hyman, 1998) and reported in case studies (Palmer, Blanchard, Stein, Mandell & Miller, 2006). Although heavy metals such as mercury have been implicated as risk factors for autism, the evidence for a relationship between low-dose exposures to methylmercury are conflicting (Palmer et al., 2006).

- **Parental age:** Maternal and paternal age may be risk factors for autism. For every 10-year increase in parental age, the relative risk for autism spectrum disorders increases by 28 percent (paternal) to 31 percent (maternal) (Croen, Najjar, Fireman, & Grether, 2007). Children born to men 40 years or older were 5.75 times more likely to have an

autism spectrum disorder compared to children with fathers younger than 30 (Reichenberg et al., 2006).

7. Power and Sample Size

Assuming 100,000 infants are born into the Study, with an exposure prevalence of 2 percent, the smallest detectable relative risk would be 2.8 for cerebral palsy and 2.4 for autism.

8. Other Design Issues

Retention of index children at least to or beyond the average age of diagnosis for these disorders (e.g., age 6 or 7 for autism) will be important to address this hypothesis with sufficient power. It is important to collect maternal serum and umbilical cord blood samples or blood samples taken early in infancy to measure cytokine concentrations. This will require coordination with medical professionals to ensure the collection of these samples.

Because the contacts with these patients will essentially fall within the scope of standard of care during the pregnancy, additional cost will be relatively minor and entail maintaining the pregnancy and infant/childhood database and the tissue sample repositories.

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GENE-ENVIRONMENT INTERACTIONS AND BEHAVIOR

1. Meta Hypothesis

Exposures to adverse psychosocial, chemical, and physical environments and other stressors during vulnerable periods of pregnancy and early childhood can interact with genotype to cause or modulate behavioral problems in childhood.

2. Specific Hypotheses

Based on the literature, specific hypotheses about gene-environment interactions and behavior can be formulated around the neurotransmitter systems and modulation by hormonal and other factors often used to account for etiology and pharmacological treatments of psychiatric disorders, including depression and serotonin, ADHD (and schizophrenia) and dopamine, and conduct (and anxiety) disorders and norepinephrine.

1. The neurotransmitter serotonin has been associated with depression in animal studies with primates and in clinical studies of pharmacologic treatment with specific serotonin reuptake inhibitors.

    1.1 Children with one or two copies of the short allele of the 5-HTT promoter polymorphism will be more likely to exhibit depressive symptoms when exposed to stressful life events than those with the same genotype, but without stressful life events.

    1.2 Children homozygous for the long variation in the 5-HTTLPR serotonin transporter gene will be at a greater risk for developing alcoholism in adolescence if they are also exposed to early life stress than those who have this same allelic variant coupled with low early life stress.

    1.3 Children with the l/s form of the 5-HTT gene will exhibit abnormal impulsivity and aggression when their early rearing environment is stressful compared with children who have the same genotype but are reared in a low-stress environment.

2. The neurotransmitter norepinephrine has been associated with antisocial and anxious behavior in animal studies and pharmacologic treatment of conduct and anxiety disorders. Based on this literature, an example of this hypothesis is that children with low monoamine oxidase A (MAOA) activity who are maltreated will be more likely to exhibit antisocial behavior than children with low MAOA who are not maltreated or maltreated children with high MAOA activity.

3. The neurotransmitter dopamine has been associated with ADHD in animal and clinical studies. Psychostimulants are known to affect dopaminergic systems and thus dopamine-related genes make plausible candidates for studies investigating gene-environment effects on ADHD. Several environmental toxicants affect dopamine as well. Example hypotheses include:

    3.1 Children exposed to prenatal tobacco smoke who are homozygous for the dopamine transporter (DAT) gene 480-bp allele are at greater risk for hyperactive-impulsive behaviors than children who have either smoke exposure or the DAT genotype alone.

3.2 Children exposed to maternal insensitivity show increased oppositional and aggressive behavior, but only if they possess at least one DRD4 allele 7-repeat allele.

4. For other psychiatric disorders without a standard pharmacologic intervention, such as autism, this candidate gene or candidate pathway approach has not been as useful. However, the serotonin transporter gene (SLC6A4) and the promoter polymorphism (5-HTTLPR) have been implicated. Differences in phenotype within and across autism genetic studies have contributed to heterogeneity of findings, with over transmission of the short allele in some studies and the long allele in others. Also, the variability of effects may depend on specific environmental conditions that interact to mask association or linkage of autism and SLC6A4. Using a nested case-control approach, the Study can be used to identify genes associated with autism conditional upon environmental factors (i.e., chemical and biological exposures) that emerge in this and other studies currently underway.

3. Background and Justification

Psychiatric disorders are responsible for significant burden related to disabilities of children and adults in the United States. The prevalence of psychosocial dysfunction in school-aged and preschool-aged children presenting for primary pediatric care is estimated to be 12 percent and 14 percent, respectively (Jellinek et al., 1999).

The effects of early exposures on adult outcomes have been addressed by Developmental Origins of Health and Disease (Gillman, 2005). This organization evolved from focused studies that followed an insightful observation of an association of birth weight and cardiovascular disease (Barker & Osmond, 1986), and its direction and purpose have been described by Gluckman, Hanson, & Pinal (2005) with a focus on obesity, insulin resistance, and other physical conditions. Similar observations have been made about psychiatric disorders, with reports of an association between low birth weight and/or premature birth and a variety of disorders, including ADHD (Lou, 1996; Linnet et al., 2006). A leading account of early origins is based on the concept of adaptive fetal or infant responses that produce "thrifty phenotypes." These adaptive responses are assumed to have an epigenetic basis (Gluckman et al, 2005). This assumption can be evaluated with the use of emerging epigenetic measures such as genome-wide methylation scans (Callinan & Feinberg, 2006) that allow for the evaluation of imprinting of genes related to some psychiatric disorders, (Luedi, Hartemink, & Jirtle, 2005), including autism (Lamb et al., 2005), schizophrenia (Francks et al., 2003; DeLisi et al., 2002), and Tourette's Disorder (Simonic et al., 2001).

Maternal genotype also has been evaluated in terms of enzymes that affect fetal exposure to toxic substances. For example, the maternal genes in the detoxification pathways related to tobacco smoke (Van Rooij et al, 2001; Shi et al., 2007) and alcohol (Jacobson et al., 2006) affect exposure of the fetus to the teratogenic effects of these substances. Both maternal and fetal genotypes may play a significant role in moderating the effects of environmental conditions that have a relatively high prevalence, such as maternal smoking (about 20 percent) and alcohol consumption (20 percent).

Research in animal models has demonstrated the influence of mothering on gene expression (Rutter, 2007). This illustrates that knowing the genotype is not sufficient for predicting phenotypic expression without additional knowledge of the psychosocial environment (Meyer, Palchaudhuri, Scheinin, & Flugge, 2000). Intracellular and extracellular environments play an important role not only in the magnitude of expression but also in the direction of expression (i.e., whether it is up- or down-regulated). The intracellular environment is a function of its genetic components and influences from extracellular factors, such as hormones, neurotransmitters, cytokines, and nutrients (Meaney, 2001b).

Variation in these constituents can determine how and when a gene is functionally expressed. Psychosocial factors are involved in these interactions through their influence on hormones, neurotransmitters, and nutrient intakes.

Several recent studies suggest genetic predisposition and psychosocial stressors interact to shape neurobehavioral outcomes. Seminal studies based on the Dunedin birth cohort of 1,000 children, (Caspi et al., 2003) found a serotonin transporter polymorphism moderated the influence of stressful life events on depressive symptoms, depression, and suicide. They also found a functional polymorphism in the MAOA gene moderated the effect of child maltreatment on subsequent antisocial behaviors and criminality (Caspi et al., 2002). Results from initial replication studies have been mixed, though study design and exposure assessments have varied (Dick et al., 2007; Haberstick et al., 2005; Young et al., 2006). This is not unexpected, because the size of the critical subgroups (defined by extreme placement in the genotype-exposure combinations) was small (i.e., approximately 20 to 30). These early mixed findings highlight the need for a definitive, large, prospective study to address these research questions. The NCS will provide a birth cohort that may produce critical subgroups of 200-300.

In the first published molecular genetic studies of ADHD, the candidate gene approach was used and statistical association was documented for the dopamine transporter (DAT) gene (Cook et al., 1995) and the dopamine receptor type 4 (DRD4) gene (LaHoste et al., 1996). These candidate genes were chosen based on dopamine theories of ADHD and the sites of action of drugs used to treat ADHD (Swanson et al., 2000). Cook et al. (1995) investigated parent-to-child transmission rates of the DAT alleles and reported an increased prevalence (0.85) and transmission (0.60) of the most prevalent 10R-repeat allele in a sample of 119 ADHD children. LaHoste et al. observed a higher than expected frequency of the DRD4 7R allele (0.28) in a group of ADHD cases, and Swanson et al. (1998) replicated this finding and extended it by showing linkage-disequilibrium in proband-parent triads. A recent meta-analysis confirmed the association of ADHD with alleles of the DRD4 gene and suggested association of ADHD with another dopamine gene, DRD5 (Li, Sham, Owen, & Lin, 2006). This meta-analysis did not confirm an association of ADHD with the allele of the 40 bp variable number tandem repeat (VNTR) of the DAT gene, suggesting this allele may not be associated with ADHD, may be in linkage disequilibrium, or interact with another polymorphism nearby. Another possibility is that the conflicting results arise from a gene-environment interaction such that the gene creates susceptibility and the environmental risk factor promotes expression. Other meta-analyses have reviewed the limited evidence of association of ADHD with non-dopamine genes (Faraone, Doyle, Mick, & Biederman, 2001). The failure to detect a strong signal does not discount the existence of genes with high risk alleles, of multiple genes that combine to confer ADHD risk, or of genes with effects dependent on interactions with environmental factors. Signals for genes in several different loci have been reported (Ogdie et al., 2003; Arcos-Burgos et al., 2004). Brookes et al. (2006) described a combination of candidate gene and genome scan approaches and confirmed association of ADHD with the DRD4 and DAT genes and also provided suggestive evidence of association of ADHD with 16 other genes. A large, longitudinal birth cohort study, such as the NCS, with multiple environmental exposures will provide a much needed database to facilitate resolution of these issues.

The recognition of autism has increased during the past few decades from a prevalence of less than one per 1,000 to more than six per 1,000 children (Centers for Disease Control and Prevention [CDC], 2007). Focused research efforts and an intense search for genes associated with autism have not identified a gene with a large effect, but linkage scans have identified some overlap of regions harboring many genes with some related to the central nervous system (Muhle, Trentcoste, & Rapin, 2004). Proposals (Herbert et al., 2006) and studies (Hertz-Picciotto et al., 2006; Hu, Frank, Heine, & Lee, 2006) have recently incorporated the evaluation of environmental influences, which has redirected efforts to address environmentally sensitive genes (Nickerson et al., 2005). The Hu et al. study of gene expression was based on only five monozygotic twins discordant for autism. This approach of using highly

informative cases can be extended using the systems biology approach to track perturbations, as described by Hood, Heath, Phelps, & Lin, (2004), related to regression of autism. Longitudinal samples obtained within the Study will allow investigators to evaluate cases using RNA from serial samples taken on the same child during normal development and after the regression of autism occurs. The CHARGE study described by Hertz-Picciotto et al. is a large, ongoing case-control study of 500 autism cases focusing on chemical and biological exposures. Using a nested case-control approach, the NCS can be used to identify genes associated with autism conditional upon environmental factors (i.e., chemical and biological exposures) that emerge in the CHARGE study and other current studies.

3.1 Public Health Importance

Prevalence/incidence

Major depressive disorder affects as many as 2.5 percent of children and 8.3 percent of adolescents, an age group in which the female-to-male ratio of depression is 2:1 (Birmaher et al., 1996). On a commentary on the mental illness epidemic among teens, Friedman noted that in 2005, the CDC estimated 17 percent of U.S. high school students seriously considered suicide and 8 percent attempted suicide at least once during the preceding year (Friedman, 2006). Physical violence also is a serious problem in adolescents. In 2003, 17 percent of American high school students carried a weapon (gun, knife, or club) and 33 percent were involved in a physical fight (CDC, 2004). The Study provides an opportunity to examine etiological factors and gene-environment interactions in the development of psychosocial problems, such as depression and violent behavior.

Childhood onset disorders such as autism and ADHD also offer opportunities for the investigation of gene-environment interactions. The prevalence of autism is low, but its recognition and perhaps even prevalence has been increasing. The 2002 prevalence estimate from the Autism and Developmental Disabilities Monitoring Network indicates autism now affects about 1 out of every 150 children in the United States (CDC, 2007). The prevalence of ADHD is much higher, and its recognition and treatment have been increasing for decades (Swanson, Lerner, & Williams, 1995; Swanson et al., 2007). An estimated 8.7 percent (7.3-10.1 percent) of U.S. children aged 8 to 15 years meet DSM-IV criteria for ADHD, and almost 15 percent of 10-year-old boys and 5 percent of 10-year-old girls carry this label (CDC, 2005).

Economic and/or social burden

According to the Global Burden of Disease Study, depression is the fourth most important cause of death and disability (Murray & Lopez, 1997; Holden, 2000). The worst outcome of depression is suicide, which accounts for 6.8 percent of all deaths in 10- to 14-year-olds and 11.9 percent of deaths in 15- to 19-year-olds (Arias, MacDorman, Strobino, & Guyer, 2003). The annual cost of depression in the United States ranged between $43.7 billion and $52.9 billion in 1990. Adjusted for inflation, this estimate would be close to $70 billion today (Greenberg, Leong, Birnbaum, & Robinson, 2003). Due to its high prevalence, the total cost attributed to ADHD is high. A recent analysis estimated the societal cost for ADHD in childhood and adolescence is between $36 billion and $52 billion annually. Autism, which has a low prevalence, also has significant economic impact and social burden. This has been recognized by special NIH programs that focus on the causes of autism and legislation that gives priority for investigations in this area.

3.2 Justification for a Large Prospective Longitudinal Study

A prospective study is required because the exposures in question precede the outcomes and because these exposures influence gene expression at different time points. The prospective design also provides more accurate and timely exposure measurements relative to subclinical and clinical outcomes.

A longitudinal design is required because it is important to trace the development of the cohort through childhood and adolescence to measure the neurobehavioral outcomes of relevance. Several reports from smaller cohorts have indicated childhood antecedents (or perhaps risk factors) can predate symptom development by long periods. In addition, the interactions under investigation require data on prenatal and early childhood exposures.

Gene expression is subject to multiple environmental influences: social connections (Meany, 2001), nutrition (Waterland & Jirtle, 2003), and toxic exposures (Jacobson, 2006; Braun, Kahn, Froelich, Auinger, & Lamphear, 2006). A large cohort is needed to provide enough power for the candidate gene approach to studying these neurobehavioral outcomes while accounting for multiple environmental influences and interactions. The use of a large cohort enables the examination of gene-environment and gene-gene interactions.

3.3 Scientific Merit

Animal research on genes associated with behavior and on the modification of gene expression by psychosocial factors has been accumulating for a number of years. This research covers a fairly broad range of factors from physiological risk to behavioral traits and has emphasized the extent to which the functional importance of a gene is determined by factors which influence expression. Intracellular and extracellular environments play an important role not only in the magnitude of expression but in the direction of expression (i.e., whether it is up- or down-regulated) (Meaney, 2001). The intracellular environment is a function not only of its genetic components but also of influences from the extracellular factors such as hormones, neurotransmitters, cytokines, and nutrients. Variation in these constituents can determine how and when a gene is functionally expressed. Psychosocial factors are involved in these interactions through their influence on hormones and neurotransmitters. This can be illustrated by the influence of stress on transcription factors. Glucocorticoids such as cortisol are part of the hormonal response to stress.

The transcription factors c-jun and c-fos are sequence-specific DNA-binding proteins that bind to DNA in a multiprotein complex that controls cell proliferation and growth. These transcription factors are extremely responsive to extracellular stimuli and have been shown to influence glucocorticoid receptor-induced transcription of proliferin, causing it to increase or decrease according to their presence together or alone in the surrounding environment (Diamond, Miner, Yoshinaga, & Yamamoto, 1990). Research in rats has demonstrated immobilization stress (physically restraining the animal) upregulates both of these transcription factors in endothelial, myocardial, and smooth muscle cells of coronary vessels (Ueyama, Yoshida, & Senba, 1999). Similarly, it has been demonstrated that immobilization stress influences gene expression in the hippocampus in rats, causing increased expression of corticotropin-releasing hormone mRNA and a decrease in 5-$HT1_a$ mRNA levels in the dentate gyrus (Givalois, Arancibia, & Tapia-Arancibia, 2000; Lopez, Lierzon, Vazquez, Young, & Watson, 1999). These latter data complement other research that has demonstrated serotonin polymorphisms differentially influence heart rate and blood pressure responsivity to stress (Barr et al., 2003) by showing stress also influences the functional expression of serotonin polymorphisms. Similar interactions have been demonstrated for the $alpha_2$-adrenoceptor variants, which influence the magnitude of stress responsivity. Animal research shows the functional expression of this gene is itself influenced by stress (Meyer et al., 2000). In addition, stress-induced expression of corticosteroid receptors has been demonstrated to vary by gender

(Karandrea, Kittas, & Kitraki, 2000). Together, these data illustrate the complexity and ongoing nature of gene-environment interactions, emphasizing that "genes or environment" is not a meaningful question. The presence of a linked allele at a locus is an indication of risk, but it is not enough to predict functional expression. Understanding function requires knowledge of the factors that influence when and in what direction it is expressed.

The Study's core hypothesis addressing the impact of maternal stress during pregnancy and risk of asthma states that excessive maternal psychosocial stress during pregnancy, in conjunction with maternal and fetal genetic susceptibilities, is reflected in specific measures of biologic function and results in an altered trajectory of fetal growth and development. During a Study-related workshop centered on this hypothesis, one of the mechanisms postulated for this influence was gene-environment interactions associated with gene expression (Gluckman et al., 2005; Gillman et al., 2006). Research on nonhuman primates has demonstrated the influence of mothering on gene expression. Research in Rhesus monkeys has demonstrated a short form of the serotonin transporter (rh5-HTTLPR) gene is associated with drinking alcohol to excess in monkeys reared in an environment with same-age peers and no mother, indicating a genetic link to alcohol abuse (Barr et al., 2003). However, monkeys with this same genotype reared together with their mothers actually consume less alcohol, indicating this same polymorphism under different circumstances confers a protective effect. These data further illustrate that knowing the genotype is not sufficient for predicting phenotypic expression without additional knowledge of the psychosocial environment.

Many diseases of interest to the NCS have complex etiologies and pathogenesis involving multiple genes and environmental factors. The disease prevalence varies from very common (e.g., 8.7 percent for ADHD) to relatively rare (e.g., 0.6 percent for autism). The risk alleles also vary from common (e.g., 0.7 for the DAT 10 repeat allele) to relatively rare. Thus, the common variant-common disorder model as well as the rare variant-common disorder model must be considered. Linkage and association studies are the primary tools used to study the role of genes and environment on disease occurrence. The main difference, in a general sense, between association and linkage studies is related to the sampling method. Association studies use the candidate gene approach, where association can be tested in populations of unrelated individuals. Linkage studies differ from association studies in that they require the recruitment of related individuals in a family. Both approaches have advantages and disadvantages, but most experts agree that linkage studies are good for identifying new genes while association methods are good for testing known ones. The opportunity to collect exposure and genetic information on parents of the children, and perhaps even on some grandparents, offers an opportunity for the Study to use linkage methods as well as association methods in studying gene-environment interactions on behavior.

The Study provides a unique opportunity to study candidate genes for disorders such as depression and antisocial behaviors, ADHD and autism, and psychosocial and environmental influences that interact with these genetic factors. As noted by Moffitt, Caspi, and Rutter (2005), incorporating these psychosocial and environmental influences may help researchers identify candidate genes for these disorders. In several studies, genotype was unrelated to disease phenotype when examined in the full cohort; however, significant genetic associations were revealed when the sample was stratified by exposure to an environmental factor. Such gene-environment interactions also provide an explanation for the inability to replicate genetic associations across studies. If an environmental exposure is a key trigger of a genetic effect, then variations in environmental exposure rates from one sample to the next could lead to inconsistent findings. The Study's detailed exposure assessments throughout the prenatal period and early childhood will be critical for illuminating environmentally contingent genetic effects and for clarifying the inconsistent results previously reported.

The Environmental Genome Project (EGP) (Goehl, 2005) has focused on genes sensitive to environmental factors that also meet other acknowledged criteria, such as variation in a coding region or evolutionary origin by conservation or selection (Nickerson et al., 2005). In the investigation of autism, the overlap in chromosome regions implicated by linkage studies and from the EGP have been identified (Herbert et al., 2006). Some of these genes are related to gastrointestinal and immune abnormalities that have systemic impact and are environmentally responsive. These genes and others related to the central nervous system (e.g., the serotonin transporter gene) will be evaluated in the Study. The polymorphism in the DRD4 gene has two characteristics used by the EGP to target genes of special interest: functional variation and signs of positive selection (Nickerson et al., 2005). The functional significance of the DRD4 risk-related polymorphism has been investigated by multiple groups. The initial unexpected results found the "risk" allele of the DRD4 polymorphism apparently has protective effects instead of conferring risk, and the authors suggested the subgroup of ADHD children with a 7R allele present had a partial syndrome characterized by behavioral excesses without cognitive deficits while the 7R-absent subgroup have the full syndrome characterized by both behavioral excesses and cognitive deficits (Swanson et al., 2000). Several independent groups have reported similar results (Manor et al., 2002; Langley et al., 2004; Bellgrove et al., 2005), which support functional variation with theoretical positive selection in the DRD4 polymorphism.

3.4 Potential for Innovative Research

This hypothesis has high potential for innovative research. Complex behaviors such as aggression, substance abuse, depression, schizophrenia, ADHD, and autism have defied attempts at simple genetic or environmental explanations. For example, the recent report on merged cohorts to evaluate autism made this point with the discovery of a new chromosome region of interest in the large sample not implicated in the multiple smaller studies (Autism Genome Project Consortium, 2007). In fact, even in areas where genetic and environmental main effects have been documented (e.g., ADHD), the emphasis now is on gene-environment effects that may account for inconsistencies or discrepancies in results across studies based on single sites or small samples.

Although there are a number of candidate genes and a number of environmental factors associated with these outcomes, none of them alone explain enough variance to suffice as targets for intervention. Complexity introduced by gene-environment and gene-gene interactions may require an approach to predict outcomes or responses in subgroups rather than to address single causes of disorders (Clark, Boerwinkle, Hixson, & Sing, 2005). The Study enables the measurement and timing of early exposures and the tracking of neurobehavioral outcomes throughout childhood and adolescence, thus providing an unprecedented opportunity to investigate gene-environment interactions.

3.5 Feasibility

This study is feasible. The size of the study provides the power for genome-wide scans of association for many childhood onset psychiatric diagnoses, including ADHD (about 5,000 cases are expected to be identified by the age of 7 years) and autism (in the Study sample of 100,000, about 700 cases are expected to be identified by the age of 3 to 5 years). These cases can be matched with nonaffected (2:1) or clinical controls (e.g., with nonautistic developmentally disabled cases) for prospective nested case-control studies. The prospective nature of the Study avoids many of the problems of retrospective case-control studies (recall bias, etc.) (Manolio, Bailey-Wilson, & Collins, 2006). In addition, if disorders are partially dependent on early developmental origins related to adverse exposures of psychological, chemical, biological, and physical factors during fetal and early childhood stages of development, then the birth cohort design of the Study is superior (Willett et al., 2007). In addition to association studies based on the prospective, nested case-control design made possible by the Study birth cohort, the opportunity to collect information on family members offers opportunities for linkage studies.

Because of the currently available molecular techniques and the number of environmental exposures to be measured in this study, investigation of gene-environment interactions is not only feasible, it can be done at reasonable cost with high benefit to the Study.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Exposure to psychosocial stressors during pregnancy:
  - Parental depression, stress, life events
  - Social support
  - Financial strain
- Exposure to smoking and alcohol during pregnancy
  - Maternal genotype and detoxification: GSTT1, NAT2, ADH2
- Genotype: Examples include 5-HTTLPR, DAT VNTR, and COMT
- Parental antisocial behaviors, psychopathology

Primary/child

- Genotype: Examples include 5-HTTLPR, MAOA, DAT, and COMT
- Psychosocial environment
  - Salivary cortisol at 6 months, 1 year, 2 years, 3 years
  - Parenting style: Nurturance, discipline techniques
  - History of child neglect and abuse: Records from child protective services
  - Quantitative and qualitative home environment assessments (e.g., the HOME inventory)

4.2 Methods

Primary/maternal

- Questionnaire
- Clinical diagnosis
- Blood for genotyping (appropriately collected and stored)

Primary/child

- Blood for genotyping (appropriately collected and stored)
- Salivary cortisol at 6 months, 1 year, 2 years, 3 years
- Parenting style: Nurturance, discipline techniques
- Measure of depression in parents
- Measure(s) of child abuse: Parental discipline style, records from child protective services

4.3 Life Stage

Primary/maternal

- Pregnancy

Primary/child

- First 3 years and beyond

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- ADHD
- Autism
- Depression
- Schizophrenia (adolescents)
- Antisocial behavior
- Other psychosocial and neurobehavioral outcomes

5.2 Methods

- DSM-IV based assessments for:
  - Depression
  - Antisocial personality disorder

- Conduct disorder
- Oppositional defiant disorder

- ADHD
- Autism
- Anxiety disorder
- Convictions for violent crimes
- Medical records review provided from parents
- School record review
- Observations of family/friends

5.3 Life Stage

- 3-5 years (disruptive behaviors), 6-12 (ADHD, oppositional defiant disorder, depression), preadolescence and adolescence (depression, conduct disorder)

6. Important Confounders, Mediators, and Effect Modifiers

- **Smoking:** Nicotine induced c-fos mRNA expression was found in several brain areas associated with cognitive function in rats. This may be associated with development of schizophrenia in adults.
- **Postnatal parental depression:** Parental depression may influence child depression and other behavior.
- **Prenatal infection:** Prenatal infection may be related to neurodevelopmental disorders (see other hypotheses) and will need to be controlled for the analysis.

7. Power and Sample Size

For any condition or disorder with a given prevalence at a given assessment point (or age of the cohort), the power to detect associated genes depends on the risk allele proportion. This is especially important for the investigation of hypotheses about common variants in common disorders. The high prevalence of disorders such as depression, anxiety, and ADHD ensure high statistical power, especially for evaluation of risk alleles that also have high expected frequencies in the population (i.e., common variants). The power to detect gene-environment and gene-gene interactions can be calculated (Gauderman, 2002a; Gauderman & Morrison, 2006), and for the combination of prevalence, allele frequency, and cases expected for the high-frequency conditions, the power provided by the Study is adequate to address multiple gene-environment and gene-gene interactions. Examples will be provided for depression, antisocial behavior, ADHD, and autism.

For depression with an expected population prevalence of 2.5 percent of children and 8.3 percent of adolescents, a hypothesized gene (serotonin transporter gene) and environment (stress) interaction was evaluated for three variants of the risk allele, three levels of stress, 0.05 probability and 80 percent power. The frequency of the "l" form of the serotonin transporter gene is about 60 percent (50-60 percent in Caucasians and close to 70 percent in African Americans). Therefore the gene frequency is 0.36 for the "ll" variant, 0.48 for the "ls" variant and 0.16 for the "ss" variant. To achieve 80 percent power, the total number of subjects required is 47,920.

For antisocial behavior (aggression), the population prevalence among adolescents ranges from 17-33 percent depending on the specific measure. A hypothesized gene (MAOA) and environment (child abuse) interaction was evaluated by considering the two most common VNTR polymorphisms, the three-repeat variant with low activity and the four-repeat variant with high activity. These alleles have a population prevalence of about 33 percent and 62 percent, respectively (Caspi et al., 2003). Child abuse was cited as 8 percent severe, 28 percent probable abuse, and 64 percent with no abuse. Power analyses based on a prevalence of 5 percent in the unexposed population (conservative according to Caspi, et al., 2003) result in a required sample size of 31,650.

For ADHD with a population prevalence of 5 percent by age 7 years, the gene (DAT) and environment (maternal smoking) interaction was evaluated by considering the homozygote genotype for the 10 repeat allele of the 40 bp VNTR, which has a population prevalence of about 50 percent. Maternal smoking was estimated to be about 20 percent. Given the prevalence of exposure and outcome discussed above, the minimum detectable odds ratio, based on 80 percent power and a 5 percent type I error is 1.27.

8. Other Design Issues

- **Ethical/burden considerations:** Human subjects issues associated with observation of certain psychosocial stressors (e.g., abuse) must be carefully addressed in the study protocol, with any planned remedial actions approved by appropriate institutional review boards.

- **Cost/complexity of data collection:** The longitudinal design is required because it is important to trace the development of the cohort through childhood and adolescence in order to measure not only the exposures but also the neurobehavioral outcomes of relevance (e.g., depression, substance abuse, antisocial behavior). Several reports from smaller cohorts have indicated childhood antecedents (or perhaps risk factors) predate symptom development by long periods.

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PRENATAL AND PERINATAL INFECTION AND SCHIZOPHRENIA

1. Meta Hypothesis

Prenatal infection and mediators of inflammation during pregnancy and the perinatal period are associated with increased risk of schizophrenia.

2. Specific Hypotheses

1. A child born to a mother experiencing an infection of toxoplasa, rubella, or other known pathogens during the first 22 weeks of gestation has an increased risk for developing schizophrenia in adulthood.

    1.1 Mothers of low socioeconomic status (SES) vs. mothers of a higher SES who experience a prenatal or perinatal infection during pregnancy will have children with an increased risk for schizophrenia as adults.

    1.2 Consuming a diet high in vitamins and minerals (i.e, folate, vitamin A, vitamin D) that are known to influence immune function and fetal development during pregnancy will reduce the affect of a mother experiencing a prenatal or perinatal infection(s) and having a child that will be at an increased risk for developing schizophrenia in adulthood.

2. The presence of specific genetic factors, such as candidate receptor genes 5-HT2a, D3, and NMDA, will increase the likelihood that a child born to a mother experiencing a prenatal or perinatal infection will develop schizophrenia in adulthood.

3. Background and Justification

Schizophrenia is a severe psychiatric disorder typically appearing in late adolescence or early adulthood. It is associated with significant long-term morbidity, occupational disability, social disadvantage, and high mortality from suicide and other causes. The burden of the disease extends to the family for whom there are major economic and social implications. Converging evidence suggests that many cases of schizophrenia are neurodevelopmental in origin, that both genes and environment play a role in the etiology of these cases, and that exposures in early gestation, in particular, infection and nutritional deficiency, may be linked to schizophrenia, (Cannon, Jones, Susser, van Os, & Murray, 2002; Susser, Brown, & Gorman, 1999).

While infectious agents have been suspected of increasing the risk of schizophrenia, their role has not been well established (Bromet & Fennig 1999; Buka, Tsuang, Torrey, Klebanoff, Bernstein, et al., 2001; Buka, Tsuang, Torrey, Klebanoff, Wagner, et al., 2001). Herpes simplex viruses (HSV) are known to cause encephalitis in infants (Corey, Whitley, Stone, & Mohan, 1988), thus latent effects of less severe infection are biologically plausible. In recent data from long-term follow-up of participants in the U.S. Collaborative Perinatal Project (a longitudinal study conducted in the 1960s), maternal serum immunoglobulins (IgG and IgM) obtained during pregnancy were associated with increased risk of psychosis in offspring (Buka, Tsuang, Torrey, Klebanoff, Bernstein, et al., 2001). However, others have not found an increased risk of schizophrenia spectrum disorders associated with IgG antibody to herpes simplex virus type 2 (Brown, Schaefer, Quesenberry, Shen, & Susser, 2006). Conflicting results among studies may be due to differences in outcome (psychosis vs. schizophrenia spectrum disorders), varying effects of exposure depending upon nutritional status of the mother, timing of infection during gestation,

or chance findings due to small numbers of cases. For example, risk for schizophrenia was increased seven-fold for influenza exposure during the first trimester, but no increased risk was observed if influenza occurred during the second or third trimester (Brown et al., 2004). HSV is just one of many infectious agents, including respiratory infections (Brown et al., 2004; Brown et al., 2001), rubella, and perhaps exposure to toxoplasmosis (Brown et al., 2006), that have been linked with risk for schizophrenia in offspring. Not only is prenatal infection important, it is possible that exposure to infections during conception is also relevant. Exposure to genital/reproductive infections during the periconception period, which has been cited to occur in 4.2 percent of pregnancies, has been associated with a five-fold increase in the risk of schizophrenia in adults (Babulas, Factor-Litvak, Goetz, Schaefer, & Brown, 2005).

Prenatal infection can either contribute directly to adverse effects on the developing brain or indirectly by increasing pro-inflammatory cytokines (Cai, Lin, Pang, & Rhoades, 2004). Cytokine polymorphisms have been identified (i.e., tumor necrosis factor-a [TNF-a] and mannose-binding lectin [MBL]) and are associated with a greater immune response to infection (Gibson et al., 2006). Whether the presence of these polymorphisms increases risk of schizophrenia with exposure to prenatal infection has not been studied in humans, although animal studies support this hypothesis (Ashdown et al., 2006). This is an example of a type of gene-by-environment interaction that may be tested within the context of the National Children's Study (NCS), and it is expected that during the next several decades specific candidate genes will be more clearly implicated in the risk to schizophrenia or with risk factors associated with schizophrenia.

3.1 Public Health Importance

Prevalence/incidence

Infections of many different types are common in the U.S. population. More importantly, some types of infections are more prone to occur during pregnancy. For example, one out of five adolescents nationwide, and similarly in adults ages 21 and older, have had a genital HSV infection (Centers for Disease Control and Prevention [CDC], 2004). Antibodies to HSV-2 have been detected in approximately 20 percent of pregnant women, although only 5 percent report a history of symptomatic infection (Brown, 1998). Physical changes during pregnancy can also incur vulnerability to respiratory disorders. Pregnant women in their second and third trimesters of pregnancy have a greater risk of influenza-related morbidity compared to nonpregnant and postpartum women (CDC, 2004). Additionally, about 1-2 percent of term pregnancies are affected by chorioamnionitis (intrauterine infection). In pregnancies ending in preterm births, the prevalence of such infection is higher (Wu & Colford, 2000). Periodontal disease is also a source of infection during pregnancy and may be associated with adverse pregnancy outcomes due to metastatic spread of infection, metastatic injury from circulating microbial toxins, or metastatic inflammation induced by microorganisms (Li, Kolltveit, Tronstad, & Olsen, 2000).

Schizophrenia is a chronic, severe, and disabling disorder that affects approximately 1 percent of people worldwide. The schizophrenia incidence rates per 10,000 person-years for males ages 15-19 is 9.4, for 20-24 is 5.6, and for 25-29 is 3.3. The incidence rates for females in the same age groups are 1.5, 1.3, and 4.1, respectively (Bresnahan et al., 2000). The cumulative risk for schizophrenia by age 38 was 0.93 percent for men and 0.35 percent for women.

Economic and/or social burden

Annual costs of schizophrenia in the United States were recently estimated at $62.7 billion (Wu et al., 2005), with a substantial portion due to disease in young adults (Genduso & Haley, 1997). Pharmacological treatment for schizophrenia can relieve many of the disorder's symptoms, but most individuals with schizophrenia live with residual symptoms for many years after diagnosis. About two

thirds of those who develop schizophrenia continue to be affected throughout adulthood (Bromet & Fennig 1999).

Individuals with schizophrenia and other mental illnesses are at increased risk for cardiovascular disease due to high rates of smoking, obesity, diabetes, and hypertriglycerdemia. Substance abuse and high-risk sexual behavior (which may partially explain the observed higher rates of HIV and infectious hepatitis in individuals with schizophrenia) also are increased in schizophrenia (Lambert, Velakoulis, & Pantelis, 2003; Goff et al., 2005).

3.2 Justification for a Large Prospective Longitudinal Study

Investigation of the prenatal origins of schizophrenia requires a large prospective cohort followed from early gestation through adulthood.

A prospective study is required to obtain precise prenatal exposure data. For the exposures of interest (e.g., prenatal infection), biological specimens are essential for precise measurement and timing. Serum and placental specimens can be collected prospectively but stored for later analysis. To conserve specimens and reduce cost, a nested case-control design will be employed.

The longitudinal design is required because it is important to trace the development of the cohort through childhood and adolescence, before the onset of schizophrenia. Several reports from smaller cohorts have indicated that risk factors occurring during childhood can predate a formal diagnosis by decades (Cannon et al., 2002). In addition, while prenatal exposures may play an important role in schizophrenia, this by no means precludes an important role for postnatal experience. Indeed, some reports have suggested that adverse childhood experiences contribute to increased risk of schizophrenia.

A large cohort is required to study this relatively infrequent outcome. As noted above, earlier studies with good prenatal data have suggested relationships of prenatal exposures to the outcome of schizophrenia, but their interpretation is limited by small numbers.

A large cohort is virtually a precondition for extending this field to investigate several types of hypothesized interactions, including gene-environment and nutrition-by-infection interactions.

There are many potential confounders, mediators, and effect modifiers, such as the presence of other developmental disabilities (ADHD, autism); race; SES; postnatal infection and trauma; and low birth weight. A longitudinal study design can consider these potential multiple interactions, inform temporality, and support causality.

3.3 Scientific Merit

The “neurodevelopmental theory” proposes that the neural basis for schizophrenia originates in prenatal brain development that begins long before any clinical symptoms are apparent, and is caused by the combination of genetic and environmental effects (Cardno et al., 1999; Singh, McDonald, Murphy, & O’Reilly, 2004). Findings in schizophrenic patients suggest that early harbingers of schizophrenia include minor physical anomalies (Waddington, 1990; Rosso et al., 2000) and motor (Walker et al., 1994), cognitive (Comblatt et al., 1999; Cannon et al., 2002, Davidson et al., 1999, Kremen, 1998), and social (Jones, Rodgers, Murray & Marmot, 1994; Done, Crow, Johnston, & Sacker, 1994; Olin & Mednick, 1996) disturbances in childhood. By onset, schizophrenia is associated with ventricular enlargement; decreased total brain, gray, and white matter volumes (Lawrie et al., 1998; Wright et al., 2000); and decreased hippocampal (Nelson, Saykin, Flashman, & Riordan, 1998), thalamus (Konick & Friedman 2001), and frontal lobe (Davidson & Heinrichs, 2003) volumes. Definitive evidence for an

environmental prenatal exposure that increases risk of schizophrenia is necessary. By providing such evidence or refuting the hypothesis, the Study will contribute in a unique and fundamental way to our understanding of the etiology of schizophrenia.

3.4 Potential for Innovative Research

The technologies used in the direct and indirect assessment of relevant prenatal exposures will include serologic analyses and placental pathology. These methods, which are common to many of the research hypotheses addressed in the study, are exceptionally useful in the context of the present hypothesis. Each provides a window on gestational experience, and holds the potential to improve the knowledge of the timing and mechanisms of exposures adversely influencing neurodevelopment and elevating risk of schizophrenia.

- **Serum samples:** In addition to basic serologic analyses for specific candidate infections (e.g., influenza, HSV-2), high throughput methods will be used to detect viral nucleic acids, antiviral antibodies, and host factors. This will permit the simultaneous measurement of multiple analytes using small amounts of sera.

- **Placenta:** Placental data can be used to indicate nutritional, infectious, toxic, and other exposures. Novel technologies of digital image capture for gross assessment of placenta and image analysis will standardize gross measures and extend what can be measured in the placenta. These advances will permit us to assess potential mechanisms for the influence of adverse exposures.

Taken together, these measures will provide unprecedented access to early gestational life and address the core of the neurodevelopmental hypothesis.

3.5 Feasibility

Due to the longitudinal nature of the study, these hypotheses can be addressed. A minimum of three visits during pregnancy will be required to investigate longitudinal changes, or trajectories, in infection and subsequent immune responses. Direct assessment of infection during pregnancy is optimal. Clinical visits and medical record abstraction will also yield valuable information. The large sample size of the study provides adequate power to detect any associations between infectious agents during pregnancy and later manifestation of schizophrenia symptoms in offspring. Using the widely cited rate of 1 percent in the population, approximately 1,000 cases of schizophrenia can be potentially identified throughout the course of the study. Prospective, nested case-control studies can be used to compare cases to controls for which the study provides a richly diverse sample to ensure adequate matching.

4. Exposure Measures

The central interest of this hypothesis is in the periconception environment and prenatal development. Early gestation is a time of unique vulnerability to the consequences of environmental insult. There is evidence that both infectious and nutritional exposures during this period profoundly affect brain development. From the perspective of exposure measurement, early gestation is therefore of critical interest in the work proposed here.

4.1 Individuals Targeted for Measurement

Primary/maternal

Maternal infection/inflammation:

- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers)
- Blood for viral nucleic acids. The specific type of viral infection may modify the risk of neurological disorders.
- Cultures/vaginal swabs
- Medical histories of fevers and infections (medicine usage)
- Assessment of dental health via interviews for potential periodontal disease before and after pregnancy

Secondary/maternal

- Retrospective medical record review

Primary/child

Prenatal infection/inflammation:

- Infection serology (lymphocytes, antibodies, cytokines/interleukins, inflammatory markers)
- Umbilical cord (antibodies, cytokines, viral nucleic acids)
- Placental pathology

4.2 Methods

Primary/maternal

- Blood samples
- Vaginal/cervical cultures
- Examinations by a medical professional
- Interviews

Secondary/maternal

- Medical and obstetrical history
- Family history

Primary/child

- Umbilical cord blood culture/pathology
- Amniotic fluid analysis, if available

4.3 Life Stage

Primary/maternal

- Repeated measures from first through third trimesters and birth

Secondary/maternal

- Enrollment, all trimesters, follow-up after birth at predetermined intervals (for family history)

Primary/child

- Perinatal period and throughout the course of the study

5. Outcome Measures

From the perspective of outcome, we are interested in childhood markers of, and risk factors for, schizophrenia. Therefore, specific postnatal assessments will be periodically conducted during childhood and adolescence. Our interest lies in the trajectory of cognitive, language, motor, sensory, and psychological and social development, as well as the appearance of specific psychiatric symptoms over time. Although the median age of onset is in the early to mid-20s for men and late 20s for women (American Psychiatric Association, 1994), screening for schizophrenia should begin in early adolescence to capture any early onset cases and detection of subclinical schizophrenic traits.

5.1 Outcomes Targeted for Measurement in Child

- Schizophrenia screening and diagnosis: Standardized screening and diagnostic instruments, e.g., Schizotypal Personality Questionnaire (screening) and KID-SCID or SCID for diagnosis at ages 18-21 or the NIMH Diagnostic Interview Schedule for Children, will be administered in a face-to-face diagnostic interview. These instruments have been extensively tested. However, one must consider the possibility that during the next several decades, the state-of-the-art diagnostic interview may require use of an instrument's version that is not currently well-validated, but will be in the future, or an instrument that may not yet be developed.

5.2 Methods

- Examination by a medical professional
- Screening and diagnostic instruments for schizophrenia spectrum disorders, social function, neurological, and psychological testing

5.3 Life Stage

Primary/child

- Screening and diagnostic instruments throughout the course of the study at predetermined intervals when the child is at an appropriate age for specified instruments

Secondary/parental

- Screening and diagnostic instruments with parental respondent throughout the course of the study (when instruments are not suitable for direct assessment of younger children)

6. Important Confounders, Mediators, and Effect Modifiers

- **Age:** Schizophrenia typically manifests itself in late adolescence or early adulthood. Men tend to have earlier onset (early to mid 20s) than women (late 20s). Early childhood onset may have unique risks (Nurnberger et al, 1994).
- **Socioeconomic status, race, ethnicity status:** Individuals with schizophrenia tend to be of a lower socioeconomic class. However, like many mental illnesses, this may be that the symptoms of the disease themselves cause a drift into a lower class.
- **Paternal and maternal age:** Paternal age at the time of birth may be a risk factor for schizophrenia (Malaspina et al., 2001).
- **Stress:** Stress may trigger disease development. Maternal unwantedness of pregnancy has been found to be a risk factor for the development of adult schizophrenia (Herman et al., 2006). Indicators of maternal stress (cortisol) will be assessed during pregnancy.
- **Residential environment:** Residential environment is associated with risk of schizophrenia. Most studies consistently report a positive association with urban birth (Lewis, David, Andreasson, & Allenbeck, 1992; Marcelis, Navarro-Mateu, Murray, Selten & van Os, 1998; Mortensen et al., 1999) and rural living younger than age 16 is negatively correlated with the incidence of schizophrenia (Kendler, Gallagher, Abelson, & Kessler, 1996). However, more research is needed to determine the underlying factors that may account for this association (toxic exposures, stress, diet, selective migration) (McGrath & Scott, 2006; Pedersen & Mortensen, 2006).

- **Smoking status:** Rates of smoking among schizophrenics are about 66-88 percent (Herman et al., 2006), two to three times that of the general population. Smoking usually starts around adolescence prior to the average age of diagnosis (Hughes, Hatsukami, Mitchell, & Dahlgren, 1986).

- **Systematic infections:** There is evidence that children with postnatal systematic infections, particularly central nervous system infections, are 4.8 more times as likely to develop schizophrenia (Rentakallio, Jones, Moring, & Von Wendt, 1997).

- **Vaccinations:** Maternal vaccinations may be a plausible option for prevention of schizophrenia by reducing the rate of prenatal infection (McGrath, 2000).

- **Nutrition and diet:** A Finnish study hypothesized that poor nutrition, but not lower caloric intake, in utero and in early childhood is an increased risk factor for schizophrenia (Wahlbeck, Forsen, Osmond, Barker, & Eriksson, 2001). Associations have been reported between schizophrenia and maternal homocysteine concentrations suggesting that folate status during pregnancy may be important (Brown et al., 2007). Other investigators have suggested that low vitamin D status also may be a risk factor (McGrath, 2001).

- **Gestational age, weight, head circumference:** Schizophrenics are more likely to be premature and have a low birth weight (Jones, Rantakallio, Hartiainen, Isohanni, & Sipila, 1998). Certain prenatal infections also are associated with preterm birth.

- **Mother's medical and obstetrical histories:** Various obstetric complications are often cited as possible causes for fetal neurodevelopmental disruption and consequent disorders (Jones et al., 1998).

- **Maternal medication and drug use:** Illicit drugs taken during pregnancy, including marijuana, PCP, and methamphetamines, increase the risk of schizophrenia among the offspring (Kelly & McCreadie, 1999).

- **Environmental exposures:** Exposure to solvents (Perrin et al., 2006) and heavy metals such as lead (Opler et al., 2004) have been found to be associated with schizophrenia.

- **Family history of mental health:** Schizophrenia has been shown through family, sibling, and twin studies to have a strong genetic component (Cannon et al., 2002).

- **Maternal and infant DNA:** Genetic polymorphisms have been identified that influence the concentration of circulating cytokines in response to a viral infection (Gibson et al., 2006). Elevated cytokine concentrations can be neurotoxic to the fetus, therefore, the presence of specific cytokine polymorphisms or genes not yet identified may modify the risk of schizophrenia given exposure to infectious agents.

- **Social, language, motor, and cognitive development:** Deficits in social and cognitive development may increase risk for psychosis (Jones et al., 1994). Cognitive and achievement test scores are lower in pre-schizophrenic children compared to healthy children (Done, Crow, Johnston, & Sacker, 1994; Davidson et al., 1999). The severity of speech and language deficits may also be associated with the age of schizophrenia onset (Hollis, 1995). Declines in general intellectual function between

ages 4 to 7 may be specific to schizophrenia and associated spectrum disorders (Kremen et al., 1998).

- **Genetic risk factors as effect modifiers:** A number of candidate genes have been extensively studied as risk factors for schizophrenia. These include COMT, DTNBP, and NRG1. These susceptibility genes may modify the risk for schizophrenia incurred by perinatal and prenatal infection.

- **Premorbid psychopathology:** Some authors have suggested that schizophrenia is part of a psychopathic progression that begins during early childhood (Rutter, Kim-Cohen, & Maughan, 2006). The design and the data collected in the NCS will enable investigators to determine whether this continuum exists.

- **Migrant and minority populations:** A higher rate of schizophrenia has been observed in migrants compared to native-born populations (McGrath et al., 2004).

7. Power and Sample Size

Although nested case-control studies are planned within the study, for simplicity power is presented within this document for the entire cohort. The use of placental data and serum from early gestation (preferably first trimester) will reduce the effective sample size, thus we assume 80,000 participants with complete measures. We assume that the lifetime risk of the outcome is 0.01 (this risk will vary according to narrow or broad definitions of schizophrenia and schizophrenia spectrum disorders). For the present purposes, we have assessed the smallest detectable risk, varying the disease probability in the unexposed (appropriate to ascertainment at varying ages), and prevalence of exposure (dichotomized).

Table 1 The smallest detectable risks for a sample size of 80,000 and power of 80 percent and alpha = 0.05:

Smallest Detectable Relative Risk			
Prob (disease in unexposed)	Prevalence exposure = .05	Prevalence of exposure = .10	Prevalence exposure = .20
.003 (age 21)	RR = 1.9	RR = 1.6	RR = 1.4
.005 (age 30)	RR = 1.7	RR = 1.5	RR = 1.3
.009 (age 40)	RR = 1.5	RR = 1.3	RR = 1.2

For more common exposures such as influenza, the power of this study would be significantly higher. A recent study suggested, based on population attributable risk estimates, that up to 14 percent of schizophrenic cases would not occur if influenza exposure during early to mid-pregnancy had been prevented (Brown et al., 2004).

8. Other Design Issues

- While a simple examination of maternal antibody titers would advance science in this area, consideration of the timing of the infection in relation to birth would be useful. Multiple maternal blood samples during pregnancy will help determine the trimester that infection occurred and the immune response.

- Vaginal swabs for viral culture would enable an evaluation of the timing and rate of viral shedding in relation to subsequent schizophrenia.

- Ascertainment of schizophrenia among children born into the study will rely on reports of clinical diagnosis. However, for a more sensitive assessment of outcome, the study will employ screening questionnaires that specifically assess participants on a continuum of dysfunction. This will enable detection of early subclinical syndromes and a spectrum of related psychotic disorders (schizophreniform, etc.).

- Because schizophrenia is generally not diagnosed until late adolescence and through the early 20s, response and retention rates of the study participants will be important for assessing this hypothesis.

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FAMILY INFLUENCES ON CHILD HEALTH AND DEVELOPMENT

1. Meta Hypothesis

Family resources and processes shape the structure and quality of children's homes, childcare, school experiences, and economic opportunities. These resources and processes affect children's developmental and health trajectories and mediate or moderate other environmental influences on children's outcomes.

2. Specific Hypotheses

1. The nature and stability of family structures, including parental unions, household composition, and living arrangements, affect child outcomes, including functioning of stress-responsive biological regulatory systems, levels of social competence and emotional regulation, and internalizing and externalizing behavior problems.

2. Families' social networks influence child health and development by providing or limiting access to instrumental or emotional support for either child or adult family members by placing demands on parents' time for helping others, by providing or limiting access to information and health-supportive resources, by exposing children to positive or abusive relationships, or by supporting healthy or unhealthy norms for health-related behaviors.

3. Family socioeconomic status (e.g., income, wealth, parents' education, and occupation) influences the health and development of children both directly and by moderating the effects of risk factors such as prematurity.

4. Children with emotionally or physically unhealthy family members are likely to be in poor mental or physical health themselves, in part because of shared genetic predispositions but also because of constrained parenting and compromised resources. Children who are exposed to negative family dynamics display more problematic health and developmental outcomes.

5. Parental promotion of healthy lifestyle behaviors through teaching and modeling contribute to better physical and mental health outcomes in children.

6. Parental monitoring of children's activities at home and in their neighborhoods enhances child health and development.

3. Background and Justification

Families are the epicenters of social-environmental influences on children's health and development (Demo & Cox, 2000; McLoyd, Cauce, Takeuchi, & Wilson, 2000). Most studies of children's health and development hypothesize that family variables have direct, mediating, or moderating influences on children's health and on social and cognitive developmental outcomes.

A review of the existing research suggests that two major domains: family resources and family processes, are primary influences on children's health and development. The family resources domain includes family structure (i.e., parental unions, household composition, and family living arrangements); family socioeconomic status (e.g., parents' and other household members' education, income, wealth, health insurance, and human capital); social resources (ties and access to supportive others); family physical and mental health; and family identity (e.g., identification with cultural norms,

attitudes, and values associated with specific racial, ethnic, religious, or other socially defined groups). The family processes domain includes management (e.g., decision making, resource allocation, parental involvement and engagement in children's school and education, seeking medical care, and engaging children in activities such as sports); parenting (e.g., parental practices such as monitoring, nurturance, protection, and guidance; parenting styles; and direct interactions between parents and children); and family climate (i.e., family cohesion, marital/spousal relationships and family violence). The links between these domains and processes operate and change as children develop.

3.1 Public Health Importance

Prevalence/incidence

Many children in the United States are living in households with potentially problematic family resources and processes. In 2005, 18.2 percent of children younger than 18 and 21.3 percent of children younger than 5 lived in poverty (U.S. Census Bureau, 2005). Children comprise the largest group living in poverty in the United States.

Estimates as recent as 2005 indicate that 68 percent of children live in two-parent families, down from 77 percent in 1980 (U.S. Census Bureau, 2005). The U.S. Census Bureau estimates that one-third of children today are born to unmarried mothers and may grow up either in single-parent families or spend significant portions of their lives living with other relatives or stepparents. Half of all children in America younger than 16 will someday live in a single-parent household (Fields, 2001).

Family violence, a critical dimension of family climate, has profound effects on children (National Research Council, 1993). In 2004, 872,000 (11.9 per 1,000) children were victims in maltreatment cases substantiated by state child protective services (U.S. Department of Health and Human Services [HHS], 2006), which is known to be a conservative estimate of the true prevalence of maltreatment.

Economic and/or social burden

Although there is substantial variability overall, adults who grew up in families with conflictual parental relationships or divorce are more likely to experience depression, adjustment difficulties, and dissolution of their own marriages during adulthood (O'Connor, Thorpe, Dunn, & Golding, 1999). The relation between parental divorce and the child's subsequent adult difficulties is mediated through the quality of childhood parent-child relationships and, therefore, is potentially malleable.

Family economic status is correlated with children's economic status in adulthood, although there is certainly elasticity in these predictions (Charles & Hurst, 2003), thus making such intergenerational patterns potentially modifiable. Efforts to better understand and minimize this reproduction of inequality would lower poverty rates and increase societal well-being.

3.2 Justification for a Large Prospective Longitudinal Study

A large prospective longitudinal study of the influence of families on child development is essential for accurate examination of how families influence the unfolding of child developmental outcomes through time.

Family processes and resources change during time, and the timing, sequence, and duration of exposures to these family characteristics may have differential effects on children's outcomes.

Socioeconomic status may vary during a child's life, having both short- and long-term effects on health and development. Research suggests that economic disadvantage during childhood has lasting effects observable in the form of health disparities during adulthood and old age (Hayward & Gorman, 2004). Family resources that affect income, however, are not static. Changes in employment, marital status, and living arrangements can produce sharp changes in household incomes, in the environments in which children live, and in their access to medical care. Cross-sectional data provide only a snapshot of exposures and outcomes and, therefore, are less useful for understanding how child health and development are affected differently by concurrent or cumulative family circumstances. Differential timing of exposure is also important. Research on family poverty (Duncan & Brooks-Gunn, 1997) identifies early childhood as a vulnerable period in which the experience of poverty has disproportionate effects on development. A longitudinal study would allow for further identification of potentially sensitive periods in child development when the effects of specific family exposures might be heightened.

A large sample size is essential for several reasons. The United States is becoming more diverse, and norms about family structure and process differ across racial, ethnic, and religious groups. Consequently, the same family exposures could have different impact within subpopulations. A large sample size will permit the National Children's Study (NCS) to assess the impact of the family on child health and development within and across diverse subpopulations. A large sample is also needed to capture the effects of low-prevalence yet high-impact family exposures such as family violence and child maltreatment. The field of child maltreatment research has struggled to estimate accurate prevalence rates, to establish adequate comparison groups when examining the effects of maltreatment on development, and to deal with issues of retrospective bias in the family relations of maltreated children. A large representative sample and a prospective study will be invaluable in reducing bias in these analyses.

3.3 Scientific Merit

Family resources

Although divorce rates have stabilized since the 1990s, the general acceptability of divorce has also contributed to structural changes in families such that children are more likely to live in a single-parent or cohabiting household at some time in their lives. Children's living arrangements have become not only more diverse but also more unstable, especially for low-income and some minority (e.g., African American) families (Wu, Bumpuss, & Musick, 2001). Evidence suggests that children who experience changes in family structure and concomitant residential instability are at risk for low educational attainment in late adolescence (Hill, Yeung, & Duncan, 2001). The effects of poverty on child health and development are also greatest among single-parent families. For example, rates of low birth weight and preterm infant births are higher among African-American single mothers living in poverty (Brooks-Gunn & Chase-Lansdale, 1995).

Children's health and both household income and parental education are well linked. Poverty, single-parent family status, and low parental education levels all contribute to children's poor health outcomes (Bauman, Silver, & Stein, 2006). There is a health gradient that persists through all income and education levels such that children from families with higher incomes and more parental education have a higher probability of being healthy and developing to their highest potential. It is still the case, however, that 66 percent of children from the poorest quintile are in excellent or very good health (Case & Paxson, 2002). It is important to understand why and how the latter group thrives despite limited resources. There is a growing understanding that family wealth (i.e., accumulated assets) has an effect on child health and psychosocial functioning above and beyond that of income (Bradley & Corwyn, 2002). Moreover, there is increasing attention to racial disparities in family wealth that exceed differences in income (Conley, 2001). Further research is needed to understand how specific types of family resources interact with each other and with family processes in influencing health and developmental outcomes.

Family social resources, including enhanced material resources (e.g., goods and services, information), emotional support (which may buffer the negative appraisal of stressful events), social engagement (connection to productive activities), and social influences (maintenance of healthy norms and behaviors) have been linked to child health and development. Research has documented associations between caregiver stress, caregiver social isolation, and child health outcomes (Wright, Rodriguez, & Cohen, 1998). Evidence also documents the significance of social support during pregnancy for fetal growth (Feldman, Dunkel-Schetter, Sandman, & Wadhwa, 2000). Much remains to be learned, however, about the social, psychological, behavioral, and biological pathways involved in these associations.

The physical and mental health of parents and other family members can impact the health and well-being of children. Physical or psychological disabilities faced by parents, caregivers, or siblings can compromise the quantity and quality of nurturing provided to children. Children whose parents suffer from serious psychological problems such as depression or alcoholism have a higher risk for ineffective or inconsistent parenting, maltreatment, placement in foster care, and homelessness (Coyne & Downey, 1991; Mowbray et al., 2000; Oyserman, Mowbray, Meares, & Firminger, 2000). More general parental life stress also impacts parenting, even if only temporarily (e.g., death in the family, chronic illness, poor relationship quality, marital or cohabitation dissolution, work stress, neighborhood crime and violence) (Ge, Conger, Lorenz, & Simmons, 1994).

Race, ethnicity, and immigrant status represent important cultural and structural factors that influence families and children. Family and culture research shows differences in the ways in which parents manage children's lives, provide supervision and guidance, and support their goals. For example, the literature suggests concepts especially salient to immigrant families are parental respect, family loyalty, pride, parental expectations, and family obligations (Fuligni, Tseng, & Lam, 1999; National Research Council, 1998). This research has made it clear that family process constructs (warmth/closeness, monitoring, involvement) among immigrants do not hold the same meaning as they do for native-born families (Garcia Coll & Patcher, 2002). Similar research has focused on racial differences in parenting practices (Jones, Zalot, Chester, Foster, & Sterrett, in press; McLoyd et al., 2000). Family also plays an important role in children's attitudes and beliefs regarding the role that race, ethnicity, and gender play in their lives (Hughes & Chen, 1997; Spencer, 1983). Identity attitudes have been linked to children's outcomes in a variety of domains including self-esteem (Smith, Walker, Fields, Brookins, & Seay, 1999), academic beliefs and performance (Witherspoon, Speight, & Thomas, 1997), friendship selections (Hamm, 2000), substance use and abuse (Marsiglia, Kulis, & Hecht, 2001; Scheier, Botvin, Diaz, & Ifill-Williams, 1997), engagement in risky sexual behaviors (Belgrave, Van Oss, & Chambers, 2000), and violence (Arborna, Jackson, McCoy, & Blakely, 1999). These social identities also play a role in buffering the deleterious impact of experiencing racial, ethnic, and gender discrimination (Sellers & Shelton, in press; Williams, Spencer, & Jackson, 1999). Thus, it is important that a study like this captures parenting processes and family climate characteristics that facilitate health and healthy behavior across different ethnic, racial, and immigrant groups.

Family processes

Parent-child relationships can have direct effects on child health and children's exposure or vulnerability to a variety of social and environmental health risk factors. Altering family resources (e.g., socioeconomic status, family structure) is not always a practical focus for intervention. In contrast, family processes (such as parenting practices) can be modified through intervention and often mediate the links between family resources and child outcomes.

Parenting style has a considerable influence on children's developmental outcomes. Models of parenting highlight the centrality of the emotional quality (i.e., level of warmth, trust) of the parent-

child relationship in determining whether parents are effective in disciplining their children, learning about their children's everyday activities, serving in the role of advisor and confidant, and conveying their beliefs and values (Darling & Steinberg, 1993). A substantial body of research documents that "authoritative" parenting styles, characterized by high levels of warmth, high expectations for maturity, and moderate levels of discipline, are linked to children's social competence, achievement, and self-regulation abilities (Parke & Buriel, 1998). When relationships are high in warmth and trust, children are also more likely to respond positively to socialization efforts. Family environments characterized by high conflict, aggression, and violence adversely affect child health outcomes (Repetti, Taylor, & Seeman, 2002).

Effective parenting also requires that children perceive parents as having power and status, because children are more likely to identify with and model adults they perceive as powerful. Parental power may come in such forms as parents' access to resources, their ability to protect their child from illness or danger, and their ability to solve common problems (Parke & Buriel, 1998). Qualitative research suggests that socioeconomic differences in how parents view the task of fostering children's development may be associated indirectly with children's health outcomes, as low-income parents engage in fewer prevention behaviors and positive interventions on behalf of their children (Lareau, 2003). Another body of work documents links between parental monitoring and well-being outcomes in childhood and adolescence, including school grades, association with deviant peers, involvement in delinquent activities, and conduct problems (Crouter & Head, 2002). The conditions under which parents are more or less effective at monitoring their children are less well understood, but the emotional quality of the parent-child relationship may be an important moderating factor (Stattin & Kerr, 2000).

3.4 Potential for Innovative Research

The NCS has the potential to provide an innovative and unprecedented body of longitudinal data that unites information on family resources and processes from before birth until early adulthood with child physical and mental health outcomes. No such comprehensive data resources currently exist.

The large, representative sample will allow the NCS to establish the nature and scope of family resources in the United States in a way that has not been possible to date, including how such resources vary across population subgroups. Family processes are often studied within small convenience samples or targeted subgroups rather than across varied populations. Initial documenting of the full range of family characteristics will be an innovation in the field of family research.

The study will examine the links between family influences and child outcomes within and across ethnic, racial, and socioeconomic subgroups. Such subgroup analyses, which have been done on limited samples, will permit a better understanding of how the same family practices have different consequences for child development within differing groups. This information will be an invaluable starting point for tailoring interventions to different types of families.

3.5 Feasibility

Family resources and processes influence child health and development outcomes throughout childhood and adolescence with key critical periods arising for specific elements of family influence (e.g., parental control over children's activities, diet, and hygiene declines with age). However, neighborhood context and family resources moderate this decline. As control declines, monitoring of the child rises and becomes more important. There are well validated and standardized assessment tools for measuring both resources and structure. Repeated assessments throughout the study will capture these progressive changes.

Relatively fixed information on the family (e.g., race, ethnicity, education of grandparents) can be measured once or twice during the study period. Relatively stable family factors can be collected regularly but at less frequent intervals than factors that may change often. For example, wealth, specific parenting practices, family climate, and family social resources might be included in this set. Information that might change more unpredictably, such as family structure, income, parental employment, child care arrangements, and residence, needs to be assessed more frequently to account for changes.

Because family resources and family process are active fields of research within the social sciences, valid and reliable measurement tools, including parental report measures, observational measures of family processes, and report measures for older children and adolescents, are available, which will ensure robust measurement of family influences. Child development outcomes, including academic achievement, social competence, and behavior problems are measurable through well-established and standardized measures, again available from multiple reporters.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Family resources

- Family structure (marital status, living arrangements, residential mobility)
- Socioeconomic status (income, employment)
- Social support
- Parental physical and mental health
- Family identity

Family process

- Parental management of child health
- Parenting (warmth, discipline, monitoring)
- Family climate
- Parental mental health

4.2 Methods

Family resource measures

- Interview
- Questionnaire
- Medical record review

Family process measures

- Interview
- Questionnaire
- Direct observation of parent-child interaction

4.3 Life stage

Family resource measures

- Prenatal through late adolescence, ongoing regular assessments

Family process measures

- Birth through late adolescence, regular questionnaire/interview assessments
- Observation of parent-child interaction during infancy, childhood, adolescence

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Social and emotional functioning
- Risk-taking behavior and aggressive behavior problems
- Child health status
- Academic achievement
- Educational attainment

5.2 Outcome Methods

- Questionnaires/interviews with parents, children, teachers (measuring child social, emotional, and behavioral functioning)
- Direct testing/observation of child (social behavior, aggressive behavior, achievement)
- Medical record review
- School record review

5.3 Life stage

- Social/emotional: Infancy through late adolescence
- Risk-taking behavior/aggression: Preschool through late adolescence
- Health status: Infancy through late adolescence
- Academic achievement: Middle childhood and late adolescence
- Educational attainment: Late adolescence

6. Important Confounders, Mediators, and Effect Modifiers

Many of the covariates listed in the other hypotheses will impact this hypothesis as the health outcomes studied are wide ranging. Some examples are:

- **Genetic influences:** Genetic susceptibility to environmental stressors or predisposition for psychopathology that may be passed from parent to child can influence both family functioning and child outcomes.
- **Demographic variables:** Age, gender, language spoken, migration history, etc., may interact with family structure and processes (some demographic information will be exposures depending on specific hypothesis).
- **Parental education level:** Highest grade attained or participation in school or training programs may interact with family structure and processes.
- **Media influences:** Frequency and content of television viewing and video and computer use may impact child mental health outcomes.
- **Neighborhood characteristics:** Geographic area of residence, as well as neighborhood characteristics, may affect family resources and processes and influence child outcomes of interest.

7. Power and Sample Size

Starting with the birth cohort of 100,000, the minimum odds ratio between measures of the child's health and development and hypothesized exposures related to family influences will depend on the measures of exposure and outcome, the prevalence of the exposure and outcome, and the age at which the assessment is completed. The age of assessment determines the number of children retained in the study for analysis. For this discussion, higher levels of exposure are assumed to contribute to higher levels of the outcome. The calculations assume a target of 80 percent power using a two-sided 95 percent confidence interval and an intraclass correlation based on the NCS sample design.

The following table shows the minimum odds ratio that can be reliably detected as a function of assumptions about the prevalence of the outcome, exposure, and age of assessment.

Prevalence of the outcome	Prevalence of the exposure	Age of assessment	Minimum odds ratio that can be reliably detected
50%	50%	5	1.06
5%	50%	5	1.15
50%	5%	5	1.15
5%	5%	5	1.35
50%	1%	5	1.36
5%	1%	5	1.81
50%	50%	20	1.07
5%	50%	20	1.16
50%	5%	20	1.16
5%	5%	20	1.38
50%	1%	20	1.40
5%	1%	20	1.88

8. Other Design Issues

- **Ethical/burden considerations:** Family privacy must be protected for the collection of sensitive information on family climate. Nonetheless, detected instances of child abuse and/or neglect must be reported to authorities. Regarding burden, because this study will combine comprehensive measurement of both health and family dynamics, the burden on families must be considered in setting limits to the scope of measurement.

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IMPACT OF NEIGHBORHOOD AND COMMUNITIES ON CHILD HEALTH

1. Meta Hypothesis

Neighborhood and community influences are associated with both protective and risk factors within the social, physical, psychological, and environmental domains. These factors impact child development and physical and mental health status and outcomes across an individual's life course.

2. Specific Hypotheses

Neighborhood factors (both positive and negative) can impact child health and development in a variety of ways. Lower income, low-resource communities are characterized by a number of risk factors, such as crime and violence; more pollutants and other toxicants; and weaker or less stable social service, education and health care infrastructures. Higher income, high-resource communities are characterized by factors and infrastructures that support child health and development. The following are examples of specific hypotheses regarding neighborhoods and communities that guide the National Children's Study (NCS):

1. Neighborhoods characterized by lack of social cohesion and efficacy, inadequate social services, and higher levels of social isolation will increase maternal stress levels and impact pregnancy outcomes.

2. Residing within high stress, low resource environments impacts a child's own levels of stress and depression and increases the incidence of conduct disorders within children.

3. Neighborhoods with high levels of environmental pollutants (e.g., lead, mold, endotoxins) within housing and other physical structures will negatively impact cognitive development as well as contribute to the onset of childhood asthma.

4. Neighborhood safety problems related to physical dilapidation and interpersonal violence increase the incidence of intentional and unintentional injury and death. Greater exposure to interpersonal violence is associated with higher levels of aggression and other conduct disorders in children.

5. Neighborhoods with limited access to recreational activities contribute to an early onset of obesity-related chronic conditions including hypertension, heart disease, and diabetes.

6. Neighborhoods characterized by poor social infrastructures, (e.g., inadequate schools, health care and social services) impact family cohesion and stability and complicate the management of health problems such as asthma and diabetes.

7. Children of non-English speaking immigrant families living in linguistically isolated neighborhoods have poorer language and literacy skills and lower academic achievement.

8. Children who live in neighborhoods with higher exposure and easier access to alcohol and illicit drugs will be more likely to develop substance abuse problems and engage in delinquent activities.

3. Background and Justification

Health outcomes for children vary across geographic areas, from smaller micro environments such as local neighborhoods to broader, more macro ones, such as urban, suburban, and rural communities. These patterns of variation are due only in part to the characteristics of the individuals and families who live in these areas; they can also be attributable to systematic differences within different "community" environments.

Neighborhoods and larger communities vary in their structure. Structural characteristics of communities include characteristics of the built environment (e.g., density, housing quality and age, the distribution of parks and recreational facilities, and neighborhood walkability), hazards (e.g., noise, traffic, and crime), and residential stability and demographics (e.g., age, class, gender, racial, ethnic composition).

Neighborhoods and communities also vary substantially in the economic and social resources available to their residents. The economic resources of communities include employment rates and stability; business presence within the community; housing value and property tax; residential mobility; the stability, quality and accessibility of core community infrastructures including schools, public services, religious institutions, community associations and volunteers, and the location and accessibility to grocery stores. Scarce resources produce competition between residents which decreases the level of social cohesion and support and increases the level of relative deprivation within families. The quality of schools is correlated with the economic resources within neighborhoods and communities. These resources affect child health and welfare.

Community social processes that impact families and children include social cohesion and isolation, social organization and infrastructure, social norms, and collective efficacy and social capital. The levels of informal and formal social control impact crime and injury rates and drug and alcohol use and abuse. Socialization, both within the family and larger community structure, impacts how socially acceptable attitudes and behaviors and informal methods of social control are transmitted. Neighborhoods with familial stability allow for the family unit to operate as a moderating variable between neighborhood and community instability (e.g., increased crime and decreased social control). Community structure, resources, and processes are interrelated. How they affect child development and health in relationship with other determinants (e.g., biomedical) are important to uncover.

3.1 Public Health Importance

Prevalence/incidence

Examples of variations in children's health outcomes across geographic areas include:

- Among the 50 largest cities in the United States in 1991, infant mortality rates ranged from 5.3 per 1,000 births in Miami to 21.0 per 1,000 in Washington, DC, and the percent of births to mothers who received late or no prenatal care in 1994 ranged from 2 percent in Honolulu to 15 percent in Washington, DC (Annie E. Casey Foundation, 1997).

- A study of women living in central North Carolina found that residing in a wealthier census tract was associated with a 40 percent reduction in the risk of preterm birth for Black women (Kaufman, Dole, Savitz, & Herring, 2003).

- Asthma mortality and hospitalization vary significantly among large cities and among neighborhoods within cities. Within cities, asthma death rates are highest in areas with higher concentrations of poor people and minority residents (particularly African Americans) (Lang & Polansky, 1994; Carr, Zeitel, & Weiss, 1992). In Rochester, MN, the relative risks of developing asthma is 60 percent higher among children living close to major highway intersections or railroads (Juhn et al., 2005). Asthma prevalence is low among Mexican American children in the Southwest and high among Puerto Rican children in the East (National Academy of Sciences Institute of Medicine, 2000).

- In a study of community characteristics and blood lead levels in 20,296 children in Monroe County, NY, the overwhelming majority of those with elevated blood lead levels lived in the city. Other community-level variables associated with increased risk of elevated blood lead levels included: lower housing value, older age of housing, higher population density, higher rates of poverty, lower percent of high school graduates, and lower rates of owner-occupied housing (Lanphear, Byrd, Auinger, & Schaffer, 1998).

- Among a national sample of adolescents, those living in rural working class neighborhoods were almost 40 percent more likely to be overweight, and those living in exurban and mixed race urban neighborhoods were about 30 percent more likely to be overweight than those living in newer suburbs (Nelson, Gordon-Larsen, Song, & Popkin, 2006).

- Data from the Project of Human Development in Chicago Neighborhoods indicate that living in an unsafe neighborhood increased aggression in girls ages 9-13 (Molnar, Browne, Cerda, & Buka, 2005). Data from this study also showed that neighborhoods with high social capital had lower aggregate death rates for total mortality and a lower death rate due to heart disease for white men and women and, to a lesser extent, Black men and women (Lochner, Kawachi, Brennan, & Buka, 2003).

Economic and/or social burden

As illustrated above, a large number of child health and developmental outcomes have been associated with neighborhood or other measures of geographic location. The economic and/or social costs of an unhealthy environment depend on the specific health or developmental outcomes being examined as well as the combination of different risk and protective factors within these environments. The potential for improving child outcomes through interventions and other change strategies at the neighborhood and/or community level is large. Because such strategies can produce effects in multiple domains and many individuals, the public health impact can be far greater than individual-level efforts. For example, strategies to strengthen collective efficacy among local parents have the potential to empower them to request core social services and develop mechanisms of informal social control to reduce aggression and risk-taking behaviors, thus improving mental health among children. By building social capital, families may increase their ability to strengthen efforts to increase neighborhood safety, reducing rates of injury, victimization, and other adverse environmental exposures. Improving neighborhood housing conditions and quality can reduce exposure to multiple environmental hazards such as toxicants, resulting in reduced levels of asthma, injury, and lead poisoning. Improved housing also has the potential to reduce levels of neighborhood instability and crime, which in turn lead to a host of social protective factors such as those linked to positive child development.

3.2 Justification for a Large Prospective Longitudinal Study

Answering questions about the impacts of neighborhoods and communities on child health requires a multidisciplinary approach to theory and measurement, and a large, clustered probability sample that is representative of the diverse social, behavioral, and physical environmental characteristics that influence child development. This broad variability and diversity is necessary if we are to investigate the complex mechanisms through which neighborhoods influence child health and well-being. These mechanisms must be tracked longitudinally through multiple stages of child development. The vast majority of existing studies addressing neighborhood effects are cross-sectional, making it impossible to detect effects that occur later in time and the effects of neighborhood change and individual mobility on health. A prospective approach is also needed to detect potential developmental time frames during which neighborhood conditions may be especially important.

3.3 Scientific Merit

Considerable research has been conducted on the effects of neighborhoods on child and adolescent developmental outcomes. Much of this research has been focused on early childhood and adolescence, with a greater focus on developmental and behavioral outcomes than on health outcomes (Leventhal & Brooks-Gunn, 2000; Sampson, Morenoff, & Gannon-Rowley, 2002). In recent years, research on neighborhood effects related to health has expanded dramatically, with particular attention to child health outcomes including pregnancy and birth outcomes (Kaufman et al., 2003; Nelson et al., 2006) physical activity and overweight (Nelson et al., 2006), asthma (Wright, 2006), mental health (Truong & Ma, 2006), and risk behaviors (Cubbin et al., 2005).

The expanding literature on neighborhood effects cannot be fully addressed here. Three recent reviews (Burton & Jarrett, 2000; Leventhal & Brooks-Gunn, 2000; 2003) summarize the literature on the effects of neighborhood characteristics on developmental, health, and behavioral outcomes. Neighborhood socioeconomic status (e.g., measured in terms of income, unemployment, and percent of managerial and professional workers) has positive effects on school readiness, IQ, and achievement in early childhood and adolescence and on overall educational attainment. In addition, these neighborhoods have better school resources, more after-school activities, and better educated faculty. Low-socioeconomic status neighborhoods have also been associated with externalizing (acting-out and aggressive) behaviors and, less consistently, with internalizing (depressive and withdrawn) behaviors, as well as with teen sexual activity and childbearing (Leventhal & Brooks-Gunn, 2000). The effects of racial and ethnic diversity on behavior problems appear to vary depending on the race of the child and the socioeconomic status of the neighborhood. Neighborhood residential instability (or turnover in the neighborhood's population) has been linked to higher rates of behavioral problems such as delinquency and crime, but one study found higher rates of alcohol use in more stable neighborhoods (Leventhal & Brooks-Gunn, 2000).

Many studies find that neighborhood effects are complex, often influencing some groups but not others or influencing the relationships between other determinants and health outcomes. For example, research by O'Campo and her associates (1997) suggests that receiving prenatal care has less influence on birth weight among women living in high-risk neighborhoods than women living in low-risk neighborhoods. One study (Simons et al, 1996) linked community disadvantage to psychological distress and conduct disorders in boys but not girls, while another (Kupersmidt et al., 1995) found that higher neighborhood socioeconomic status acted to reduce levels of childhood aggression among children from single-parent families.

Research on neighborhood effects on obesity has increased rapidly in recent years. While many studies have pointed to the importance of the built environment (e.g., Gordon-Larsen et al., 2006), others suggest it is not just the built environment but also the interactions among land use, infrastructure, and social factors that create obesigenic environments (Lopez & Hynes, 2006; Boehmer et al., 2006; McNeil et al., 2006). Sallis and Glanz (2006) point out, however, that definitive evidence showing causal linkages between environmental factors and obesity remains elusive.

Theoretical models for linking individual behaviors to neighborhood effects have been proposed by several scientists. Jencks and Mayer (1990) propose five alternative mechanisms: (1) neighborhood institutional resources, (i.e., the availability of financial, social, and organizational resources that affect the ways in which a young person enters adulthood); (2) collective socialization, (i.e., the transmission of attitudes and behaviors through role models, supervision, and monitoring and other aspects of community social organization); (3) social contagion, (i.e., the spread of norms, values, and behaviors among residents of neighborhoods); (4) competition among neighborhood residents for scarce community resources; and (5) relative deprivation, (i.e., individuals' and families' assessment of their own well-being compared to the average economic level of the neighborhood). Leventhal and Brooks-Gunn (2000) suggest three complementary mechanisms: (1) availability of institutional resources (learning, health, recreation, etc.); (2) the mediating effects of parental relationships and support networks; and (3) the influence of community formal and informal institutions and norms that serve to guide and monitor behaviors.

In studies of child health and developmental outcomes, the role of the family in mediating and moderating neighborhood influences is crucial (Burton & Jarrett, 2000). Areas with few job opportunities would be expected to influence child outcomes through the effect of job scarcity on parental income, work, and stress. Also, areas that are high in crime may have differing effects on child outcomes depending on the parenting strategies parents adopt in relation to this environmental threat. Children may be affected minimally if they are closely supervised, but strongly if they are not.

Research on the mechanisms responsible for observed neighborhood effects is still in its infancy (Leventhal & Brooks-Gunn, 2000). The Project on Human Development in Chicago Neighborhoods has provided evidence that high levels of collective efficacy, defined as the extent to which neighborhood residents feel empowered to act together toward a common goal, can reduce rates of violent crime within neighborhoods (Sampson, Raudenbush, & Earls, 1997). Many other studies have similarly explored specific effects related to neighborhood resources, structure, and process. However, the research base is still too limited to determine which pathways are most important.

3.4 Potential for Innovative Research

By following children from birth to adulthood, the NCS offers an unprecedented opportunity not only for studying how differences in neighborhood and community factors impact both child development and individual and community health and well-being, but for examining how changes in these factors (whether from transformed communities or through mobility) can affect a child's outcome trajectory over time. These effects can be identified within context and across time, which is fundamental to understanding how influences on child development related to social, psychological, and biomedical factors vary throughout the developmental trajectory. Integrating these domains into one study will allow for a comprehensive understanding for how child development and health status are impacted within real-life settings.

3.5 Feasibility

The research design and study protocol for the NCS offer a variety of both quantitative and qualitative methodologies and instruments for obtaining the necessary data to measure the multiple dimensions of neighborhood and community. Many of these measurements will pose little inconvenience to the study subjects because much of the data can be collected independent of the study subjects. For instance, household location in relationship to crime and injury rates; population density; community infrastructure (including churches, schools, and grocery stores); drug and alcohol "hot spots" and many other characteristics can be captured using global positioning system (GPS) technology. Data available from the Census and the American Community Survey provide information on population characteristics, road networks and transportation grids kept by transportation departments, the locations of schools, commercial establishments and recreational facilities typically maintained by local planning boards, land-use patterns developed from remote imagery, and administrative data on local crime rates and enforcement. The linkage of these coordinates with other data is cost-effective and provides a dynamic multi-factor "map" to characterize local neighborhoods and larger communities. Once the links have been made, it is possible to measure neighborhood characteristics using several different operationalizations of "neighborhood" or "community." It will even be feasible to embed the smaller neighborhoods or communities into larger ones to understand how these smaller contexts are, in turn, influenced by the larger contexts. It will be important to record GPS household location each time the child moves in order to update the change in context as the child develops and passes through developmental milestones from infancy to adulthood. There are other measures, such as ethnographic observation and neighborhood canvassing, within the protocol that will also be of little inconvenience to the study subjects or residents of the targeted neighborhoods and communities. In addition, there are instruments within the protocol that will assess social and collective efficacy; social isolation and social capital; stability of and access to community infrastructure; and formal and informal social control.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/familial

- Neighborhood and community characteristics, including:
  - Structure (e.g., age, racial and ethnic composition, population density, housing stocks/quality, and health status of population)
  - Resources (e.g., income, quality of community organizations such as schools, recreational facilities, commercial outlets, public services, religious organizations, and employment opportunities)
  - Processes (e.g., social interaction, crime levels and law enforcement, and political activity)
  - Family's perceptions of the community and neighborhood; residence history (neighborhood cohesion, collective efficacy)
  - Physical observations of neighborhood condition (e.g., Superfund/hazardous waste sites, dilapidation), recreational facilities, sidewalks, public facilities (e.g., libraries), stores, etc.

4.2 Methods

Primary/familial

- Household surveys
- Direct observation
- Existing state and local databases (e.g., Census Bureau data)
- In-depth local studies or ethnographies (adjunct studies)

4.3 Life stage

- Familial: Prenatal and at moderate intervals (every two to three years) during childhood and adolescence

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- This hypothesis relates area of residence (neighborhood and community characteristics) to multiple health outcomes. For specific information on measurement issues, see hypotheses associated with:
  - Pregnancy and birth outcomes
  - Weight throughout childhood
  - Asthma incidence
  - Blood lead levels
  - Cognitive (executive function, IQ, etc.) and behavioral outcomes
  - Injury
  - Obesity and physical development

5.2 Methods

- Anthropometry
- Blood sample
- Direct testing/observation of child: Neuropsychological, cognitive, and behavioral tests; autism screening and diagnostic tests
- Injuries: Questionnaire/interview of parents, medical record review

- Asthma:
  - Examination, interview, and testing by medical professional (e.g., history of asthma symptoms, lung function tests, skin prick test, exhaled breath for NO, etc).
  - Medical record review
  - Cord and blood samples

5.3 Life Stage

- Anthropometry: Birth through age 21
- Blood sample: Birth (cord blood), periodically through age 21
- Direct testing/observation: Dependent on testing to be done; periodically through age 21
- Injuries: Periodically through age 21
- Asthma: Periodically through age 21

6. Important Confounders, Mediators, and Effect Modifiers

To differentiate the effects of neighborhoods from those of other exposures, analyses will need to adjust for confounders. Each health outcome is associated with an independent and unique set of covariates and confounders, although some overlap in individual factors, such as components of socioeconomic status, may occur. For example, analyses of the influence of neighborhood indoor and outdoor air pollution on asthma incidence and prevalence should be adjusted for exposures related to child care location if not in the neighborhood, and for genotype. The influence of neighborhood factors (e.g. educational infrastructure) on cognitive performance must be adjusted for parental education and IQ, socioeconomic status, and parenting practices. The influence of neighborhood on unintentional injury should be adjusted for type of child care arrangements as well as parenting practices. It is also important that the influence of neighborhood recreational facilities on individual obesity be adjusted for nutrient and caloric intake as well as genotype. The influence of neighborhood language isolation on language development and school readiness should control for parental and child IQ, socioeconomic status, language spoken by the child care provider, and whether or not the child attended pre-school.

7. Power and Sample Size

Given an exposure measure such as presence of a community characteristic, for example, parks), and a categorical outcome measure, for example, obesity, the relationship between exposure and outcome can be measured using an odds ratio. Starting with the birth cohort of 100,000, the minimum odds ratio that can be reliably detected in the NCS will depend on the specific measures of exposure and outcome, the prevalence of the exposure and outcome, and the age at which the assessment is completed. The age of assessment determines the number of children retained in the study for analysis. For this discussion, higher levels of exposure are assumed to contribute to higher levels of the outcome. The calculations assume a target of 80 percent power using a two-sided 95 percent confidence interval and an intraclass correlation based on the NCS sample design. The intraclass correlation for neighborhood effects is assumed to be 0.33 and the intraclass correlation for outcome is assumed to be 0.04.

The following table shows the minimum odds-ratio that can be reliably detected as a function of the age of assessment and assumptions about the prevalence of the outcome and exposure. For the calculations, the assumed outcome is either the presence of asthma at age 7 (with a reported prevalence of 8.5 percent) and the presence of infant mortality as of age 1 (with a reported prevalence of about 0.7 percent). Three values for the exposure prevalence are assumed to illustrate how the minimum detectable odds ratio varies with exposure prevalence.

Assumed outcome	Prevalence of the outcome	Age of assessment	Prevalence of the exposure	Minimum odds-ratio that can be reliably detected
Asthma at age 7	8.5%	7	50%	1.27
			15%	1.37
			5%	1.63
Infant mortality	0.7%	1	50%	2.28
			15%	2.52
			5%	3.55

For continuous outcome variables, such as IQ, the power can be measured by the difference in the mean IQ between the exposed and unexposed groups. This difference depends on the standard deviation of the observations in each group. The following table shows the minimum exposure-related difference in the mean IQ that can be reliably detected for exposures with prevalence of 5 percent, 15 percent, and 50 percent.

Assumed outcome	Standard deviation of the outcome	Age of assessment	Prevalence of the exposure	Minimum difference in the mean that can be reliably detected
IQ	15	18	50%	1.01
			15%	1.41
			5%	2.32

8. Other Design Issues

- **Ethical/burden considerations:** Data on neighborhoods and communities need to be handled carefully to reduce the risk of identifying specific participants.

- **Cost/complexity of data collection:** Neighborhood-level data needs to be collected using existing resources (e.g., Census Bureau data, other extant databases) and possibly new data collections. The study will need this data for neighborhoods in which participants originally live and those into which study participants move over time. Measures for various types and sizes of geographic unit will likely be required (e.g., states, metropolitan areas, school districts, census block groups). Measuring the social environment may also involve the collection and integration of information on the local areas in which participants live; community surveys of values, attitudes, and social processes; and observational studies of schools, religious organizations, and day care centers.

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IMPACT OF MEDIA EXPOSURE ON CHILD HEALTH AND DEVELOPMENT

1. Meta Hypothesis

Exposure to mass and electronic media from stationary and mobile sources can have both positive and negative short- and long-term effects on children. Home- or school-based media include television, video, and interactive media such as electronic games and the Internet. Multimedia mobile devices, including cellular phones, portable digital music players, and portable computers, integrate with traditional radio, television, print, and film media. The amount, type, content, and context of media exposure from infancy through adolescence influences brain and neurological development; cognitive and social development; and risk-behavior factors related to aggression, injury, substance use, sexual health, obesity, and other aspects of physical development. Exposure to specific media content will lead to developmental trajectories along a continuum of prosocial to antisocial behavior.

2. Specific Hypotheses

1. Exposure of children to educational media content enhances the development of cognitive skills and increases academic achievement. The effect of exposure to English-language educational media content (such as "Dora the Explorer" and "Blue's Clues") will be maximized in the developmental period of 2-5 years old, especially when other sources of cognitive support and stimulation are minimal and when English is not the first language.

2. The context and amount of exposure to violent media content (in television, movies/videos, and interactive video games) influences the risk of desensitization (including acceptance of interpersonal violence) and aggressive behavior (including injury by a firearm). This effect will be moderated by poverty, exposure to violence in the neighborhood or home, developmental delay or brain injury, and peer group aggression. A subhypothesis is that exposure to news and other "realistic" media violence increases the propensity for children to experience chronic fear and anxiety. This effect is increased by a predisposition for anxiety that may be genetically linked, reflective of living in high-crime neighborhoods, or developed through personal experience with victimization.

3. Background and Justification

Many claims exist regarding the impact of the mass media and especially the newer, more interactive, electronic media, on the health and development of American children. Some suggest media constitute a developmental risk factor, while others point to opportunities for enhancing children's positive development (American Academy of Pediatrics [AAP], 1999). Recent studies of early childhood exposure to media have concerned many in the public health community because America's youngest children are increasingly immersed in an electronic culture, yet there is no clear understanding of the impact of this media exposure on child health and development (Christakis & Zimmerman, 2006). From concern for the potential deleterious effects of early media exposure on neurobehavioral development and despite the absence of much scientific evidence at this point, the AAP recommended children younger than 2 not watch television and that children 2 and older be limited to 1-2 hours of educational "screen media" a day (AAP, 1999). Pre-existing and current research that focuses on the effects of broadcast television is becoming outdated with the increasing accessibility of competing portable technologies that integrate Internet, video games, traditional and satellite radio, music videos, and digital film. Given the ubiquitous presence of media in children's lives, it is imperative to document: (a) what aspects of contemporary media have positive and negative influences on development; (b) at what ages the media

have those effects; and (c) what individual and contextual aspects of younger children's lives put them at risk or opportunity for such developmental pathways. The outcomes for which media effects are hypothesized are central to children's health and development and to the National Children's Study priorities.

3.1 Public Health Importance

Prevalence/incidence

From infancy, American children are exposed to large amounts of media, including videos and television, computers and computer games, and printed media, although the amount and type of this exposure varies. Screen media use begins early, with 61 percent of children younger than 1 watching more than an hour a day and 90 percent of 4- to 6-year-olds watching more than two hours a day. Within this age group, daily time spent with books is considerably less than with screen media at about 40 minutes a day (Rideout, Vandewter, & Wartella, 2003). Beyond early childhood, media use generally increases in time spent, types of media used, and breadth of content. American children between the ages of 2 and 18 spend an average of five and one-half hours each day using media (often using more than one kind simultaneously) (Roberts & Foehr, 2004).

The presence in the media of many potentially noxious images has been well documented. The average child who watches about two hours of television daily will see about 10,000 violent acts per year (National Television Violence Study, 1997-98). Virtually all children experience some exposure to violent media content prior to age 10; those in the top quintile of exposure are considered most at risk of long-term effects (Lefkowitz, Eron, Walder, & Huesmann, 1977).

Studies have found that 70 percent of episodes on primetime television feature alcohol use (Christensen, Henriksen, & Roberts, 2000), and smoking is portrayed in almost all top-grossing movies (Dalton et al., 2002). The amount of sex on television has doubled since 1998, and only about 10 percent of sexual scenes in television programs popular among teens in 2005 contained a sexual precaution message (Kunkel, Eyal, Finnerty, Biely, & Donnerstein, 2005).

Studies have also linked adolescent and young adult health outcomes and behavior with music and music video preferences (Wingood et al., 2003). Music as a cause, indicator or predictor of sexual behavior (Martino et al., 2006), violence, and suicide (Martin, Clarke, & Pearce, 1993; Scheel & Westefeld, 1999) exhibits differentially in young males and females and among racial groups.

Each of these kinds of media content has been linked with corresponding negative health outcomes, including increased anxiety and fear (Cantor, 2001), aggressive behavior, physical assault and injury (Anderson et al., 2003), cigarette smoking (Sargent et al., 2005), and early initiation of sexual intercourse (Brown et al., 2006). Exposure to frequent advertising for non-nutritious foods and the time children spend using the media may also contribute to the epidemic of obesity (Institute of Medicine of the National Academies, 2006). A recent longitudinal birth cohort study conducted in New Zealand estimated that in 26-year-olds, 17 percent of overweight, 15 percent of raised serum cholesterol, and 15 percent of poor fitness could be attributed to watching television for more than two hours a day during childhood and adolescence (Hancos, Milne, & Poulton, 2004).

Because media influences are so widespread, even small effects of media on children's and adolescents' health and development can impact public health outcomes such as these:

- In 2002, 32 percent of violent crimes (including threatened and completed acts of violence) were committed by persons aged 20 or younger. Aggressive behavior can also result in unintentional injury (e.g., from motor vehicle accidents). Motor vehicle crashes are the leading cause of death for 15- to 20-year-olds; aggressive driving is implicated in two-thirds of fatal crashes (U.S. Department of Justice, 2003).

- Recent underage alcohol use was reported by 29 percent of youth aged 12 to 20 (Substance Abuse and Mental Health Services Administration, 2004).

- Obesity is a growing epidemic in America, particularly among children. About one-third of children aged 2-19 are at risk of overweight or are overweight (17 percent) (Ogden et al., 2006).

- More than one-third (34 percent) of U.S. ninth-graders and 63 percent of 12th-graders have had sexual intercourse (Centers for Disease Control and Prevention [CDC], 2006). Young people ages 15-24 acquire half of all new sexually transmitted infections (STIs) each year in the United States and most remain undiagnosed and undetected (Weinstock, Berman, & Cates, 2004). If untreated, STIs can lead to serious health consequences, such as infertility and death (Chesson, Blandford, Gift, Tao, & Irwin, 2004).

Economic and/or social burden

Many of the potential outcomes of media exposure have long-term health costs. For example, incarceration for violent crimes results in earnings loss between 10-30 percent, and decreasing wage growth over the lifecycle that is also associated with lower levels of mental and physical health and lower life expectancy (ChildTrends Data Bank, n.d.). It is estimated that teen childbearing costs taxpayers at least $9.1 billion a year (Hoffman, 2006). The total estimated burden of the nine million new cases of STIs that occurred among 15- to 24-year-olds in 2000 was $6.5 billion (Weinstock et al., 2004).

Preventability/malleability

The negative effects of media can be ameliorated and the positive effects enhanced primarily through education of parents and children. Media exposure is potentially malleable, especially when children are young because media are used at the discretion of parents. One intervention that encouraged parents and children to reduce the amount of time the children spent watching television and videotapes and playing videogames resulted in lower body mass index among elementary school children (Robinson, 1999). Research suggests parental mediation can reduce the negative effects of media use, but it is not clear what strategies are most effective (Vandewater, Park, Huang, & Wartella, 2005).

3.2 Justification for a Large Prospective Longitudinal Study

Much of the prior work on media effects has been cross-sectional rather than longitudinal, which makes it difficult to assess the role of media in growth and development through time. Some recent longitudinal findings have been based on samples in other countries that could be similar to the United States (Hancox, Milne, & Poulton, 2005). Although some important exceptions exist in the United States, (e.g., research on the effects of violent television viewing [Huesmann & Eron, 1986]; research on educational television viewing [Anderson, Huston, Schmitt, Linebarger, & Wright, 2001]); and the PSID

Child Development Supplement Data, CDS I and II, [http://www.psidonline.isr.umich.edu/CDS/]), little is known about how long-term media use affects children. Since no studies have had the capacity to study the effects of media exposures on children's health and development starting at birth, the National Children's Study could measure the effects of media exposures controlled by parents and not children. Most of the longitudinal studies relied on convenience samples, locally random samples, or some combination of the two, obviating the ability to assess prevalence and differences in impacts across population groups. The National Children's Study (NCS) will provide the opportunity to track media diets early and through time, examine how uniform these patterns are across subgroups, and assess multiple prosocial and antisocial outcomes.

3.3 Scientific Merit

The underlying conceptual model is that exposure to electronic screen media until age 2 influences attention and cognitive development negatively, and exposure after age 2 produces results dependent on type of content. Exposure to educational/prosocial content across different kinds of media predicts compliant behavior, school readiness, and school achievement. Exposure to violent/antisocial content predicts noncompliant behavior, antisocial behavior, and aggression.

Media exposure may affect children's development beginning in infancy through a number of pathways. Although studies have consistently shown book exposure is associated with positive consequences, particularly for the development of literacy, the effects of early exposure to electronic media are less clear. Some studies have indicated exposure to video at those younger than 2 is associated with attention deficit symptoms and slower cognitive development (Thakkar, Garrison, & Christakis, 2006). Current theory suggests both direct interaction of children with screen media and distractions caused by exposure to background media may affect neurodevelopment. Increasingly, television programs and videos (e.g., "Teletubbies" and "Baby Einstein") are created for children younger than 2. Although this content is promoted as educational for infants and toddlers, recent studies have indicated children younger than 2 show little to no learning from video as compared to equivalent live demonstrations (Kuhl, Tsao, & Liu, 2003). Time spent watching TV may also displace other potentially more valuable activities basic to neurodevelopment, such as active play or parental interactions.

As cognitive abilities mature, media may influence children's perception of normal and abnormal social behavior, social reality, and cultural norms. Most evidence suggests that the kind of content matters, and different media diets will lead to different outcomes. Short-term prospective studies, for example, have shown adolescents with aggressive tendencies tend to seek out violent content in action films, video games, and Internet sites, and subsequently behave more aggressively than adolescents exposed to less violent content. The bidirectional process may result in the development of antisocial behavior (Slater, Henry, Swaim, & Anderson, 2003). Some theory suggests the amount of exposure to the narrow and typically violent view of the world offered by television results in the adoption of that world view (Gerbner, Gross, Morgan, Signorielli, & Shanahan, 2002).

Much of the research on the effects of media on social behavior based on Bandura's cognitive social learning theory (Bandura, 2001) suggests children learn how to behave by watching attractive adult role models, both in real life and vicariously through the media. Desensitization theory suggests young people exposed to certain types of graphic media depictions (e.g., sex and violence) may become less reactive to these stimuli, and, in the case of violence, more indifferent to the plight of others (Molitor & Hirsch, 1994). The effects of media on social development (e.g., sharing, showing empathy, etc.) has been best demonstrated in the area of children's educational programs (Friedrich & Stein, 1973). Shows such as "Sesame Street" and "Barney" have been successful in eliciting specific types of prosocial behaviors when those behaviors were modeled in the program. We expect that the more consistently

children are exposed to prosocial content across media (e.g., television, computer/video games, Web sites), the more they will behave in prosocial rather than antisocial ways.

3.4 Potential for Innovative Research

The cohort of children and the measurement of social, psychological, and biological development makes possible the examinations of complex interactions involving multiple aspects of the child's social environment, biological predispositions, and dynamic media diets that have not been possible. The longitudinal design provides an unparalleled ability to demonstrate associations between media exposures and developmental outcomes in children. The types of media to which children are exposed are expected to continue to change throughout the Study. Most research focuses on television. Although it is expected television will continue to be a central medium in children's lives, the proliferation of new media provides a ripe area for new research. The majority of existing research is focused on school-age children and adolescents and on one outcome at a time; young children are often excluded. As discussed, even young children are now spending time with electronic media. The study provides a unique opportunity to examine the short-term and long-term effects of such early media exposure on multiple outcomes.

Prior research indicates aggressive antisocial behavior is increased by exposure to violent content and decreased by educational and prosocial content whereas academic achievement is enhanced by exposure to educational and prosocial content and diminished by violent content. A media diet rich in prosocial/educational content has been associated with increased high-school grades, greater literacy, and reduced aggressiveness during adolescence (Hoffman, 2006). Although findings of this research suggested different developmental trajectories, there is little information about whether particular subgroups or ages are more or less susceptible to media influence and to what extent antisocial behavior and academic achievement are mutually influencing (Chesson et al., 2004). It is also not clear if negative trajectories can be interrupted. Research conducted throughout the NCS has the potential to fill these gaps in understanding and may shed brighter light on how both positive and negative media exposure impacts children's development.

3.5 Feasibility

Critical periods for exposures and outcomes

The first two years of life are thought to be critical for media effects on neurodevelopment. The preschool years are key ages for cognitive development and the development of other abilities necessary for school readiness. Social development may be affected by the media throughout childhood. Engagement in risk behaviors is likely a function of media exposures throughout childhood and adolescence.

Availability of needed subgroups, settings, strategies

There are no special needs. The large sample should provide sufficient variation in exposure and outcomes.

Measurement tools

Valid and reliable measures of most exposure and outcome variables of interest exist, although some have not been tested with all age groups or do not exist in languages other than English. Types of media exposure and measurement tools will be updated as the child, new media and measurement technologies develop. Interview time is the primary burden for parents and children. The

use of new technologies for monitoring media use may be helpful in reducing that burden. Parents typically appreciate being asked about their child's media use because they are interested in the media's prosocial and antisocial effects (Rideout & Hamel, 2006), and older children and adolescents enjoy thinking about their own use of the media.

4. Exposure Measures

Exposure will be defined as time exposed to particular categories of content: educational (subdivided into primarily prosocial or academic), violent (titles that contain one or more instances of violent or antisocial behavior), and other (neither educational/prosocial or violent/antisocial) (Hoffman, 2006).

4.1 Individuals Targeted for Measurement

Primary/parental

- Audiovisual media: Frequency and content of television viewing, video games, and computer use
- Print media: Frequency of reading to child (up to age 5)

Primary/child

- Stationary audiovisual media: Frequency and content of television/movie viewing, video game and computer use at home, school, library or other neighborhood sources (age 6 and periodically through adolescence)
- Print media: Frequency of reading for pleasure and school (age 6 and periodically through adolescence)
- Use of mobile multimedia devices: Frequency and content of Podcasts, Internet, radio, games and movies (age 6 and periodically through adolescence)

4.2 Methods

Primary/parental

Interview parent attitudes toward media (prenatal, 2, 5, 7, 9, and 12 years) and parental mediation of media use (24 and 36 months, 5, 7, 9, 12, and 16 years) (Valkenburg, Krcmar, Peeters, & Marseille, 1999). Another potential measurement tool could be a family media diary, to be maintained for one weekday and weekend day. A parent will record a child's media use until the child is old enough to accurately record personal media consumption. Minutes of media use for each family member will be noted along with type of medium (text, Internet, computer game, TV program, or DVD) and for the child, the title of the media program.

Primary/child

Assessments will include interviews (after age 9), direct observation, household surveys of available media, and media-use diaries tied to analysis of content. In the sixth and later years, it is expected the child's exposure to electronic audio and audiovisual media can be automatically monitored by a pendent or bracelet device worn by the child. Several commercial entities are developing such items

to assess media exposure. The technology makes use of an inaudible signal embedded in the audio track that provides the title. The technology will automatically record the title, time, and duration of exposure. The data can then be linked to content analysis to calculate each child's exposure to prosocial and antisocial content.

4.3 Life Stage

Parent attitudes toward media and parental mediation of media use

- Primary/parental (periodically, prenatal through adolescence)

Family media diary

- Primary/child (periodically, birth through adolescence)

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Although the primary outcomes will be any major antisocial behavior (as indicated by criminal record) and scholastic achievement (obtained from high school and post-high school academic records), subcategories of outcome will include aggressive behavior and language and cognitive development measured starting in preschool. Violence, sexual health, and attitude outcomes will be measured from preadolescence through early adulthood.

5.2 Outcome Methods

Measures of neurological, cognitive, social, and physical development of the child will be collected for various other study hypotheses and should include school records, medical records, and criminal records (in adulthood).

5.3 Life Stage

Periodically, birth through late adolescence and early adulthood.

6. Important Confounders, Mediators, and Effect Modifiers

A number of mediating and moderating variables will affect both media exposure and outcomes. From an ecological perspective, these include:

- Factors associated with neighborhoods/school environment such as likelihood of exposure to violence, child care, school quality
- Factors associated with the home environment such as socioeconomic status, number of adults and children present, languages spoken, exposure to domestic violence, exposure to alcohol and drug abuse
- Factors associated with the parents such as stability of marital union, parenting style, maternal depression, parental co-viewing and media discussion

- Factors associated with the child such as race/ethnicity, genetics, learning disabilities, psychological predispositions (e.g., sensation seeking, anxiety), conduct disorders, trauma and brain injuries, sleep disorders, language development, social alienation

7. Power and Sample Size

For the specific hypothesis that children from non-English speaking families regularly exposed to English-language educational programming during the preschool years will be 1.5 times more likely to be school-ready than children from non-English speaking families who are not regularly exposed to English-language educational programming, there is a .05 level of significance for a difference in odds of 1.5, (17 percent of children who are regularly exposed to English language educational programming will be school-ready relative to 12 percent of those who are not exposed to English language educational programming) with power of .90.

For the specific hypothesis that children who are in the top quintile of exposure to violent media will be 1.5 times more likely to experience a firearm injury during the period from birth to age 21 than are children who are less exposed to violent media, there is a .05 significance level for a difference in odds of 1.5 (top quintile = .008, other quintiles = .005) with a power of .90.

8. Other Design Issues

- **Cost/complexity of data collection:** A unique aspect of data collection for this hypothesis includes gathering and archiving samples of various media types including television programs, videos, and video games.

- **Cost of sample analysis:** Media content analysis (i.e., coding, categorizing, etc., the samples of videos, television programs, video games) can be costly but can be archived for future analysis using separate funds.

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SOCIAL INSTITUTIONS AND CHILD HEALTH AND DEVELOPMENT

1. Meta Hypothesis

Interactions between children and families and the formal child care, school, and religious institutions in their communities influence children's cognitive, social, and emotional development.

2. Specific Hypotheses

1. The physical and social environments of nonparental child care settings influence child health and cognitive and social functioning. Variations in the quality of child care affect child outcomes. Child care mediates some family influences on child outcomes.

2. Children's participation in schools affects social, emotional, and physical development. Provision of health services and of curricula targeting health promotion directly influences children's health and mental health outcomes. Child, family, and community factors interact with structural and functional aspects of schools to shape child development.

3. Family participation in religious organizations during early and middle childhood (ages 3-10) results in better emotional health and fewer health-compromising behaviors during middle adolescence (ages 14-15). These effects are stronger in female children, ethnic minority and immigrant families, in impoverished areas, and when the religious organizations provide effective mechanisms for integrating adolescents into the life of the religious community.

3. Background and Justification

Formal institutions available to children and families in a community are key contextual variables that influence children's cognitive, social, and emotional development. Although a number of institutions are influential for child health and development, child care, schools, and religious institutions are particularly influential among very broad sectors of the population. In more limited studies, these institutions all have been found to have a significant influence on children's physical and mental health outcomes.

Although there have long been avenues of inquiry addressing institutions as important contexts for development (particularly for emotional adjustment and mental health outcomes), there rarely have been opportunities to examine the interactive influence of multiple institutions or the interactions between institutions and other important developmental contexts, such as family or neighborhood.

Many of the institutions encountered by children and families are publicly supported organizations that serve large numbers of individuals. They represent settings that are potentially modifiable in ways that enhance children's development or settings where specific interventions or treatments could be delivered. Almost all children attend school for the majority of their childhood years; large numbers also are involved in preschool and child care programs. The identification of the factors within these programs that influence developmental pathways is critical to efforts to reform or reshape the organizations that serve children.

To date, there have been no population-based, prospective longitudinal studies that examine the influences of child care on children's health and development. The most comprehensive study, the NICHD Study of Early Child Care and Youth Development, followed about 1,300 children from birth through age 15 at 10 sites across the country. The participants, however, were not representative of the

current U.S. population and low income children were underrepresented. Similarly, no research of this scope has been conducted on the impact of school characteristics and family religion on children's health and development. Although the Department of Education has undertaken a number of studies on schools, none has been a longitudinal study that follows children from birth through high school, and thus none has had the capacity to look at complex interplay over time between early family and neighborhood contexts, schools, and child and adolescent outcomes. This research will permit examination of interactions between diverse family and institutional environments as predictors of children's cognitive, social, and emotional development.

3.1 Public Health Importance

Prevalence/incidence

Examples of the prevalence of exposure to child care, school, and religious institution influences and the nature of those exposures include:

- **Child care:** Estimates from the 2001 National Household Education Survey showed that 41 percent of infants younger than age 1 were in regular nonparental child care arrangements; by age 4, nearly four-fifths of all children were in child care (Mulligan, Brimhall, & West, 2005).

- **Schools:** Almost all American children attend school outside the home, and most of those children attend public school. School facilities may be a significant source of environmental hazards. The U.S. General Accounting Office (GAO) estimated in 1995 that about 60 percent of the nation's schools were in need of major repairs (GAO, 1995). Apart from the physical risks, deteriorating school buildings detract from the learning environment and require the diversion of resources from the school's instructional mission. In 2001, the American Public Health Association raised concerns about children's exposure to lead, radon, mold and moisture, asbestos, inadequate plumbing, poor lighting, and indoor air pollution in school buildings (American Public Health Association, 2001).

- **Religion:** Among adolescents aged 13-18, 87 percent are affiliated with an organized religion, 80 percent pray, and 40 percent pray daily (National Study of Youth and Religion, 2002). More than half of all American teens attend religious services at least monthly, with 38 percent attending every week (National Study of Youth and Religion, 2002). More than half are involved in religious youth groups at some point during their high school years. Both religious affiliation and practice vary significantly by region, gender, race, and urban/rural residence (National Study of Youth and Religion, 2002; Wilcox, Rostosky, Randall, & Wright, 2001).

Economic and/or social burden

There is a tremendous burden being borne by families, communities, and the nation, for detrimental social, emotional, and cognitive child outcomes, although exact impact depends on the specific outcome being examined. The annual cost of depression alone in the United States ranged between $44 billion and $53 billion in 1990. Adjusted for inflation, this estimate would be close to $70 billion today (Greenberg, Leong, Birnbaum, & Robinson, 2003). The rate of depression among adolescents may be of particular concern, as some population estimates suggest that adolescent depression

has a prevalence of more than 8 percent (Birmaher et al., 1996), which is higher than the rate among adults.

Societal costs associated with juvenile aggression and delinquency are difficult to estimate but include both immediate financial costs and the long-term costs of each individual's contributions to society. By age 17, 37 percent of juveniles report that they have engaged in vandalism, 27 percent report assaulting someone with the intent to injure, and 8 percent report belonging to a gang. Less supportive family structure and a lack of engagement in school are both related to negative outcomes. However, in some situations, school may pose its own risks, with 4 percent of teens in 2003 reporting they had been injured during a fight at school (Snyder & Sickmund, 2006).

These patterns are complex, and determining both burden and avenues for intervention requires a multifaceted understanding of the influences. Nonetheless, the quantity and nature of social institutions to which children and families have access can be directly influenced by targeted policies. Because most children attend public schools, and because child care is becoming an increasingly public issue, understanding the role of social institutions in children's development is a worthwhile undertaking.

3.2 Justification for a Large Prospective Longitudinal Study

A large, longitudinal study will permit the kind of exploration of the interplay between levels of influence in a child's life that has not been possible in smaller cross-sectional studies.

Exposure to social institutions may vary considerably across families, communities, and regions. Different forms of child care may be preferred, religious involvement and affiliation varies, and schools have diverse characteristics. Individuals who have experience with particular combinations of institutions can cluster into population subgroups too small to be studied adequately in most research. Only a large, representative sample like that in the NCS can hope to document fully the range of supports and risks posed by the intersection of family and neighborhood qualities and their social institutions.

These patterns of environment-environment interaction may also be extremely complex, and the same type of institutional experience, for example, time spent in a center-based child care during infancy, might have different influences on children depending on both familial characteristics and subsequent school contexts.

To be best understood, institutional influences must be studied prospectively over time. Although institutions themselves may continue to exist over time, capturing their characteristics or an individual child's experience of the institutions retrospectively would involve fundamental methodological biases. Different institutions also become important for children at different developmental periods, such as the sequencing of early child care and subsequent school. Religious institutions may play a different role for youth as they enter adolescence than they did during childhood. In addition to these direct exposures at particular times in development, institutions may have indirect influences on later development as they are mediated through or moderated by family characteristics.

3.3 Scientific Merit

Child care: Quality of child care arrangements is consistently found to be associated with child outcomes in cognitive, social, and health domains (Deater-Deckard, Kinkerton, & Scarr, 1996; NICHD Early Child Care Research Network, 1998). In particular, the nature of the relationships and interactions between children and their caregivers is an essential aspect of quality shown to be related to developmental outcomes. In addition, structural aspects of the setting, including group size, staff-to-child ratios, and staff training are consistently shown to be associated with health and developmental outcomes,

including incidence of infectious disease, attachment, social orientation, and peer competence (Clarke-Stewart, Gruber, & Fitzgerald, 1994). The influences of child care have been demonstrated even when controlling for family-level factors that are likely to influence development (Clarke-Stewart, Gruber, & Fitzgerald, 1994). The effects, however, appear to be stronger for certain groups, such as high-risk children and low-income children. The NCS will permit an exploration of child care at a level of diversity of both the child care context and subgroup effects that has not yet explored through research.

Schools: Schools provide many direct services and offer curricula that influence physical and mental health. These include heath and physical education programs, special education services, counseling and therapeutic services, and programs aimed at the prevention of risky behaviors. Moreover, structural and interactional features such as lack of strong instructional practices; class size; school climate; teacher expectations; student perceptions; promotion of cooperation or competition; involvement of parents and community members; and feelings of safety and security are among the many variables that may influence health more indirectly through cognitive and social development (Roeser & Eccles, 2000). School facilities are themselves a potentially significant source of environmental hazards, including lead, radon, mold and moisture, asbestos, inadequate plumbing, poor lighting, and chemical toxins, such as cleaning and instructional supplies (American Public Health Association, 2001). Therefore, there is a need to examine longitudinally the influences of schools and school experiences on students' health and development.

Religious institutions: Research on the determinants and consequences of children's individual involvement in religious organizations is lacking as their primary involvement with religion seems to be through engagement of the entire family, but there is a significant body of research that links adolescent health behaviors to religiosity. Focus groups have found that parents feel that church involvement of their children prevents high-risk behaviors (Sim, Jordan-Green, & Wolfman, 2005) and that churches may play an important role in promoting healthy attitudes (Smith, Faris, Denton, & Regnerus, 2003). Adolescents who regularly attend church services and say religion is important to them are more likely to use seat belts, exercise, maintain a healthy diet and sleep habits, and have positive self-esteem; and they are less likely to initiate sex at an early age, smoke, drink, and engage in delinquent behaviors (Regnerus, 2003). Religious organizations may influence parents and children via social control, social support, and values and identity (Wallace & Williams, 1997). Family participation in religious organizations and religious practice influence children's health and development indirectly through parenting, the child's social context, and the child's internalization of values, and these influences cumulate over the course of development. Further, influences are modified by gender, race, ethnicity, poverty, immigrant status, and characteristics of the religious organization (Roeser & Eccles, 2000).

3.4 Potential for Innovative Research

Bio-ecological theories of human development (Bronfenbrenner, 2005) have long posited that human development unfolds over time as a consequence of a synergistic set of contextual influences. These influences exist on multiple levels and include, but are not limited to, the individual's biological and social characteristics, family relationships, social institutions, community, and society. This theoretical view has generated substantial research over time, but few studies have been able to examine the interplay of even the first three elements of human ecology on a large scale. Because of limitations in study scope and sample size, most research has been limited to a single type of social institution (e.g., school) or even a subtype of institution (e.g., kindergarten classrooms).

There have been few opportunities for observing developmental trajectories within multiple contexts, and studies focusing on children in context often have not taken social institutions into account. Moreover, the extent to which organizations interact with one another remains largely unexplored, although there are examples from the prevention literature that suggest that systems-level interventions

may have significant impacts. There has been little prospective longitudinal research that has examined the simultaneous influences of family, child care, and schools on children's health and development, and no major study that has used a representative population. Research on the effects of family religious practices on children's health and development has been even more limited in scope, often covering individual subgroups of religion and rarely examining complex environment-environment interactions.

3.5 Feasibility

It is important to narrow the research questions and choose carefully what to measure. Measurement of institutional influences will be required at several time-points during childhood. Over the course of development, children's interactions with institutions will change and broaden, with different institutions coming into prominence during different developmental periods, and different dimensions of those institutions becoming more salient at different points in the course of childhood.

Because of the broad nature of the sample, a wide variety of child care arrangements, school settings, and religious institutions will be represented in the National Children's Study (NCS). This will permit evaluation of characteristics of many settings and many subgroups of institutions.

Assessment of child social, emotional, and cognitive outcomes will be addressed using a multi-method approach, obtaining data from parents, directly from children, and from reporters external to the family such as teachers or observers. Multi-method research ensures better psychometric properties of constructs than would be obtained through single measurements. Assessment tools for all of these outcome domains exist already within the field of child development research, and show excellent internal consistence and test-retest reliability as well as convergent validity across reporters and measurement modalities. Measurements will also be repeated over time, ensuring that trajectories of child health and adjustment can be tracked as various institutions exert their influences over time.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary maternal/paternal/child

Family measures

- Religious affiliation, religiosity, religious practice
- Family structure
- Parenting practices
- Social Support from religious institutions and other sources

Primary maternal/paternal/child

Social institution measures

- Child care characteristics
  - Structural aspects (presence of a stimulating environment, group size, staff ratios, etc.)
  - Functional aspects (caregiver interactions, continuity, curriculum, policies, etc.)
  - Timing of placement in childcare, number of hours spent per day, etc.
- Child care sub-study
  - The child care substudy development group has proposed that a subgroup of participants will be assigned to the child care assessment cohort prior to the first standard data collection point. Approximately 79 percent of that cohort is expected to use some level of regular nonparental child care at sometime during childhood. At each regular visit with this cohort, permission to seek information from the child care provider would be obtained. For children who are in regular child care outside the home for a specified time and duration, for example 30 or more hours per week in the past month, a site visit would be conducted at the child care location. For children in regular care for a lesser time and duration, 10 percent of the cases would have site visits conducted. Consequently, hypotheses that involve data collected at the regular child care location are anticipated to be collected on a subset of the NCS participants.
- School characteristics
  - Programs and policies (e.g., learning disabilities, health promotion, breakfast and lunch programs, violence/drug use/high-risk sexual behavior prevention, etc.)
  - Structural (e.g., class and school size, instructional weaknesses, safety)
  - Health and mental health counseling services
  - Facilities (condition, indoor air quality, cleanliness, safety hazards, exposure to toxics)
- Religious organization characteristics
  - Teachings, policies
  - Social cohesion
  - Activities for children, youth, and adults
  - Size

4.2 Methods

Primary maternal/paternal/child

Family measures

- Interview
- Observation of parent-child interaction

Primary maternal/paternal/child

Social institution measures

- Interviews and questionnaires (child, parents, child caregivers, teachers); administrative/school records; direct observation

4.3 Life Stage

Primary maternal/paternal/child

Family measures

- Prenatal and ongoing through adolescence

Primary Maternal/Paternal/Child

Social institution measures

- Child care: Annually during the preschool years; once during year that the child enters school; once during the 6-8 year period; once during the 8-11 year period
- School: Upon entry into school; during middle childhood; as children enter adolescence; as children prepare to leave high school
- Religious institutions: Preschool years; middle childhood; middle adolescence

5. Outcome Measures

5.3 Outcomes Targeted for Measurement in Child

- Social and emotional function
- Risk-taking behavior and aggressive behavior
- Cognitive function (also see measurement issues associated with neurodevelopmental and behavioral outcomes in hypotheses focused on neurodevelopment and behavior)
- Religious involvement

5.4 Methods

- Questionnaires/interviews with child, parents, caretakers (measuring child cognitive, social, and behavioral characteristics)
- Direct testing/observation of child (neuropsychological, cognitive, and behavioral tests; social function)
- School records review (grades/performance/behavior)

5.4 Life Stage

- Timing varies depending on the specific outcome: Preschool years, middle childhood, and middle adolescence

6. Important Confounders, Mediators, and Effect Modifiers

- Family factors (parental education level, number of siblings, socioeconomic status, shared family genetic characteristics, etc.)
- Neurotoxic environmental exposures (lead, pesticides)
- Environmental exposures to neurotoxicants have been shown to affect neuro-psychological function including alterations in cognition, sensory, motor, social, emotional development and executive function
- Injury (repeated head trauma)
- Neighborhood attributes (e.g., noise, community violence, etc.)
- Media factors
- Prenatal infection

7. Power and Sample Size

The following calculations assume a birth cohort of 100,000 children, 98 percent retention per year, and a target power of 80 percent using a two-sided 95 percent confidence interval. The calculations also have some assumptions about the intraclass correlation (a measure of clustering) of the outcome and exposure data.

With subjects taking an achievement test at age 18, the NCS would be able to detect a score decrement when comparing high to low exposure quartiles of approximately 0.61 points (where the population standard deviation is 15).

For categorical data, the minimum odds ratio between measures of the child's health and development and hypothesized exposures related to social institutions will depend on the measures of exposure and outcome, the prevalence of the exposure and outcome, and the age at which the assessment

is completed. The age of assessment determines the number of children retained in the study for analysis. For this discussion, higher levels of exposure are assumed to contribute to higher levels of the outcome. The following table shows the minimum odds ratio that can be reliably detected (i.e., with a power of 80 percent) as a function of assumptions about the prevalence of the outcome and exposure. All assessments are assumed to occur at age 18.

Prevalence of the outcome	Prevalence of the exposure	Age of assessment	Minimum odds-ratio that can be reliably detected
80%	50%	18	1.09
80%	10%	18	1.15
80%	5%	18	1.21
50%	50%	18	1.07
50%	10%	18	1.11
50%	5%	18	1.16
10%	50%	18	1.11
10%	10%	18	1.19
10%	5%	18	1.27
1%	50%	18	1.39
1%	10%	18	1.62
1%	5%	18	1.87

The calculations above assume the exposure and outcome measurements are individual measurements on each child and assume an intraclass correlation of 0.04, believed to be appropriate for such measures from the NCS sample design. When the exposure is a community characteristic, such as a school characteristic, the intraclass correlation will be larger. However, an appropriate value is difficult to estimate. On the assumption that a value of 0.33 is reasonable, the following provides approximate results when the exposure is a community variable.

With subjects taking an achievement test at age 18, the study would be able to detect a score decrement when comparing high to low exposure quartiles of approximately 1.07 points.

For categorical variables, the following table shows the minimum odds-ratio that can be reliably detected (i.e., with a power of 80 percent) as a function of assumptions about the prevalence of the outcome and a community level exposure.

Prevalence of the outcome	Prevalence of the exposure	Age of assessment	Minimum odds-ratio that can be reliably detected
80%	50%	18	1.18
80%	10%	18	1.34
80%	5%	18	1.52
50%	50%	18	1.14
50%	10%	18	1.25
50%	5%	18	1.36
10%	50%	18	1.25
10%	10%	18	1.42
10%	5%	18	1.59
1%	50%	18	2.01
1%	10%	18	2.47
1%	5%	18	3.09

8. Other Design Issues

- **Interview and confidentiality:** Assessment interview time is the major burden for parents and children. Teachers and child care providers, particularly in less formal and unregulated settings, may experience data collection as intrusive and have concerns about confidentiality.

- **Religious diversity and sensitivity:** Care will be taken to treat religious differences with sensitivity.

- **Cost/complexity of data collection**: Some training will be required for administration of assessments, but it will not be extensive. Efforts will be made to select reliable, valid, and low-cost instruments requiring limited training.

- **Need for community involvement**: Measuring the social environment will involve the collection and integration of information on the local areas in which participants live. Gathering administrative data will require the agreement of the respondents as well as the cooperation of agencies that hold data.

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INFLUENCES ON HEALTHY DEVELOPMENT

1. Meta Hypothesis

Positive influences and protective factors in children's development, including family processes and parenting, biologically based child characteristics, and access to and use of high quality community services, have direct and indirect positive effects on development. These positive influences promote competence and buffer the negative effects of social, environmental, and biological risk (e.g., poverty, stress, birth weight/gestational age, integrity of cognitive, sensory, and motor systems, genetic polymorphisms) on development, leading to healthy cognitive, social, and physical child outcomes.

2. Specific Hypotheses

1. Good parental marital quality and positive parenting behavior will promote positive social outcomes for all children but particularly those at risk for poor outcomes due to low birth weight, family stress, or poverty.

2. Heritable or biologically based child characteristics, such as intelligence and temperament, will lead to positive outcomes in the face of adversity in both academic and socioemotional domains.

3. High quality child care experiences will promote positive cognitive, social, and language development, both among children in general and particularly among children at risk for poor developmental outcomes due to poverty.

4. Parental access to and use of developmentally-oriented health and social services will promote better physical, social, and academic success in children who have social or biological risk factors.

3. Background and Justification

While some research does focus on healthy development in children and youth, traditional research emphasis has been on assessing the presence or absence of pathological outcomes rather than the presence of positive outcomes. There is an important need to be able to measure the impact of a range of environmental influences not just on specific pathological outcomes but also on individual differences in positive development. A thorough understanding of healthy development in all children, and particularly in children at risk for poor developmental outcomes, informs scientific understanding of both healthy and maladaptive developmental trajectories (Cicchetti & Rogosch, 1996) and permits a more informed approach to initiatives and interventions that promote healthy development.

The core outcome concept in positive development is competence. Competence is defined as successful adaptation and healthy functioning (Masten, 2001; Masten & Coatsworth, 1998; Waters & Sroufe, 1983). Competent children and adults are able to use the resources available to them in the environment to adapt successfully to life's demands and challenges (Waters & Sroufe, 1983) and to be productive members of society. Competence as an outcome in research may be conceptualized as specific to a particular age period, specific to a domain of functioning, or some combination of the two (Masten & Coatsworth, 1998).

3.1 Public Health Importance

Prevalence/incidence

Early healthy functioning is one mechanism that can put children on trajectories of further competence and healthy adaptation. Children who show early signs of social competence tend to become even more prosocial with development (Baillargeon et al., 2007). Developmental outcomes that fall within a healthy range are predominant, but research on predictors of children's relative placement within that healthy range is still needed.

Data from the first wave of the Early Childhood Longitudinal Survey—Kindergarten Cohort indicate that 18-42 percent of children demonstrate a significant lag in their approach to learning (West, Denton, & Germino-Hausken, 2000; Zill & West, 2001). Such children who are not ready for the challenges of the school environment have often faced earlier adversities that derail their progress (Hair, Halle, Terry-Humen, Lavelle, & Calkins, 2006). Nonetheless, most children enter kindergarten with at least basic skills in the key domains of reading, mathematics, and social relationships.

Some children do achieve healthy, competent functioning despite the presence of early adversity and risk factors. In the realm of behavior and conduct, approximately 12 percent of infants and toddlers have significant behavioral or emotional problems (Briggs-Gowan, Carter, Skuban, & Horwitz, 2001). Research indicates, however, that less than 50 percent of children with conduct problems during the toddler or preschool period continue to have significant problems 1-2 years later (Baillargeon et al., 2007; Lavigne et al., 1998). Although this is encouraging, the absence of significant problems cannot be equated with optimal healthy functioning. Exact prevalence of resilient outcomes is an elusive statistic as the particular competency being studied and the particular risk or adversity challenging the development of that competency will affect the likelihood that a child will manifest a resilient outcome.

Economic and/or social burden

Many children in the United States are living in households with circumstances that could put them at risk for poor developmental outcomes. In 2005, 18.2 percent of children younger than 18 years old, and 21.3 percent of children younger than 5 years old, lived in households below the national poverty line (U.S. Census Bureau, 2005). Estimates from 2005 indicate that only 68 percent of children are currently living in two-parent families, down from 77 percent in 1980 (U.S. Census Bureau, 2005). Poverty, stress, family difficulties, health problems, developmental or physical disability, and learning or behavioral problems can put children at risk for decrements in their functioning over time (Schoon, 2006).

Studies show that children with deficits in emotional and social skills at school entry are likely not only to have ongoing behavioral and emotional problems but also to suffer in academic achievement in the long-term (Wentzel & Asher, 1995). Prospective longitudinal research has demonstrated that psychosocial factors prior to school entry, such as poor quality home environments and negative early caregiving experiences, can predict later high school dropout (Jimerson, Egeland, Sroufe, & Carlson, 2000). Yet the mechanisms that change these negative trajectories over time or those that could be implemented to move children onto more positive trajectories are not well understood.

Preventability/malleability

Because healthy, competent outcomes are desirable, the concepts of preventability and malleability can be best conceptualized as efforts to promote competence and health in children at risk due to adversity. Resilience can be a natural process through which children's interpersonal and intrapersonal resources assist them achieve optimal outcomes despite the presence of threats to

development (Luthar & Cicchetti, 2000). Resilience, however, can also be promoted through bolstering systems such as parenting behavior, home environment, and child cognitive and social functioning. Intervention studies with premature infants such as the Infant Health and Development Program (IHDP, 1990) have sought to promote resilient outcomes through broad-based intervention services. Programs such as Head Start serve children from low-income families for preschool-based intervention designed to promote school-readiness and subsequent school success (U.S Department of Health and Human Services, Administration for Children and Families, 2005). A greater understanding of the pathways to competence and resilience would permit greater tailoring of programs to the particular risks and needs of children and their families.

3.2 Justification for a Large Prospective Longitudinal Study

Research on influences on positive outcomes and resilient outcomes requires tracking of a variety of protective or ameliorative exposures and experiences over time. Exposures that predict positive child outcomes, such as marital quality and parenting behaviors, child care experiences, access to and utilization of child health or intervention services, and child characteristics, will all be assessed by the NCS at multiple time points. Elements of biological or social risk whose effects on child outcomes might be moderated by these exposures will also be assessed on the NCS including, but not limited to, poverty, low parental educational attainment and literacy, prematurity, and developmental disability.

The tracking of exposures over time is essential because the influence of the exposures may differ across developmental periods. For example, the specific timing of the onset of non-parental child care may be relevant for promoting individual differences in positive childhood adjustment and adaptation at some ages, but not at others (NICHD Early Child Care Research Network, 1998). Many of these exposures will also change in their quality or quantity over time, and the changes themselves may be pertinent to child adjustment. Finally, the duration and cumulative levels of exposures over time, both for risk factors and for exposures that might ameliorate risk and promote healthy outcomes, may be important for child competence.

Repeated assessments of child outcomes, including physical, cognitive/academic, and social outcomes, are also essential to an understanding of healthy development. Examining the predictors of an absolute level of child adjustment at a given time point is certainly valuable. Nonetheless, because healthy development is an ongoing process rather than an event (Yates, Egeland, & Sroufe, 2003), only through identifying improvement or decrement of child functioning over time can we fully understand which exposures promote positive trajectories of development. Complex statistical techniques such as growth curve modeling utilize multiple time points of child outcomes to demonstrate trajectories of functioning over development (Burchinal, Nelson, & Poe, 2006).

The large sample size of the NCS will be important for the examination of rare risk factors and of interactive effects of risks and exposures on child outcomes. Subgroups of individual or overlapping risks (e.g., poverty, very low birth weight, developmental disability) can only be found in sufficient numbers in a large sample, and interactions with protective or ameliorative exposures may follow different patterns depending on the nature of the risk. Interactions between risk and protective factors are, by definition, the only way to examine processes of resilience in child development (Luthar & Cicchetti, 2000). Additionally, some exposures that promote healthy development in all children may act synergistically such that the greatest magnitude effect is seen only when two exposures interact.

3.3 Scientific Merit

Healthy development, and specifically competence, can be conceptualized as comprising successful navigation of developmental tasks appropriate to a particular phase of life, such as secure

attachments with caregivers in infancy, school readiness in preschool, or social and academic competence during the school years. It can also be conceptualized as concerning more specific domains of functioning (social competence, cognitive competence) as they develop throughout the lifespan (Masten & Coastworth, 1998). Competence may be achieved through unimpeded progression of competent functioning over time or through the more challenging attainment of competence despite adversities that threaten healthy development, described as "resilient outcomes" (Masten, 2001).

The processes involved in resilience are neither unusual nor extraordinary. They are normal adaptive human systems at work. These are the fundamental, innate behavioral and physiological systems that promote successful functioning. The broad systems that promote healthy, competent outcomes include, but are not limited to, family and caregiver-child relations and child characteristics such as adaptive cognitive functioning and intelligence (IQ), self-regulatory systems for emotion and behavior, and the desire to explore and learn (Masten, 2001). When these systems function well, they can protect the functioning of children who face environmental, social, and biological risk factors. In addition to these interpersonal and intrapersonal adaptive systems, societal resources such as child care and preschool experiences and health care access and services can ameliorate risk and facilitate the functioning of adaptive systems either directly or indirectly.

Family process and caregiver-child relationships

Within the domain of family functioning, both marital and caregiver-child relationships serve as protective factors for children's development. Marital relationships provide social and emotional support to caregivers, enabling them to approach parenting with greater ease and facilitate competence in their children. For parents of toddlers and preschoolers, harmonious marital relationships predict child attachment security with mothers (Goldberg & Easterbrooks, 1984) and attachment security and interactive competence with fathers (Frosch, Mangelsdorf, & McHale, 2000; Goldberg & Easterbrooks, 1984). Even within groups of highly stressed mothers, those who report high levels of marital harmony have children who are more likely to be securely attached to them (Kazui, 1997). Research shows that mothers who report high levels of love and support in their marriages have infants who score higher on tests of cognitive and language development, and display better emotion regulation skills (Porter, Wouden-Miller, Silva, & Porter, 2003). High quality marital relationships also provide models of harmonious interpersonal functioning for older children. Parents who engage in constructive behaviors during disagreements, as compared to those who engage in destructive conflict behavior, have children who show higher levels of positive emotionality (Cummings, Goeke-Morey, Papp, & Dukewich, 2002).

Social support within the family can also modify the effects of genetic polymorphisms on children's development (Fox et al., 2005). Children who have a short allele for the promoter region of the serotonin transporter gene (5-HTT) are at risk for a behavioral tendency toward fearfulness, shyness, and inhibition in the face of novelty. Yet, when such children are raised in families with high levels of support, they exhibit normal, competent functioning in these areas (Fox et al., 2005).

Whereas most theoretical models suggest that marital quality acts indirectly on child development, parent-child relationship quality has a more direct influence with responsive, nurturing, and authoritative parenting promoting competence and well-being in children (Borkowski, Ramey, & Bristol-Meyer, 2002). Early positive parenting has been shown to predict subsequent socioemotional and language competence in young children (Belsky & Fearon, 2002), and to predict increases in academic and social competence from childhood to adolescence (Masten et al., 1999).

Among children at biological risk for poor developmental outcomes, positive parent-child relationship interactions predict higher levels of competence than would be expected given the compromises to their early physiological functioning. Among mothers of preterm children, those who

exhibit high levels of maternal responsiveness during infancy have children who have higher than predicted levels of social and language competence during preschool (Beckwith, & Rodning, 1996). Additionally, very low birth weight infants whose mothers adapt interactions responsively to their children's limitations and changing abilities show increases in social skills over time at a rate faster than those infants whose mothers are less responsive (Landry, Smith, Miller-Loncar, & Swank, 1998).

Children in families with high levels of social risk also show resilient outcomes when they experience high quality parent-child relationships. In low socioeconomic status, high stress families, children often have difficulty in the domains of academic achievement and social behavior. Nonetheless, research has shown that high quality parent-child relationships can promote better-than-expected trajectories of development. Young children whose families have high levels of social and contextual risk fare better on preschool expressive language abilities and social competence if their mothers are nurturing and supportive than if their mothers are rejecting or emotionally distant (Belsky & Fearon, 2002). Likewise, in high social-risk families, early supportive and stimulating home environments predict greater-than-expected increases in academic achievement through childhood and adolescence (Jimerson, Egeland, & Teo, 1999).

Child characteristics

Some child characteristics have been identified in the literature as protective factors, such that children with particular qualities function better than expected in the face of biological or social risk. High IQ, which may permit greater cognitive flexibility, is associated with resilient outcomes. Children who face particularly high levels of adversity yet have high IQs score significantly higher on measures of both social and academic competence in adolescence than do their counterparts with lower IQs (Masten et al., 1999). Child characteristics associated with resilient outcomes, however, are not narrowly restricted to highly heritable features such as IQ. Among children at biological risk (born preterm), better neonatal neurobehavioral organization also predicts subsequent social and academic competence in middle childhood and adolescence above and beyond variance accounted for by levels of socioeconomic risk (Cohen, 1995).

A variety of child temperamental characteristics have also emerged as relevant to resilient outcomes. In families at risk due to high levels of stress and low socioeconomic status, children with the best social competence skills are those who have low temperamental emotional reactivity and high levels of sociability (Smith & Prior, 1995), even when the temperamental qualities are assessed 7 years earlier (Mathiesen & Prior, 2006). Among children attending Head Start, those who combined the temperamental qualities of adaptability, flexibility, and willingness to approach novelty were among the most competent socially, well beyond what would be predicted given the levels of adversity their families face (Mendez, Fantuzzo, & Cicchetti, 2002).

Child care experiences

Experience in a high quality child care or preschool can promote positive child functioning in the domains of cognitive, language, and social development. High quality child care is characterized by a safe, health-promoting environment; cognitive and social stimulation; and sufficient staffing by caring, responsive adults (Votruba-Drzal, Coley, & Chase-Lansdale, 2004). Research on the effects of child care has focused primarily on the quality of care, although the age of onset and the duration of care have also been studied.

High quality care experiences promote better cognitive and language development in infancy and early childhood (Burchinal, Roberts, Nabors, & Bryant, 1996; Caughy, DiPietro, & Strobino, 1994; NICHD Early Child Care Research Network, 2002; Peisner-Feinberg et al., 2001); better social

competence in early childhood (NICHD Early Child Care Research Network, 2002; Peisner-Feinberg et al., 2001; Volling & Feagans, 1995; Vortruba-Drzal et al., 2004); and better social and language outcomes into middle childhood (Belsky et al., 2007; Peisner-Feinberg et al., 2001). In addition to overall quality of child care being important to child outcomes, caregiver characteristics, such as training/qualifications and warmth with the child, and care environment characteristics, such as child-staff ratio, have emerged as particularly important features in predicting child outcomes (Burchinal et al., 1996; NICHD Early Child Care Research Network, 2002).

Because families select the child care they use, family characteristics are often confounded with child care effects. Nonetheless, child care does seem to have effects on child development above and beyond what can be accounted for by family characteristics (NICHD Early Child Care Network, 2002; Peisner-Feinberg et al., 2001). Research has also examined the family characteristics that might interact with child care quality to promote resilient child outcomes and has found that the positive effects of high-quality child care seem to be the greatest for children who come from low-income families and children whose home environments provide lower levels of cognitive and social stimulation (Caughy et al., 1994; Peisner-Feinberg et al., 2001).

Access to developmentally-oriented child services and health care

Healthy development may be promoted in children at risk for poor health and developmental outcomes through early intervention services and access to health care services. The 2004 Individuals with Disabilities Act (Public Law 108-446), Part C mandated public funding of early intervention services for children under age 3 with developmental disabilities or who are at risk for developmental delay. Children at risk for poor developmental outcomes due to poverty and social risk are also eligible for publicly funded early intervention programs such as Head Start and Early Head Start. Access to this array of programs has been variable, however, with some parents unaware of eligibility criteria or how to obtain needed services (Peterson et al., 2004; Shannon, 2004).

Many of these programs have been effective in promoting at least short-term gains in positive child outcomes. The Infant Health and Development Program (IHDP, 1990) was an intervention program designed for families and their infants who were at risk for developmental delays and difficulties due to low birth weight and preterm birth. Services provided included home visitation from birth to age 3, educationally-oriented child care at ages 2-3, and links to community resources. Long-term follow-up showed that families who participated for more days had children who showed greater increases and sustained advances in cognitive development (Hill, Brook-Gunn, & Waldfogel, 2003). Other early intervention programs showing positive effects on cognitive outcomes have focused on nutrition (Worobey, Pisuk, & Decker, 2004) or on broad family and child services (Reynolds, & Ou, 2004).

Head Start and Early Head Start are federally funded programs that provide intervention services to children and families at risk due to poverty. Early Head Start provides services during pregnancy and up to child age 3, including a combination of home-visitation and center-based services addressing child health and development. At age 3, children who participated in Early Head Start scored better than a control group on cognitive, language, and social development outcomes (Love et al., 2005). Head Start, available for preschool-age children at risk due to poverty, provides an enriched preschool experience to foster positive child health and development. Recent evaluation findings show that Head Start children show gains on pre-literacy skills, socialization skills, access to health care, and health status (U.S. Department of Health and Human Services, Administration for Children and Families, 2005).

In addition to these intervention services, access to regular health care services reduces the likelihood of children having medical needs that are not addressed. Children living with both parents, children whose parents had at least high school degrees, and children whose families were more than 200

percent above the poverty threshold are the most likely to have their medical needs met, most likely to have timely health care without regard to cost, and most likely to have health insurance (Bloom, Dey, & Freeman, 2006).

3.4 Potential for Innovative Research

Healthy outcomes are not static phenomena. They are a process of positive adaptation to ever changing environments and intra-individual developmental changes. Although much research has been conducted on resilient and healthy outcomes, little research has been able to examine resilience as the process unfolds from birth until early adulthood. Additionally, the complexity and depth of such research has been limited by relatively small samples usually found in longitudinal research on child outcomes. The NCS will provide the rare opportunity to uncover these trajectories in greater depth, including statistical approaches such as growth curve analyses that permit modeling of individual differences in non-linear trajectories of development over time.

Because healthy outcomes do change over time, the scope and duration of the NCS will provide an opportunity to examine longitudinally the exposures that help maintain healthy outcomes, and those that erode competence. For example, early intervention can promote short-term gains, but a better understanding is needed of the conditions that cause those gains to be lost again (Zigler & Styfco, 2001). Understanding what interferes with resilience and when children are most susceptible to additional risk will allow interventionists to design ongoing program follow-up to counter these risks.

The NCS will also permit a more effective exploration of gene-environment interactions and their effects on genetic expression and resilience outcomes. Studies to date have either been retrospective or with relatively small samples, limiting the range of analyses. The greater sample size and range of data on the NCS will allow for more power and more complexity in analyses, thus opening the potential to identify more varied mechanisms through which genetic risk might be modified to produce healthy outcomes.

3.5 Feasibility

Although infancy and early childhood risks may be particularly critical for resilience research, the most complete view of healthy development and competence requires tracking of risk, protective factors, and competence throughout development. Periodic assessment of the developmentally appropriate manifestations of these domains will be possible on the NCS.

Resilience analyses, which look at factors that promote healthy development despite risk factors, require representation of subgroups in the sample. Relevant risk subgroups, such as families living in poverty, children born preterm or at low birth weight, and children with developmental disabilities, should be adequately represented in the NCS.

Valid and reliable measures of child competence exist in various domains, including social, physical, and cognitive development, and are widely available due to extensive prior research in these fields. Measures of exposures, such as family functioning, child characteristics, experiences in child care, and use of developmental services, have also been assessed in previous research, and valid and reliable measures are available using a range of methodologies. Such measures are minimally intrusive, and costs per administration are reasonable.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/parent and family

- Marital quality
- Parenting/parent-child relationship (warmth, nurturing, sensitivity)
- Life stress
- Use of developmentally-oriented health or social services

Primary/child

- Child temperament
- Child IQ
- Child neurobehavioral organization
- Child care (quality, age at entry, hours, stability)
- Child care substudy
    - The child care substudy development group has proposed that a subgroup of participants will be assigned to the child care assessment cohort prior to the first standard data collection point. Approximately 79 percent of that cohort is expected to use some level of regular non-parental child care at some time during childhood. At each regular visit with this cohort, permission to seek information from the child care provider would be obtained. For children who are in regular child care outside the home for a specified time and duration, for example 30 or more hours per week in the past month, a site visit would be conducted at the child care location. For children in regular care for a lesser time and duration, 10 percent of the cases would have site visits conducted. Consequently, hypotheses that involve data collected at the regular child care location are anticipated to be collected on a subset of the NCS participants.

Secondary/parent and family

- Socioeconomic status (income, employment)

Secondary/child

- Gestational age/birth weight
- Developmental delay/disabilities
- Genetics

4.2 Methods

Primary/parent and family

- Interview (marital quality, parenting style, stress, use of services)
- Observation (parent-child interaction)

Primary/child

- Parent interview (temperament, child care)
- Direct child assessment (IQ, neurobehavioral organization)
- Observation (temperament)
- Interview with child care provider/observation (substudy)

Secondary/parent and family

- Interview (socioeconomic status)

Secondary/child

- Medical record review (gestational age, preterm birth, disabilities)
- Direct assessment of child (developmental delay)
- Blood for genetic analysis

4.3 Life Stage

Primary/parent and family

- Beginning after birth and continuing periodically through adolescence (marital quality, parenting, stress, use of services)

Primary/child

- Birth (neurobehavioral organization)
- Infancy/childhood (temperament)
- Periodically through infancy and childhood (child care)
- Childhood (IQ)

Secondary/parent and family

- Periodically prenatal through adolescence (socioeconomic status)

Secondary/child

- Birth (gestational age, preterm birth, disability, blood)
- Infancy/childhood (developmental delay/disability)

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Social competence
- Cognitive development/achievement
- Physical and motor development
- Health status

5.2 Outcome Methods

- Interviews with parent, child care provider, teacher
- Direct assessment and observation of child

5.3 Life Stage

- Periodically from birth through age 21

6. Important Confounders

- Exposure to environmental toxins (including lead or other toxins), either at home or in a child care setting, has the potential to reduce the child's competence (Hubbs-Tait, Kennedy, Droke, Belanger, & Parker, 2007) even in otherwise enriched settings.
- Parental IQ might account for some exposures because higher IQ parents could be more skilled at parenting and at organizing and managing the child's environment (Bradley et al., 1993; van Bakel & Riksen-Walraven, 2002).
- Genetics might link competence across generations (Kim-Cohen, Moffitt, Caspi, & Taylor, 2004) as some components of competence (including but not limited to IQ) are passed down from parent to child.
- Service provision (intervention, child care) effects might be confounded with parenting variables, as parents who are more engaged with their children might seek intervention or choose different features of the service environment (Burchinal & Nelson, 2000; McCurdy & Daro, 2001).

7. Power and Sample Size

There are many possible outcome measures and exposures discussed as part of this hypothesis. Many different analyses could be conducted, each with different power characteristics. The

following illustrates the power using several possible analyses. For this discussion, power is defined as the smallest difference between the exposed and non-exposed groups that could be reliably detected using the NCS data, i.e., the minimum effect size. In this case "reliably detected" corresponds to a power of 80 percent for detecting a significant difference based on a two-sided 95 percent confidence interval. The calculations include a design effect based on the sample design of the NCS and assume year-to-year retention of 98 percent and an assessment at age 10. In addition, the calculations assume that a complete set of exposure and outcome values are available for 80 percent of the children still in the study at the time of the statistical assessment.

For the power calculations below, the outcome and most exposure variables are assumed to be continuous. The continuous variables are assumed to have a distribution that can be reasonably approximated by a normal distribution. For the purposes of illustration, the outcome can be assumed to be child's social competency and the primary exposure is parenting quality. The child's social competence will be measured at several different times during the Study.

The relationship between the exposure and outcome can be described by the slope, which is the change in the outcome measure associated with a one-unit change in the exposure. The minimum effect size is then the minimum slope that can be reliably detected. The slope depends on the standard deviations of the outcome and exposure variables. To simplify the presentation and calculations, the outcome and exposure variables are assumed to be scaled to standard deviations of 1.0. As a result, the slope is the change in the outcome measured in standard deviation units associated with a one standard deviation increase in the exposure. For example, the minimum detectable slope might be 0.20. This means that a one standard deviation increase in the exposure is associated with a 0.20 standard deviation increase in the outcome.

In the most simple example, the child's social competency (Y) measured at one time might be predicted by parenting quality (X) using linear regression. The following table shows the minimum slope that can be reliably detected and the associated r-square.

Minimum effect size for the model Y = X.

Parameter	Minimum value that can be reliably detected
X	0.0166
r-square	0.00028

In most cases, other covariates will also be included in the model. The following table shows the minimum effect sizes assuming three independent variables (X, W, and Z), all normally distributed and having pair-wise correlations on 0.20. In general, higher correlations among the variables will result in larger minimum effect sizes.

Minimum effect size for the model Y = X W Z. The value of r-square corresponds to a model where each parameter is equal to the minimum effect size.

Parameter	Minimum value that can be reliably detected
X	0.0172
W	0.0172
Z	0.0172
r-square	0.00028

Many models will also include an interaction. The following table shows the minimum effect sizes assuming a model with two independent variables (X and Z) and the interaction of X and Z. X and Z are assumed to be all normally distributed and have a correlation on 0.20.

Minimum effect size for the model Y = X Z X*Z. The value of r-square corresponds to a model where each parameter is equal to the minimum effect size.

Parameter	Minimum value that can be reliably detected
X	0.0169
Z	0.0169
X*Z	0.0163
r-square	0.00091

The interaction term may be a categorical variable, such as an indicator of children that were born preterm. The following table shows the minimum effect sizes assuming a model with two independent variables (X and C) and the interaction of X and C, where C is coded as 1 for children born preterm and 0 otherwise. For the calculations, 12 percent of children have C = 1. Also the correlation between C and X is 0.15. Using these assumptions, the minimum effect sizes are shown below.

Minimum effect size for the model Y = X C C*Z, where C is a categorical variable. The value of r-square corresponds to a model where each parameter is equal to the minimum effect size.

Parameter	Minimum value that can be reliably detected
X	0.0178
Z	0.0536
X*Z	0.0519
r-square	0.0015

Having determined that there are significant differences between preterm and other children, the analyst can fit separate models to both groups of children. The following table shows the minimum effect sizes for a linear regression model predicting Y from X applied to the 12 percent of the children with C = 1 using the correlation assumptions above. Because there are fewer children in the analysis, the design effect is slightly lower.

Minimum effect size for the model Y = X fit to a subset of 12 percent of the children.

Parameter	Minimum value that can be reliably detected
X	0.0322
r-square	0.0012

The models fit to these data are likely to have many covariates and interactions. The minimum effect sizes will depend on the number of parameters and the correlations among the variables. Larger minimum effect sizes are generally associated with more dependent variables, including interactions, and higher correlations among the dependent variables. In general, the NCS will provide adequate power for assessing relationships among the anticipated dependent and independent variable. More complex models can also be fit to the data, including growth curve models with or without latent components. The minimum effects sizes for these models are likely to be larger.

8. Other Design Issues

Direction of effects in analyses must be considered and controlled. Rather than effects going directionally from exposure to child outcome, there could be reverse effects or reciprocity over time. For example, highly competent children might foster better parenting and more harmonious marital relations, and this may then lead to greater competence in children. Both longitudinal analyses and controlling for earlier values of the variables in longitudinal analyses will be important for ruling out or understanding reverse-causation.

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THE ROLE OF PRENATAL MATERNAL STRESS AND GENETICS IN CHILDHOOD ASTHMA

1. Meta Hypothesis

Prenatal, maternal stress increases the risk of childhood asthma. Genetic and environmental factors that influence immune development and lung growth/airway inflammation in early life modify the association between maternal psychological stress and the development of asthma.

2. Specific Hypotheses

1. Children of mothers with high psychological stress levels during pregnancy will have an increased risk of asthma/wheezing.

2. Prenatal stress exposure will modify the association of prenatal exposure to oxidative toxins (tobacco smoke and air pollution, in particular) in predicting subsequent risk of asthma.

3. Polymorphisms in maternal genes will interact with prenatal exposures to stress, smoking, and air pollution in predicting subsequent asthma risk.

    3.1 Children with mothers with high stress exposure during pregnancy will vary in their increased risk of asthma/wheezing in part related to variation in genes in the corticosteroid pathway (i.e., beta 11 hydroxylase and the glucocorticoid receptor).

    3.2 Children with mothers with high stress exposure during pregnancy will vary in their increased risk of asthma/wheezing in part related to genetic variation in maternal toxin metabolizing genes (e.g., glutathione S transferase M1, T1, and P1 and Cytochrome P450 1A1).

3. Background and Justification

Asthma prevalence and morbidity has increased in the United States. This disproportionately affects poor minority children living in urban environments and remains a paradox largely unexplained by accepted environmental risk factors. This suggests the existence of as-yet-unidentified factors or unmeasured interactions (Wright, Rodriguez, & Cohen, 1998; Wright, 2005). This paradox, in part, has led to reconsideration of the overlap between biological determinants and psychosocial factors (i.e., life stress). Growing understanding of the complexity of asthma epidemiology underscores the need to examine interactions between established risk factors with genetic factors (Lemanske, 2002) as well as interactions between concurrent exposure to prevalent environmental factors (Cloughterty et al., in press). These hypotheses examine biologic pathways that established environmental risk factors may interact with genetic risk factors and with each other. The interactions, if proven, will help establish the mechanisms by which asthma occurs.

3.1 Public Health Importance

Prevalence

Asthma is the most common chronic illness among children in the United States (National Academy of Sciences, 2000; American Lung Association Epidemiology and Statistics Unit, 2006). In 2004, 6.5 million children younger than 18 had asthma. During the past two decades, asthma prevalence

increased approximately 75 percent among U.S. children. Similar or greater increases in the prevalence of asthma during the second half of the 20th century were reported from other countries (Eder, Ege, & von Mutius, 2006). Though asthma prevalence has plateaued in many groups, black children and adolescents have continued to experience increased prevalence (American Lung Association Epidemiology and Statistics Unit, 2006). Hospitalization and death rates in children have also stabilized (Akinbami, 2001). Physician visits for asthma by children continued to increase after 2000 (Akinbami, 2001), with an increase in the use of preventive (controller) medications such as inhaled corticosteroids (Stafford, Ma, Finkelstein, Haver, & Cockburn, 2003; Suissa, Ernst, Benayoun, Baltzan, & Cai, 2000; Suissa, Ernst, & Kezouh, 2002).

Economic and social burden

In 2004, the direct health care costs of asthma was estimated at $11.5 billion while indirect costs (lost productivity) were estimated at $4.6 billion for a total of $16.1 billion (American Lung Association Epidemiology and Statistics Unit, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. One study estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs (Malone, Lawson, & Smith, 2000). Asthma also poses a substantial and increasing public health burden from school absence and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Prospective studies that incorporate strategies for studying stress reactivity during pregnancy, infancy, and early childhood (De Weeth & Buitellar, 2005; Jones, Holzman, Zanella, Leece, & Rahbar, 2006; Harville et al., 2007; DiPietro, Cotigan, & Gurewitsch, 2003; Egiliston, Maman, & Austin, 2007) are needed to continue to elucidate the mechanisms underlying the links between stress, other vulnerability factors, and asthma development. The prospective nature of the National Children's Study enables this research as stress questionnaires will be administered prior to phenotype expression (childhood asthma), thus limiting the possibility of recall bias. Similar concerns regarding bias in smoking and air pollution exposure measures are mitigated by the prospective design. The size of the cohort will assure sufficient statistical power exists for testing the interaction hypotheses.

Interactions with other factors (e.g., genetic, bioaerosols, lifestyle factors) necessitate a large sample. Accurate exposure and phenotypic data are needed to assess the importance of many asthma and allergy genotypes interacting with many exposures in relation to complex and variable asthma phenotypes. A large sample will help evaluate the susceptibility to the adverse effects of certain pollutants and their sources in specific genotype-phenotype clusters. Also, data are needed from children of different ethnicities living across the United States under varied housing, socioeconomic, and geographic conditions. These factors may be correlated with variations in exposures, and a large sample size will be necessary to allow adjustments to account for this.

3.3 Scientific Merit

Theory and mechanisms

Asthma is a developmental disease with an estimated half of all cases diagnosed by age 3 and two-thirds diagnosed by age 5 (Reed, 2006; Reed, 2006). The early onset of disease suggests adverse early life experiences, including prenatal exposures, are relevant to asthma risk. Despite what is commonly believed, children do not necessarily "grow out of asthma" with a significant reduction in symptoms into adulthood. Almost two-thirds of children diagnosed with asthma continue to have symptoms in puberty and adulthood, and the remaining one-third experiences a persistence of lung

function abnormalities even if they appear to have clinical remission (NIH, 2004). Some prenatal exposures, such as environmental tobacco smoke (ETS), have been demonstrated to increase the risk of childhood asthma, and the greatest risk is among children with a family history of asthma, which suggests a gene-environment interaction (London, James Gauderman, Avol, Rappaport, & Peters, 2001). Stress is a highly prevalent environmental exposure also demonstrated to increase asthma risk (Wright et al., 2002). Several lines of evidence suggest prenatal maternal stress may be particularly relevant to asthma risk. Two potential pathways are proposed which may work in tandem. The first is that prenatal stress will increase polarization of the immune system to the TH2 phenotype via dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. The second is that stress may enhance airway inflammation through its role as a pro-oxidant.

HPA axis and polarization of the immune system

The HPA axis seems susceptible to early life programming. Maternal and fetal stress stimulates placental secretion of corticotrophin-releasing hormone (CRH), which is elevated in the neonatal circulation (Seckl, 1997, 2001). This may stimulate the fetal HPA axis to secrete glucocorticoids and amplify fetal glucocorticoid excess. These in utero responses may increase the risk of disease in later life (Wellberg & Seckl, 2001). Disturbed regulation of both the HPA axis and the sympathetic adrenomedullary system due to chronic stress suggests immune function, which is modulated by these systems, may also be dysregulated in these individuals. In this context, it is notable that early life caregiver stress has been linked to repeated wheeze (Wright et al., 2002) and dysregulation of immune function in a birth cohort predisposed to atopy (Wright et al., 2004). There is growing evidence these cytokine patterns are already present in the first year of life (Lemanske, 2002) and may have their roots in utero (Miller et al., 2001). Gestational exposure to maternal stress has been shown to alter the development of humoral immunocompetence in offspring as well as their hormonal and immunologic responses to postnatal stress (Barker, 2001; Egiliston et al., 2007). Evidence in rhesus monkeys suggests stress experienced during pregnancy impacts the infant monkeys' response to antigens at birth (Coe, Lubach, & Karaszewski, 1999).

Another study (Wright, Franco-Suglia, Staudenmayer, & Cohen, 2007) examined the relationship between diurnal salivary cortisol expression and total IgE among 89 pregnant mothers enrolled in the Asthma Coalition on Community, Environment, and Social Stress project, a prospective cohort designed to study the effects of in utero and early life stress on urban childhood asthma risk. Higher levels of maternal total IgE were associated with blunted HPA functioning. Other evidence suggests that elevated maternal IgE in utero may potentiate fetal sensitization to allergens and enhance atopic risk in infancy (Liu et al., 2003). Stress-induced altered activity of the maternal HPA axis may have immunomodulatory effects that influence expression of IgE during pregnancy which, in turn, may impact fetal sensitization and childhood allergy and asthma risks. These findings warrant further study taking into account concurrent environmental exposures and genetic susceptibility.

Pro-oxidant properties of stress

Stress may also enhance airway inflammation through its role as a pro-oxidant (for a detailed overview see Wright, Cohen, & Cohen [2004]). Air pollution and ETS are associated with asthma morbidity and have oxidative properties. Stress activates various P450 enzyme systems involved in the biotransformation of environmental oxidative toxins, such as ETS and air pollutants. Several animal and human studies demonstrate chronic stress will produce increased oxidative toxicity due, in part, to a decreased ability to detoxify exogenous toxins.

In a birth cohort in China, women with both occupational stress and benzene exposure had an average reduction of 184 grams in the birth weight of their children. However, subjects with only

stress or benzene exposure had less than a 20-gram reduction in birth weight. Benzene is activated and ultimately detoxified by the hepatic P450 enzyme system in conjunction with phase 2 enzymes. The interaction might therefore be due to induction of benzene activation by maternal stress. In the same cohort, mothers with the P450 system CYP1A1 HincII polymorphism AA and occupational exposure to benzene had a decreased gestational period. CYP1A1 is a phase I enzyme that activates polycyclic aromatic hydrocarbons. The association between the AA genotype and benzene exposure was postulated to be caused by an increased activation of benzene in the AA genotype mothers. The similar interaction between stress and benzene may be due to increased oxidation by stress mediating induction of P450 enzymes.

Other human and animal studies support the concept that psychological stress has pro-oxidant properties and alters biotransformation (Capel, Dorrell, & Smallwood, 1983; Kosugi, Enomoto, Ishizuka, & Kikugawa, 1994; Tomei, Kiecolt-Glaser, Kennedy, & Glaser, 1990; Irie, Asami, Nagata, Miyata, & Kasai, 2000). Spiteri, Bianco, Strange, and Tryer (2000) proposed that differences in host detoxification provide the basis for either resolution or progression of inflammation in individuals with airway hyper-responsiveness after exposure to an environmental trigger (e.g., tobacco smoke, air pollutants) that augments oxidative toxicity.

Maternal genotype

A second innovative approach of these hypotheses is to determine the role of maternal genetics on in utero environmental exposures. While there are no studies of maternal genetic polymorphisms interacting with in utero exposures in predicting respiratory outcomes in children, some studies in birth defects illustrate this concept (Van Rooij et al., 2001; Shi et al., 2007). Because stress is a pro-oxidant, interactions between maternal stress during pregnancy and in utero exposure to ETS and air pollution may be associated with asthma/wheeze in children, similar to the study by Wang et al. (2000). Mechanistically, stress may be is biologically similar to alterations in detoxification produced by genetics and leads to the expression of disease by the same biologic pathway.

3.4 Potential for Innovative Research

Several aspects of these hypotheses are innovative. The role of prenatal stress exposure on subsequent childhood asthma has not been extensively studied. This is important because the role of in utero exposures on the risk of subsequent diseases has been elucidated for environmental toxins (ETS and asthma) and nutrition (decreased fetal nutrition and increased subsequent risk of hypertension and cardiovascular disease). The extension of this critical window concept to a psychosocial exposure, such as psychological stress, may help determine asthma causation.

The concept of critical windows of exposure is well established, but the role of genetic susceptibility during these exposure windows has not been well studied. Reliance on quantitative exposure data without regard to the timing of the exposure may introduce bias into measures of gene-environment interaction. Furthermore, the role of maternal genetic factors that may interact with in utero fetal exposures has been investigated in some fields (e.g., reproductive epidemiology and birth outcomes), but this clearly deserves consideration for health outcomes, such as asthma, in later life.

3.5 Feasibility

While studies of interaction require large sample sizes, these hypotheses can be tested in the National Children's Study. Given the prevalence of the outcome/phenotype (asthma) and the exposures (stress, smoking, air pollution), and the highly polymorphic nature of candidate genes (the glucocorticoid

receptor, 11 beta hydroxylase enzyme, P450 and phase II biotransformation enzymes, and many others), these hypotheses will be testable within the framework of the study.

The data for prenatal exposures and phenotype classification will be collected. All have well established, validated measurement instruments. The frequency of family contact within the framework of the overall study design and protocol will provide opportunities to collect the exposure and outcome data to test these hypotheses. The two pathways proposed in the hypotheses (polarization of immune system by HPA dysregulation and pro-oxidant effects of stress on biotransformation enzymes) can be tested by collection of biomarkers (maternal and cord blood IgE, prenatal maternal salivary cortisol, prenatal maternal CRH, maternal urinary cotinine levels), questionnaires (Perceived Stress Scale, Parenting Stress Index, Family Environment Scale, qualitative and quantitative smoking history), environmental measures (air pollutant exposures), and DNA from both mother and child.

To test these hypotheses, an initial nested case-control design could be employed with random control sampling (matching for age) after the cohort has reached age 3-5 when early childhood wheezing will have been manifested. Nested case-control studies at future times will also be possible when the cohort is older and asthma phenotypes defined by trajectories through time can be used to identify cases.

Ethical considerations pertaining to consent for genetic testing will not differ from standard genetic testing of polymorphisms not specifically causing a known disease (i.e., none of the polymorphisms proposed will be disease-causing genotypes, such as the cystic fibrosis gene, and will not require special genetic counseling).

Important covariates (stress exposure, postnatal smoke exposure, allergen exposure, postnatal air pollution) are likely to be measured as part of the overall cohort goals and will not require additional costs.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Prenatal and postnatal stress

Primary/child

- Prenatal and postnatal stress

4.2 Methods

Primary/maternal

- Biomarkers (maternal blood IgE, prenatal maternal salivary cortisol, prenatal maternal CRH, maternal urinary cotinine levels)
- Questionnaires (Perceived Stress Scale, Parenting Stress Index, Family Environment Scale, qualitative and quantitative smoking history)

- Air pollutant exposures
- Genetic measures

Primary/child

- Biomarkers (cord blood IgE, salivary cortisol, CRH, urinary cotinine levels)
- Household smoking and air pollutant exposures
- Genetic measures

4.3 Life Stages

- Periodic including prenatal, birth, infancy, childhood, adolescence

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Asthma, wheezing, pulmonary function/dysfunction
- Immune system function

5.2 Methods

- Asthma or wheezing: Interview for medical history, medical reports from interactions with the health care system, medication review
- Pulmonary function: Pulmonary function tests and spirometry
- Immune system function: Cytokines, total and specific IgE, possibly lymphocyte subsets

5.3 Life Stage

- Asthma or wheezing: Periodic starting during infancy and throughout the Study
- Pulmonary function: At selected clinic visits starting at approximately age 7; Handheld spirometry may be performed more frequently.
- Immune system function: Starting at birth with cord blood and periodically throughout the study

6. Important Confounders, Mediators, and Effect Modifiers

- Health care access: Poor health care access in inner cities, especially among African Americans and Hispanics, exacerbates the risk of asthma attacks and deaths (Lara et al., 2003; Sin, Bell, & Man, 2004; Wallace, Scott, Klinnert, & Anderson, 2004). This and other disparity factors could be behind the greater asthma prevalence, especially among African Americans. These issues are more thoroughly discussed in hypothesis 4 and in the review by Gold and Wright (2005).

- Lower socioeconomic status and some minority groups such as blacks and Hispanics are likely to live in areas with increased air pollution such as near freeways or industrial sites. Careful assessments of these characteristics will be important to properly control for their influence on outcomes.

- Diet, physical activity, bioaerosols, stress, and infections, which will be the topic of other hypotheses, could interact with or confound associations of asthma onset with air pollutants

- For the analysis of gene-environment interactions, it will be important to include polymorphisms in genes involved in oxidative stress responses to air pollutant exposures and candidate genes

7. Power and Sample Size

The smallest detectable relative risk is approximately 1.2. This power estimate assumes a sample size of 100,000 at age of diagnosis, an asthma incidence of 5 percent and a cutoff value for "high" exposure based on the upper fifth percentile of National Children's Study subjects (i.e., a proportion exposed is 0.05). It assumes only a main effects model based on exposure to a single factor (e.g., a single pollutant) without consideration of interactions with other exposures, genetics, family history, etc. (National Children's Study Interagency Coordinating Committee, 2003).

Assessment of effect modification will be carried out by including multiplicative interaction terms for the potential effect modifier and the pollutant exposure into each regression model. For brevity, we present power analyses for a generic effect modifier. In the following, we assume a total sample size of N = 100,000 and that the incidence of diagnosed asthma by age 6 will be 5 percent. We use a fixed interval proportional hazards model to assess the association between pollutant exposure and the risk of asthma. In this case, minimum detectable hazard ratios associated with the interaction term (multiplicative increase in the effect of the pollutant attributable to the effect modifier) correspond to a specified power point that can be estimated via the usual two-sample proportional hazards formula given by Schmoor et al. (1990). The formula assumes unequal initial sample sizes because sample sizes are determined via the prevalence of the effect modifier. Based upon this formula, Table 1 presents minimal detectable hazard ratios associated with the interaction term corresponding to the 90 percent power point of a level 0.05 two-sided test.

Table 1. Minimum detectable hazard ratios associated with interactions. Estimates correspond to the 90% power point of a level 0.05 two-sided test

Prevalence of effect modifier	Minimum detectable hazard ratio associated with interaction term
2%	1.387
5%	1.234
10%	1.165
20%	1.121
30%	1.105
40%	1.100

8. Other Design Issues

- **Ethical/burden considerations:** The study also will need to have a formal strategy and process to communicate the results of environmental monitoring to the child's parents along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings. Repeated tests are potentially burdensome.

- **Cost/complexity of data collection:** Repeated waves of environmental exposure assessment can be costly and burdensome.

- **Need for community involvement:** Daycare and school cooperation would be required for some of the intended measures.

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EXPOSURE TO INDOOR AND OUTDOOR AIR POLLUTION, AEROALLERGENS, AND ASTHMA RISK

1. Meta Hypothesis

Exposures to indoor and outdoor air pollution, aeroallergens, and other environmental agents are associated with increased risk of asthma onset and progression in children and modified by genotype and other risk factors.

2. Specific Hypotheses

The following hypotheses address early and later developmental windows of vulnerability to air pollutants, aeroallergens, and other environmental agents and will require evaluation of exposure-outcome relationships at various points during the life of children. In addition, the hypotheses address how exposure to indoor and outdoor air pollution may interact with exposure to aeroallergens and other environmental, genetic, and family factors. Asthma is a complex and multifactorial disease. A myriad of environmental and familial factors are associated with asthma development, progression, and severity in children. Some factors are causative and others are protective, and these may change or vary by age.

The within-child repeated measures in The National Children's Study protocol allow for the use of time-of-exposure as a factor in the analyses of the proposed hypotheses. In Section 7 below, details of statistical tests and power analysis will be described to demonstrate how repeated measures can be used to address interactions with time-of-exposure in the onset of asthma.

1. Exposure to indoor environmental tobacco smoke, outdoor and indoor air pollutants, in-vehicle air pollutants, ozone and related photochemicals will be associated with the development of asthma.

    1.1 Exposure to indoor environmental tobacco smoke (ETS) will be associated with the development of asthma.

    1.2 Exposure to in-vehicle air pollutants will be associated with the development of asthma.

    1.3 Exposure to outdoor and indoor toxic air pollutants will be associated with the development of asthma. Examples of such pollutants include ozone, fine and ultrafine particles, NO2, ozone, volatile organic compounds (VOCs), carbonyl compounds (e.g., formaldehyde), semivolatile organic compounds from outdoor and indoor combustion sources (e.g., polycyclic aromatic hydrocarbons), and from pesticides and home products such as those containing plasticizes (phthalates, phenols).

2. For the development of asthma, a critical window may exist for exposure to indoor and outdoor air pollutants, other chemical exposures, and aeroallergens. Critical windows are age dependent on the development of the immune system and may differ depending on the outcome related to onset of asthma versus the progression and severity of the disease.

    2.1 For many of the above hypothesized associations, the greatest window of vulnerability for the architecture of the disease may be in utero and early life (0-2 years). The progression and severity of asthma, however, may be impacted by exposures throughout childhood.

    2.2 Allergic sensitization to allergens will be enhanced by exposures to air pollutants and will increase the risk of atopic asthma. The expectation is that exposure to specific outdoor and

indoor allergens and endotoxin and the interaction of these aeroallergens with air pollutants will result in varying susceptibility asthma onset and progression depending on the magnitude and timing of exposures during development as well as genetic predisposition.

2.3 The hypothesized associations with air pollutants will be modified by dietary antioxidants (see dietary antioxidants and asthma risk hypothesis), by lower respiratory infections by polymorphisms in genes involved in oxidative stress responses to air pollutant exposures, and by polymorphisms in candidate asthma and allergy genes involved in innate and adaptive immunity.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Asthma is the most common chronic illness among children (National Academy of Sciences Institute of Medicine). Data on asthma prevalence from the National Health Interview Survey (NHIS), analyzed recently by the American Lung Association, show the prevalence of asthma increased 85 percent from 1982 through 1996 to an estimated 14.6 million persons (55.2 per 1,000 persons) (American Lung Association Epidemiology and Statistics Unit, 2006). This increase was 76 percent in children younger than 18 years old or 4.43 million in 1996 (62.0 per 1,000 persons). This trend paralleled increasing asthma hospitalization and death rates in children (American Lung Association Epidemiology and Statistics Unit, 2006; Akinbami, 2006). Similar or greater increases in the prevalence of asthma during the second half of the 20th century were reported from other countries (Eder, Ege, & von Mutius, 2006).

Beginning in 1997, when questions addressing asthma in the NHIS were changed, the measures of asthma prevalence were also altered. It was not possible to compare to past trends until 2001, when a key question was added back into the questionnaire. Current asthma prevalence increased until 2001, later reaching a plateau in most groups except Black children and adolescents, who have continued to experience increased prevalence annually (American Lung Association Epidemiology and Statistics Unit, 2006). Hospitalization and death rates in children have also reached a plateau or decreased (Akinbami, 2006). Despite a plateau in prevalence, nonambulatory visits for asthma to physician offices by children have continued to increase after 2000 (Akinbami, 2006), possibly due to increased public and health provider awareness. For instance, it is likely that the main force mitigating increases in hospitalization and mortality have been the dramatic increase in the use of preventive (controller) medications such as inhaled corticosteroids (Stafford, Ma, Finkelstein, Haver, & Cockburn, 2003; Suissa, Ernst, Benayoun, Baltzan, & Cai, 2000; Suissa, Ernst, & Kezouh, 2002).

In 2004, 30.2 million Americans (104.7 per 1,000 persons) had been diagnosed with asthma by a health professional at some time during their lifetime. This estimate included 6.5 million children younger than age 18. Almost 4 million children younger than 18 were estimated to have experienced an asthma attack in 2004. Prevalence data in the United States from both the 12-month prevalence (before 1997) and 12-month attack prevalence of asthma (since 1997) were highest among children aged 5-14 years, Blacks compared with whites, and females compared to males (American Lung Association Epidemiology and Statistics Unit, 2006; Akinbami, 2006). Approximately 38 percent of the asthma hospital discharges in 2004 were in those younger than 15, however, only 21 percent of the U.S. population was that age.

Economic and social burden

In 2004, the total cost of asthma was estimated at $16.1 billion, including $11.5 billion in direct health care costs and $4.6 billion in indirect costs (lost productivity) (American Lung Association Epidemiology and Statistics Unit, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. One study estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs (Malone, Lawson, & Smith, 2000). Asthma is also a substantial and increasing public health burden because it contributes to school absence and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Currently available databases are useful to study acute exacerbations of existing asthma but cannot directly answer questions about asthma incidence and the effects of exposures to chronic outdoor and indoor air pollutants and the interactions with exposures to aeroallergens. These questions require the collection of new data and are best studied in a large prospective cohort study design that collects data in utero, postnatally, through childhood, and into adulthood. A large sample size and prospective approach is needed to accurately assess the timing of exposures, particularly during critical windows of vulnerability (e.g., specific trimester of pregnancy, early vs. later postnatal periods, etc.) on the onset of asthma in childhood. Identification of children at risk for developing the severe forms of asthma will have a clear public health impact. A large prospective study is needed to have sufficient numbers to identify risk factors to determine which children with asthma will develop the severe, persistent variety.

Interactions of air pollutants and aeroallergens with other factors (e.g., genetic, lifestyle factors, etc.) addressed by the other asthma hypotheses contribute to the requirement for a large sample. Accurate exposure and phenotypic data are needed to assess the importance of many asthma and allergy genotypes interacting with many exposures in relation to complex and variable asthma phenotypes. A large sample will be required to evaluate the susceptibility to the adverse effects of certain pollutants and aeroallergens and their sources in specific genotype-phenotype clusters. Also, data are needed from children of different ethnicities living in varied housing, socioeconomic, and geographic conditions. These factors may be correlated with variations in exposures, and a large sample size will be necessary to allow adjustments to account for this.

Landrigan et al. (2006) discuss in more detail the rationale and strengths for such a study, which is expected to direct the development of prevention strategies for both diseases of childhood and chronic diseases of adulthood whose developmental origins likely begin in childhood.

3.3 Scientific Merit

Theory supporting hypothesis

Many organic compounds such as polycyclic aromatic hydrocarbons (PAH) and quinones as well as transition metals are associated with products of fossil fuel combustion and tobacco smoke. PAH and transition metals have adjuvant effects on cytokine-mediated airway inflammation. This occurs, in part, through oxidative stress mechanisms involving the production of reactive oxygen species (ROS) by pollutants and resultant antioxidant, inflammatory, and cytotoxic responses in the human lung (Behndig et al., 2006; Nel, Diaz-Sanchez, & Li, 2001; Li et al., 2003; Xia et al., 2004; Xiao, Wang, Li, Loo, & Nel, 2003). This process has been linked to the enhancement of allergic respiratory responses to airborne allergens and may be involved in the onset of atopy (Diaz-Sanchez, Proietti, & Polosa, 2003).

Evidence for this has come primarily from studies that have used diesel exhaust particles (DEP) as a model exposure since this source is particularly rich in redox cycling compounds (Xiao et al., 2003). However, diesel exhaust in the environment is also comprised of important volatile and semivolatile (nonparticle-phase) organic compounds and metals, and automobile exhaust produces nearly the same set of components, including PAHs, which are semivolatile organic compounds. Emission factor measurements from on-road studies show that gasoline vehicles comprise the majority of mobile source particulate matter (PM) emissions in the United States (Gertler, 2005).

Atopy has repeatedly been shown to be a major risk factor in the development of asthma in children (Holt, Macaubas, Stumbles, & Sly, 1999; Hoffjan, Nicolae, & Ober, 2003; Bel, 2004). In addition to atopy, other identified risk factors include parental asthma, sibling asthma, recurrent chest infections at ages 1 and 2, parental smoking, male gender, obesity, eczema, allergic rhinitis, and outdoor pollutants (Taussig et al., 2003; King, Mannino, & Holguin, 2004; Arshad, Kurukulaaratchy, Fenn, & Matthews, 2005). Sensitization to multiple inhalant allergens (pollen and common environmental) in adults has been shown to increase the risk of having physician-diagnosed asthma, allergic rhinoconjunctivitis, and wheezing in a dose-dependent relationship (Pallasaho, Ronmark, Haahtela, Sovijarvi, & Lundback, 2006). Early (assessed at 6 months) exposure to cats increased the risk of atopic sensitization and the later development of asthma through age 10 in a dose-dependent manner except for the most highly exposed infants. This latter group showed a reduced risk of sensitization and wheezing (Lau et al., 2005). There are age-dependent changes in cytokine production by Th cells, and the blunting of these changes may not occur until age 5. Thus, these exposure/response associations in young children whose immune system is developing complicates the understanding of potential effects that a large prospective study may help resolve. Any investigation into the causative factors of asthma should not ignore the closely related causative factors of atopic sensitization and other atopic diseases as the factors leading to these conditions appear to overlap greatly and could even be independently synergistic with other risk factors for asthma. For example, higher exposures to fine particles may increase the risk of allergic sensitization to common aeroallergens.

The putative role of air pollutants in mixtures such as ETS or vehicular exhaust in allergic sensitization, asthma incidence, and increased asthma severity is hypothesized to be based, in part, on a failure of the T cell population to mature adequately to T helper 1 (T_H1) over T helper 2 (T_H2) subtype early in life. This drives an imbalance toward T_H2 immunity (Macaubas et al., 2000), which has been associated with atopy, asthma, and increased production of proinflammatory cytokines (namely IL-4, IL-5, and IL-13). This process begins prenatally (Macaubas et al., 2000). In experimental studies, increases in cytokines have been consistently linked to airway inflammatory and IgE-mediated allergic responses to DEP (Pandya, Solomon, Kinner, & Balmes, 2002). Experimental data has shown that DEP can induce primary sensitization to an allergen compared with no sensitization upon exposure to the antigen alone (Diaz-Sanchez, Garcia, Wang, Jyrala, & Saxon, 1999). These responses are hallmarks of allergic asthma where eosinophils among other cells play a key role. Effects of air pollutants on innate immunity mechanisms instead of IgE-mediated acquired immunity is largely unknown but may be important in explaining the incidence and ongoing morbidity of nonallergic or non-eosinophilic asthma (Douwes et al., 2006).

The adjuvant effects of air pollutants on shifts in either acquired or innate immune pathways may begin in utero. Continued exposure during early development is expected to increase further the risk of asthma. This is because most human lung development is complete by ages 6-8 (Burri, 1997), and the developing lung is highly susceptible to immunological and structural changes from inhaled toxicants (Finkelstein & Johnston, 2004; Pinkerton & Joad, 2006). In addition, repeated lower respiratory infections during the preschool years have been associated with the onset of asthma, but this is likely modified by genotype and early life immune phenotype (Friedlander et al., 2005).

Current scientific understanding

Asthma is a complex disease of the lower airways variably characterized by reversible airways obstruction, airway inflammation, and airway hyper-responsiveness. This is clearly reflected in the data from gene association studies which indicate a complex inheritance pattern involving perhaps hundreds of genes governing the expression of varying asthma and atopy phenotypes (Ober & Hoffjan, 2006). Asthma phenotypes that emerge from ages 1-6 have been predictive of persistent asthma symptoms and long-lasting decrements in lung function in cohort studies (Stein & Martinez, 2004). However, those studies did not evaluate the influence of air pollutants, aeroallergens, and pollutant-allergen interactions on the expression of these phenotypes or their impact on future morbidity. The National Children's Study will assess the risk of asthma-related phenotypes (e.g., transient early wheeze) and exposure-response relationships as hypothesized for diagnosed asthma. It is important to recognize that the Study's focus is on diagnosed asthma that persists into later childhood.

There is substantial evidence that air pollutants and aeroallergens can exacerbate existing asthma. Air pollutants showing acute effects have included ozone, secondary organic aerosols, and some primary constituents of particulate matter from fossil fuel combustion as well as transition metals (Gilmour, Jaakkola, London, Nel, & Rogers, 2006; Peden, 2005; Trasande & Thurston, 2005). However, the role of these air contaminants in the induction of asthma is less clear primarily due to a paucity of research.

Epidemiologic research to support the putative causal relationship of air pollutants with asthma onset in children is sparse. It is also unclear whether repeated acute biological responses to air pollutants early in life leads to the later development of allergy and asthma and which specific types of acute responses are important. Furthermore, there are major gaps in knowledge concerning the relative toxicity of air pollutant mixtures from various sources, which requires more attention to finer spatial monitoring with source tracers (Schlesinger, 2006). To answer these questions, prospective cohort studies are needed with detailed exposure and response data beginning in pregnancy.

To date, most prospective birth cohort studies of asthma onset have been small and used retrospective recall of exposures during pregnancy and early life, which can lead to exposure error. A pregnancy cohort in New York is the only U.S. study that aims to examine the effects of prenatal and early postnatal air pollution exposures on neurocognitive development, asthma etiology, and cancer risk. Early findings include PAH exposures that have adverse effects on birth weight and head circumference (Perera et al., 2003) and adverse effects on cognitive development (Perera et al., 2006). A study conducted in the Czech Republic evaluated lymphocyte immunophenotypes in cord blood at birth among 1,397 deliveries (Hertz-Picciotto et al., 2005) and suggested that the fetal immune system might be altered by maternal exposure to air pollution. Personal PAH exposure during pregnancy in Krakow, Poland, was positively associated with increased risk of respiratory infections and symptoms in children during the first year of life (Jedrychowski et al., 2005). A birth cohort study in the Netherlands found predicted traffic-related exposure to $PM_{2.5}$, $PM_{2.5}$ absorbance, and NO_2 were associated with incidence by age 2 of wheezing, physician-diagnosed asthma, ear/nose/throat infections, and flu or serious colds (Brauer et al., 2002). These findings were confirmed and strengthened at age 4 (Jedrychowski et al., 2005). They also found a positive association between air pollution and sensitization to food allergens (egg and milk) by specific IgE. Exposure estimations were based on measured pollutants from 40 sites selected on variability in traffic then linked with traffic near subject homes using geographic information systems (GIS) (Brauer et al., 2003). Other studies have used GIS-based exposure model estimates of traffic-related air pollutants and found increased exposures were associated with cough without infection and dry cough at night at age 1 in Munich, Germany (Gehring et al., 2002), with incident cough without a cold at ages 3-5 in Leicester, England (Pierse et al., 2006), and with prevalence of wheezing without a cold in Cincinnati, OH (Ryan et al., 2005). In a Southern California cohort of school children, the prevalence of

asthma in children who had no family history of asthma was significantly increased the closer the residence was to a major road (McConnell et al., 2002). In another cohort of Southern California school children followed for 8 years until age 18, both ambient levels of air pollutants including NO_2, $PM_{2.5}$, and elemental carbon (Gauderman et al., 2004) and proximity to traffic (Gauderman et al., 2007) were associated with reduced lung function. This suggests that air pollutant impacts on lung health may persist into adulthood.

Multiple types of exposures (e.g., aeroallergens, air pollutants, viral infections, diet, etc.) are often not assessed in these cohorts. In addition, most have generally relied on questionnaire-reported prevalence of physician-diagnosed asthma and a varied but incomplete set of data on asthma-related clinical phenotypes collected at one time, usually well past disease onset. Such phenotypes include atopic sensitization, airway inflammation, bronchial hyper-responsiveness, lung function, symptom type and frequency, medication use, etc., all of which will be measured in The National Children's Study. The resulting inaccuracy in exposure assessment and disease characterization can make it difficult to identify susceptible individuals based on genetic or phenotypic characteristics. This is particularly problematic given the likely dependency of susceptibility on the magnitude and developmental timing of environmental exposures (Martinez, 2000; Schwartz, 2006a; Schwartz, 2006b; Zaas & Schwartz, 2003).

There are few examples of studies of asthma or atopy where timing of environmental exposures during early development was fully assessed. Evidence to support the study of exposure to the fetus and to the child during prenatal and postnatal periods comes from studies of ETS. Children prenatally and postnatally exposed to ETS have a significantly higher risk of sensitization to food allergens by the age 3 (Zaas & Schwartz, 2003). A pediatric cohort study in Southern California showed increased risk of asthma and wheezing among school children with a history of in utero exposure to ETS but not later exposure. The association was found only among subjects with a homozygous glutathione S-transferase gene deletion polymorphism, which is expected to reduce the ability of cells to counter the proinflammatory effects of ROS (Gilliland et al., 2002).

3.4 Potential for Innovative Research

The National Children's Study offers the unique opportunity to simultaneously examine multiple risk factors for asthma and to explore possible interactive and synergistic effects between different risk factors. Perhaps the greatest opportunity for innovation would be the examination of genetic or other markers of individual susceptibility to specific environmental agents.

Few cohort studies are examining effects of indoor or ambient air pollution and aeroallergen exposures during pregnancy and early childhood on the occurrence of childhood asthma. The Study will consider these exposures and the impact of regional ambient air pollution (McConnell et al., 2002), traffic-related air pollutants and proximity to roadways (Brauer et al., 2002), and indoor sources of VOCs or semi-VOCs (e.g., cleaning agents, solvents, paints, plastics, cars in garages). The current literature on many of these compounds in relation to asthma in children is limited compared to that on occupational asthma (Delfino, 2002; Dales & Raizenne, 2004; Rumchev, Spickett, Bulsara, Phillips, & Stick, 2004; Jaakkola & Jaakkola, 2006). A recent review identified multiple studies showing associations between asthma diagnosis or other respiratory outcomes and indoor residential chemical emissions (Mendell, in press). Most of these studies were small, not large cohort designs, and not conducted in the United States. Several prospective cohort studies, however, collected data during pregnancy or the postnatal period on exposures to indoor residential emissions.

Indoor and outdoor sources that contribute to levels of a particular chemical will drive concentration of other correlated pollutants that may be unmeasured. For example, findings showing associations of acute asthma outcomes with ambient VOCs (Delfino, Gong, Linn, Hu, & Pellizzari, 2003)

and exhaled breath VOCs (Delfino, Gong, Linn, Pellizzari, & Hu, 2003) have been suggested to serve as surrogate markers for a mixture of more causally active components, particularly from traffic-related sources. These components would include many particle-bound, semivolatile and volatile components, some of which are anticipated to emerge as potentially important casual agents from future toxicological data and can be assessed from archived environmental samples. From the standpoint of asthma prevention, controlling the source will be the key to success.

Traffic is a major source of outdoor air pollution exposure most likely to be encountered on a nearly daily basis by a majority of pregnant women and children in America. Many epidemiologic studies using retrospective data or cross-sectional methods have shown associations between traffic near the home and asthma and atopy prevalence or morbidity (Delfino, 2002; Heinrich & Wichmann, 2004; Sarnat & Holguin, 2007). However, there is limited data on asthma incidence and local air pollution (Brauer et al., 2002). In addition, in-vehicle exposure to toxic air pollutant components may dominate total exposures during the work week of expectant mothers. It has been estimated that more than 50 percent of black carbon and ultrafine particle (UFP) exposures for nonsmoking urbanites in Los Angeles comes from time in vehicles (Fruin, Winer, & Rodes, 2004; Fruin, Westerdahl, Sax, Fine, & Sioutas, 2005).

The potential for assessing individual susceptibility to air pollutants, including genetic, is great with the Study. Most asthma genetic studies employ similar and highly accurate genotyping methods. However, they generally employ widely divergent and generally inaccurate methods of exposure assessment. This has likely been responsible for an inconsistent literature on asthma gene association studies, as well as missed, biased, or ignored gene-environment interactions.

3.5 Feasibility

Outdoor air pollutant exposures at home, work, or preschool during pregnancy and early childhood can be estimated using land use regression and related modeling techniques based on traffic, microenvironmental, neighborhood, and ambient monitoring data (Brauer et al., 2003; Jerrett et al., 2005; Ross et al., 2006). Indoor air pollutant and allergen measurements have been collected successfully in several large surveys and include measures collected by study participants. Personal exposures for most air pollutants and other chemicals are usually correlated with indoor measurements. Outdoor air concentrations and estimates can be compared with measurements made at homes and with information about in-home sources to provide estimates of indoor concentrations over longer time-periods. In larger primary sampling units (PSUs), the data for use in model development to predict outdoor home exposures can be collected at low cost in targeted areas given that the populations will be recruited from relatively small geographic areas (neighborhoods). An air-monitoring network exists across the United States, and populations could be supplemented with community-level outdoor air measurements. The monitoring network can be used to characterize exposure to children who move to another location. Combined with air models, this will allow investigators to characterize a wide range of exposures during prenatal, neonatal, early childhood, and later childhood periods.

The national probability sample for the Study, combined with selection of communities within each PSU, should allow for appropriate gradients in exposure to air pollution on both a regional and local scale. In addition, because pollutants such as ozone vary with climate, temporal variability must be included.

ETS exposure has been successfully assessed using questionnaires validated against particle measurements and cotinine measurements in saliva, urine, or blood plasma. This will continue to be the case in The National Children's Study.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Exposure to indoor and outdoor air pollution and aeroallergens

Primary/child

- Exposure to indoor and outdoor air pollution and aeroallergens

4.2 Methods

Primary/maternal

- Environmental sampling for indoor and outdoor air pollution measures (e.g., PM, elemental carbon, NO2, and O3)
- Time-activity patterns (questionnaires, diaries, or GPS) for use in estimating exposures and contributions to total pollutant exposures from emission sources near the home and work
- Blood samples; urine samples; other physical sampling
- Interview/questionnaire information
- Dust samples will be collected for later measurement of mold, endotoxin, and common environmental antigens

Primary/child

- Environmental air and dust samples inside and outside home, school and daycare (e.g., PM, criteria pollutant gases, allergens, endotoxin, environmental tobacco smoke, VOCs, sVOCs)
- Other exposure information (housing and neighborhood characteristics, product usage in home, child time-activity patterns [questionnaires, diaries, GPS], local agency air quality reports) for use in estimating exposures and pollutant contribution to total exposures from emission sources near the home (mobile sources, including diesel and automobile traffic, and stationary sources, such as industrial emissions)
- Biological samples (exposure markers in blood, urine, etc.)

4.3 Life Stage

Primary/maternal

- Prenatal through birth

Primary/child

- Periodic, birth through year 21

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Airway hyper-reactivity
- Lung function
- Airway inflammation (exhaled NO)
- Immune system function (e.g., lymphocyte subsets, cytokines, total IgE)
- Allergic sensitization (specific IgE)
- Symptom history and medication use
- Respiratory infection history

5.2 Methods

- Examination, interview and testing by medical professional (e.g., history of asthma symptoms, lung function tests, skin prick test, exhaled breath for NO, etc.)
- Medical record reviews
- Cord and blood samples

5.3 Life Stage

- Periodic, birth through year 21

6. Important Confounders, Mediators, and Effect Modifiers

- **Poor health care access in inner cities:** Especially among Blacks and Hispanics, poor health care access exacerbates the risk of asthma attacks and deaths (Lara et al., 2003; Sin, Bell, & Man, 2004; Wallace, Scott, Klinnert, & Anderson, 2004). This and other disparity factors could be behind the greater asthma prevalence, especially among Blacks. These issues are thoroughly discussed in the disparities in asthma hypothesis and in the review by Gold and Wright (2005).
- **Socioeconomic status:** Lower socioeconomic status and some minority groups, such as Blacks and Hispanics, are likely to live in areas with increased air pollution, such as near freeways or industrial sites. Careful assessments of these characteristics will be important to properly control for their influence on outcomes.

- **Environmental exposures:** Diet, physical activity, bioaerosols, stress, and infections, which will be the topic of other hypotheses, could interact with or confound associations of asthma onset with air pollutants (example hypotheses 2.2 and 2.3). Though the following list is not exhaustive, some important factors associated with atopy and asthma include demographic group (age, race, income, or education), familial characteristics (number of siblings, parent and sibling phenotypes for asthma), daycare attendance, pet ownership, living on a farm, body mass index, immunizations, several dietary factors, respiratory infections, and diabetes.

- **Genetics:** For the analysis of gene-environment interactions, polymorphisms in genes involved in oxidative stress responses to air pollutant exposures and candidate asthma and allergy genes will be included (example hypothesis 2.3). Ultimately, gene-environment interactions may dictate the effect any factor has on disease outcome.

7. Power and Sample Size

Power analyses will have a cut-off value for "high" exposure of the pollutant predictor of interest based on the upper fifth percentile of NCS subjects (i.e., a proportion exposed is 0.05). In addition, we assume a total sample size of N = 100,000 and that the incidence of asthma by age 6 will be 5 percent. A fixed interval proportional hazards model was used to assess the association between pollutant exposure and the risk of asthma. The minimal detectable hazard ratios associated with the pollutant corresponding to power points of 80 percent, 90 percent, and 95 percent for a level 0.05 two-sided test are 1.199, 1.234 and 1.264, respectively. The estimates assume only a main effects model based on exposure to a single factor without consideration of interactions with other exposures, genetics, family history, etc. (National Children's Study Interagency Coordinating Committee, 2003).

The overall size of effects of indoor and outdoor air pollution on the onset of asthma may be small due to interactions with time-of-exposure and other factors (e.g., lifestyle and genetic). To exemplify this, in the following we seek to quantify effect modification by subject genotype or co-exposure to endotoxins and indoor allergens. Assessment of effect modification will be carried out by including multiplicative interaction terms for the potential effect modifier and the pollutant exposure into each regression model. For brevity, we present power analyses for a generic effect modifier with varying prevalences and assume the cutoff value for "high" exposure of the predictor of interest is based on the conservative upper fifth percentile of NCS subjects. Again, we assume a total sample size of N = 100,000 and that the incidence of asthma by age 6 will be 5 percent. We used a fixed interval proportional hazards model to assess the association between pollutant exposure and the risk of asthma. In this case, minimum detectable hazard ratios associated with the interaction term (multiplicative increase in the effect of the pollutant attributable to the effect modifier) corresponding to a specified power point that can be estimated via a modification of the usual two-sample proportional hazards formula given by (Schmoor, Sauerbrei, & Schumacher, 2000):

$$\lambda = \exp\left\{ \left(z_{1-\alpha/2} + z_{1-\beta}\right) \left(\sum_{i,j} 1/p_{ij} \right)^{1/2} \Big/ \left[n \Pr(\delta = 1) \right]^{1/2} \right\}.$$

Table 1. Minimum detectable hazard ratios associated with interactions. Estimates correspond to the 80 percent, 90 percent, and 95 percent power points of a level 0.05 two sided test

Prevalence of effect modifier	Minimum detectable hazard ratio associated with interaction term for selected power points		
	80%	90%	95%
2%	3.66	4.49	5.32
5%	2.30	2.62	2.92
10%	1.83	2.02	2.18
20%	1.58	1.69	1.79
30%	1.49	1.58	1.67
40%	1.45	1.54	1.61

8. Other Design Issues

- **Ethical/burden considerations:** The study also will need to have a formal strategy and process for effectively communicating the results of environmental monitoring to the child's parents, along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings. Repeated tests are potentially burdensome.

- **Cost/complexity of data collection:** Repeated waves of environmental exposure assessment can be both costly and burdensome, but are necessary to minimize exposure error that would result from reliance on ambient monitoring systems or the administration of larger questionnaires that would be needed to characterize these exposures. The size, intrusiveness, and time needed for indoor measurements will be minimized and complemented by community-level monitoring.

- **Need for community involvement:** Daycare and school cooperation would be required for some of the intended measures.

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DIETARY ANTIOXIDANTS AND ASTHMA RISK

1. Meta Hypothesis

Intake of antioxidants in diet affects the risk of asthma.

2. Specific Hypotheses

1. Maternal intake of antioxidants during pregnancy reduces the incidence of asthma in their offspring.

2. Intake of antioxidants during childhood will reduce the frequency and severity of asthma in children by age 5.

3. Genes involved in immune pathways will interact with childhood antioxidant intake to produce gene-environment interactions.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Asthma is the most common chronic illness among children. (National Academy of Sciences Institute of Medicine, 2000). U.S. data on asthma prevalence from the National Health Interview Survey (NHIS), The Centers for Disease Control and Prevention's [CDC] National Center for Health Statistics (NCHS), show the prevalence of current asthma increased 85 percent from 1982 through 1996 to an estimated 14.6 million people (5.52 percent of the U.S. population) according to the American Lung Association (American Lung Association Epidemiology and Statistics Unit, 2006). This increase was 76 percent in children younger than age 18 or 4.43 million children in 1996 (6.20 percent).

Between 1997 and 2000, the NHIS used a different definition of current asthma prevalence, which suggested an abrupt decrease, but this difference made comparison to past trends inappropriate. To rectify this, in 2001 a question was added back to restate the prevalence of current asthma regardless of attack occurrence in the past year, which made comparisons to years before 1997 appropriate. It was then apparent that current asthma prevalence had continued to increase until 2001, later reaching a plateau in most groups except Black children and adolescents, who have continued to experience increased prevalence annually (American Lung Association Epidemiology and Statistics Unit, 2006).

In 2004, 30.2 million Americans (10.47 percent of the population) had been diagnosed with asthma by a health professional. This estimate included 6.5 million children younger than 18. Almost 4 million children younger than 18 were estimated to have experienced an asthma attack in 2004. U.S. prevalence data from both the 12-month prevalence (before 1997) and 12-month attack prevalence of asthma (since 1997) were highest among children ages 5-14, Blacks compared with whites, and females (American Lung Association Epidemiology and Statistics Unit, 2006; Akinbami, 2006).

Hospitalization and death rates in children have also reached a plateau or decreased (Akinbami, 2006). Despite a plateau in prevalence in 2001, nonambulatory visits for asthma to physician offices by children have continued to increase (Akinbami, 2006), possibly due to increased public and

health provider awareness, which improved treatment. For example, there was a dramatic increase in the use of preventive (controller) medications, chiefly inhaled corticosteroids (Stafford et al., 2003; Suissa et al., 2000, 2002), which may have mitigated increases in hospitalization and mortality.

In the United States, approximately 38 percent of the asthma-related hospital discharges in 2004 were children younger than age 15, even though only 21 percent of the U.S. population was younger than that age. This trend paralleled increasing asthma hospitalization and death rates in children (American Lung Association Epidemiology and Statistics Unit, 2006; Akinbami, 2006).

Similar or greater increases in the prevalence of asthma during the second half of the 20th century were reported from other countries (Eder, Ege, & von Mutius, 2006) and increases may still be occurring, as seen in a recent birth cohort study in Oslo, Denmark, where every fifth child is now affected by asthma. (Lodrup Carlsen et al., 2006).

Economic and social burden

In 2004, the total cost of asthma was estimated at $16.1 billion, including $11.5 billion in direct health care costs and $4.6 billion in indirect costs (lost productivity)(American Lung Association Epidemiology and Statistics Unit, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. Malone, Lawson, and Smith (2000) estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs. Asthma also poses a substantial and increasing public health burden from school absence and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Opinions differ on which study designs are ideal to use to address complex common disorders such as asthma. In a recent literature debate, Willett et al. (2007) supported the retrospective use of available databases to study common disorders such as asthma, while Collins and Manolio (2007) supported a new large cohort study mainly of adults (Manolio, Bailey-Wilson, & Collins, 2006). The commentary in both articles covered the strengths and weaknesses of both approaches. Willet et al. and Collins and Manolio praised the strengths of a design based on a large birth cohort, but they questioned the feasibility of a large prospective cohort study design that incorporates in utero and early postnatal data as well as data from stages of development from infancy through childhood and into adulthood. However, the NCS could provide valuable data that could be used to accurately assess the timing of exposures, particularly during critical windows of vulnerability (e.g., specific trimester of pregnancy, early vs. later postnatal periods, etc.) on the onset of asthma in childhood and persistence into adulthood.

Accurate exposure and phenotypic data are needed to assess the importance of many asthma and allergy genotypes interacting with numerous exposures in relation to complex and variable asthma phenotypes. Interactions of maternal and fetal genotypes contribute to the requirement for a large sample. A large sample will be required to evaluate the susceptibility to the adverse effects of certain exposures and their sources in specific genotype-phenotype clusters.

Also, data are needed from children of different ethnicities living in varied housing, socioeconomic, and geographic conditions. These factors may account for some of the ethnic disparities related to asthma, which may be correlated with variations in exposures. A large sample size will be necessary to allow for statistical adjustments to account for this. Landrigan et al. (2006) discuss the rationale and strengths for a large prospective study, which is expected to direct the development of prevention strategies for both diseases of childhood and chronic diseases of adulthood whose developmental origins likely begin in childhood.

3.3 Scientific Merit

Seaton et al., (1994) proposed that the increases in asthma occurring at that time might be due to changes in diet (i.e., decreased vegetable consumption) rather than an increase in air pollution as others had proposed. They reasoned that decreased antioxidant consumption had increased population susceptibility and had contributed to the increases in asthma incidence and prevalence, and proposed a mechanism that related decreased dietary antioxidant intake to reduced lung antioxidant defenses, which increased airway susceptibility to oxidant damage and resulted in airway inflammation and asthma. Support for this hypothesis has grown (Kirkham and Rahman, 2006; Rahman, Biswas, & Kode, 2006; Romieu et al., 2006; Romieu et al., 2002).

Interest in the relation between dietary antioxidant consumption and risk of asthma arises from several sources. One hypothesis proposed that the effect of antioxidants on asthma is related to an effect on the developing immune system of the fetus (Cunningham-Rundles, McNeeley, & Moon, 2005). Another hypothesis proposes that antioxidants protect against exposure that leads to oxidative stress and its adverse effects on lung function (Romieu et al., 2002). General population-based health surveys have demonstrated modest direct associations between pulmonary function and antioxidant consumption and serum levels (Schunemann et al., 2002; Schwartz & Weiss, 1990). Also, oxidative damage markers are found at higher concentrations in the exhaled breath condensate of children with asthma compared to those without asthma, while markers of antioxidant status are higher among children without asthma (Corradi et al., 2003). Deleterious pulmonary inflammatory response to ozone exposure, thought to be mediated at least in part by oxidative damage to airway tissues, may be modified by oral intake of vitamins C and E (Samet et al., 2001; Romieu et al., 1998).

Little direct evidence exists for the relationship between antioxidant consumption and the development of asthma, especially in relation to exposure to potential oxidative stressors such as ozone, nitrous oxides, or environmental tobacco smoke. Although the mechanisms that might link oxidative insult with the onset of asthma have not been fully elucidated, there is some evidence that pollution plays a role, either as gas (e.g., ozone) or as ultrafine particles (Elder, Gelein, Finkelstein, Cox, & Oberdorster, 2000; Kim & Nadel, 2004; Klebanoff et al., 2005; Frampton et al., 2006).

Examination of the temporal relationship between antioxidant consumption and subsequent outcome and assessment of genetic variation in immunologic response to oxidative stress and the potential modifying influence of antioxidant exposure will be important in understanding potential intervention techniques, whether related to asthma incidence or treatment (National Children's Study Interagency Coordinating Committee, 2003). A recent review (Kalantar-Zadeh, Lee, & Block, 2004) echoed this with a recommendation for more epidemiological studies and a specific proposal to evaluate the intake of antioxidants in children ages 2-6, interactions with genetic and environmental risk factors that may lead to the development and persistence of asthma, and the relationship between degree of antioxidant deficiency and the course of asthma and atopy.

Recent cohort studies of maternal intake of antioxidants during pregnancy

Devereux and Seaton (2005) reviewed a decade of research on the evidence of diet as a risk factor for asthma and dietary interventions. They noted that studies of lung function in adults (e.g., Hu & Cassano, 2000) and children (e.g., Gilliland et al., 2003) showed independent effects of positive association of antioxidant nutrients [including vitamins C and E] and measures of lung function (forced expiratory volume). However, intervention studies with children were mostly negative, and increases in antioxidants in the diets of children did not reduce the risk for asthma. The authors concluded dietary intake of antioxidants most likely were exerting effects during pregnancy or early childhood.

The theory of developmental origins of health and disease, suggesting that fetal effects could have profound effects on later outcomes, contributed to a recent change in the design of studies investigating the impact of antioxidants on asthma (Devereux & Seaton, 2005). Instead of focusing just on the effects of child intake of antioxidants, recent studies have focused on maternal intake of antioxidants during pregnancy and later effects on the risk of childhood asthma in the offspring. For example, multiple reports about maternal intake of antioxidants on childhood wheeze and asthma have been published based on birth cohorts established in Aberdeen, United Kingdom (Martindale et al., 2005; Devereux et al., 2006, 2007) and in Boston, MA (Litonjua et al., 2006; Camargo et al., 2007). These studies have focused specifically on two components of maternal antioxidant intake during pregnancy (vitamins E and D).

Based on the Aberdeen cohort, Martindale et al., (2005) evaluated 2,000 women and followed 1,924 children born to the cohort at 6, 12, and 24 months of age. Maternal vitamin E intake during pregnancy was negatively associated with wheeze (in the absence of "cold") and eczema (in atopic mothers) in 1,253 offspring at 2 years of age, and vitamin C intake was positively associated with wheeze during childhood. Devereux et al. (2006) evaluated 1,120 children born to this cohort at age 5 and reported a continuation of the negative association of vitamin E intake during pregnancy with wheeze in the offspring. This research also showed a similar negative association of vitamin E intake during pregnancy with several other phenotypes in offspring, including the persistent wheeze-without-cold phenotype and doctor-confirmed asthma and asthma plus wheeze at age 5. Maternal intake of zinc during pregnancy was also negatively associated with asthma and eczema at age 5. An additional report by Devereux et al. (2007) on outcome at age 5 revealed that increased maternal intake of vitamin D was associated with a decrease in the risk for wheezing in childhood, but another report of asthma at age 5 showed no association with maternal intake of vitamin E, vitamin D, or zinc that was suggested by the earlier reports (Willers et al., 2007).

Based on the VIVA cohort in Boston, Litonjua et al. (2006) evaluated 1,290 mother-child pairs in a cohort study of maternal diet and reported a negative association of maternal intake of vitamin E and zinc with wheezing in their offspring at age 2. In a separate report on this cohort, Camargo et al. (2007) documented a negative association of maternal intake of vitamin D with recurrent wheeze at age 3. Analysis of both child and maternal intake of vitamin D revealed that a child's intake was not related to recurrent wheezing in childhood, and the protection provided by high maternal intake of vitamin D was present for low and for high vitamin D intake of the child.

A mitochondrial antioxidant hypothesis of asthma

The focus on antioxidants suggests an involvement of the mitochondria, which produce reactive oxygen species (ROS) as a byproduct of cellular energy production. Wallace, Lott, and Procaccio (2007) developed a variant of the antioxidant hypothesis of asthma based on the involvement of mitochondria (see Appendix A). They contrasted two perspectives about the pathophysiology of asthma (the beta adrenergic theory [Israel et al., 2004], and the ROS-inflammatory theory [Corradi et al., 2003; Kinnula and Crapo, 2003]), that are consistent with the mechanism of action of common pharmacologic treatments of asthma, such as long-acting beta agonists and inhaled corticosteroids (Sin et al., 2004). Wallace, Lott, and Procaccio reasoned that these different pathophysiological mechanisms converge on a single disease process that leads to asthma. They proposed this "third factor" was the mitochondrion, which is shared by the beta adrenergic and ROS-inflammatory systems of the lung and affected by antioxidants (Shoffner & Wallace, 1994; Wallace, 2005). This innovative theory directs the search for genetic contributions to asthma and for evaluation of the effects of environmental factors (antioxidant intake) on the incidence and prevalence of asthma.

An evaluation of variation of mtDNA and the risk for asthma is lacking in the literature, but Wallace, Lott, and Procaccio hypothesized that common variants in mtDNA may predispose an individual to asthma. They reviewed the sources of genetic variation in mtDNA and identified three classes of mutation: maternally inherited deleterious mutations, ancient adaptive polymorphisms, and somatic mutations. The maternally inherited and ancestral mutations that have emerged in the human species require a search for thrifty genotypes, which have resulted from positive selection in former environments that now increase risk for asthma due to "environmental mismatch." The mtDNA haplotypes that emerged in evolutionary history and adaptation to cold climates (Wallace, 2005) produce clear haplogroups, and the most prevalent haplogroup (designated as H) is carried by about 45 percent of individuals with European heritage. In the NCS sample, it would be possible to evaluate whether this common haplogroup is associated with asthma in the context of the planned evaluations of variation in nuclear DNA using the nested case-control approach.

Evaluations of somatic mutations that occur in mtDNA and accumulate during a person's life require a different strategy. In the NCS, repeated samples will be obtained, and epithelial cells can be obtained from urine samples collected at each visit. Cells from these samples can be assayed to determine the percentage of somatic mutations at each point in time. Typically, somatic mutations are investigated for diseases in the elderly. As a result of more mutations accumulating through time, little information exists about variation in children that may be associated with common diseases. Since somatic mutations are clearly related to environmental exposures and conditions that increase ROS, a high load may predispose to asthma. Most of the endogenous ROS is from mitochondrial oxidation of calories via oxidative phosphorylation, and antioxidants affect ROS production. The mitochondrial antioxidant hypothesis of asthma would suggest that antioxidant deficiency may result in an increased rate of somatic mutations that would contribute to asthma. A simple test of this would be to determine in the case-control design whether somatic mutations in mtDNA are elevated in cases with asthma. Based on the mitochondrial antioxidant hypothesis of asthma, the intake of antioxidants may have an effect on the rate of accumulation of somatic mutation over time, and the repeated samples specified in the NCS would allow for the test of this hypothesis of an interaction of an environmental exposure (antioxidant intake) and a mitochondrial genetic factor (somatic mutation).

3.4 Potential for Innovative Research

Vulnerability to particular risk factors is determined not only by the genome acquired at conception but by the nature of the environmental influences during critical periods of development. As a result, a longitudinal assessment of the environment from before conception through pregnancy, fetal life, birth, and infancy could provide an opportunity to understand time-dependent susceptibility to asthma. For example, the review by Saglani and Bush (2006) emphasized the importance of the first three years of life and concluded that fetal and maternal genotype interact with environmental exposures to establish a risk phenotype, which interacts with antenatal exposures to set by age 3 the life-long course of chronic asthma and lung impairment.

The recent studies of maternal intake of antioxidants and the effects on the risk of asthma in their offspring based on the Aberdeen cohort (Martindale et al., 2005; Devereux et al., 2006, 2007) and the VIVA cohort (Litonjua et al., 2006; Camargo et al., 2007) indicate that fetal exposure to vitamins E and D has an impact on asthma in childhood. However, the VIVA and Aberdeen cohorts are relatively small (approximately 1,000) and were probably underpowered to address the effects on rare outcomes (e.g., asthma rather than wheeze) and interactions of different antioxidants (e.g., separate and combined effects of vitamins C, D, and E) and combination of maternal and child dietary intake of antioxidants. The sample participating children of 100,000, collected by the National Children's Study, will provide an opportunity to test hypotheses derived from these groundbreaking studies.

Also, the interplay between genes and environment is characterized by dynamic modifications to the genome, such as epigenetic modifications to nuclear and mitochondrial DNA that have been hypothesized as likely mechanisms for gene-environment interactions. The consideration of mitochondrial involvement in asthma (Wallace et al., 2007) offers an innovative approach to the investigation of genetic and environmental etiologies of asthma that may be relevant to the understanding of variability in response to common treatments (Sin et al., 2004) and to rare side effects of treatment (Israel, 2005).

3.5 Feasibility

The NCS protocol specifies the collection of biological samples that could be the source of DNA for traditional genetic analysis of candidate genes and for gene discovery based on genome-wide association scans. The anticipation of chip-based genotyping of all participants based on current technology (with an estimated cost of $30 million to $100 million), or complete sequencing of all individuals at some point in the future (at an expected cost of less than $1,000 per person), will provide extraordinary detail about genetic variation of nuclear DNA. The inclusion of markers for mtDNA would add little to the cost of this genotyping. Along with the planned definition of cases based on asthma phenotypes, this provides an opportunity to use the efficient nested case-control design for subsets of the sample or the proportional hazard design for the entire sample to evaluate effects of genes, environments (including intake of antioxidants), and their interaction.

The NCS protocol also specifies collection of biological samples at multiple points through time, which provides the opportunity to evaluate epigenetic changes proposed to be the molecular mechanisms of some gene-environment effects (Gluckman & Hanson, 2005). The epigenetic assays for environmental effects of methylation and chromatin status are rapidly evolving, and current methods will improve as a result of extensive current (Callinan & Feinberg, 2006) and future work (NIH Epigenetics Workshop, 2007).

Finally, the mitochondrial antioxidant hypothesis of asthma (Wallace et al., 2007) offers innovative and highly feasible approaches due in part to the small number of mitochondrial genes compared to the number of human genes. The assessment of mtDNA haplogroups in a case-control design would add little to the cost of the genomewide scans proposed for nuclear DNA. The methods for assessment of somatic mutations in mtDNA over time are already in place (Wallace, 2005).

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Antioxidant consumption and antioxidant levels

Primary/child

- Antioxidant consumption and antioxidant levels

4.2 Methods

Primary/maternal

- Interviews/questionnaires (diet/nutrition assessment, vitamin use): Standard food frequency questionnaires (FFQs) will be adjusted to account for the substantial addition of antioxidants to common foods such as fruit juices and cereals. Unless this is done, the FFQ tool will miss a sizeable source of antioxidants in daily diet from foods readily available in most supermarkets.
- Blood samples
- Urine samples
- Breast milk

Primary/child

- Urine samples
- Blood samples
- Interviews/questionnaires (diet/nutrition assessment, vitamin use)

4.3 Life Stage

Primary/maternal

- Prenatal through infancy

Primary/child

- Periodic, 0-3 months to year 21

5 Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

- Decrease the risk of asthma measured via allergy; child asthma index; airway reactivity

5.2 Methods

Primary

- Direct observation by medical professionals; medical record reviews; interviews/questionnaires; blood samples; urine samples

5.3 Life Stage

Primary

- Periodic, birth to year 21

6. Important Confounders, Mediators, and Effect Modifiers

- **Smoking** which is related to higher levels of oxidative stress, may reduce the effect of an antioxidative diet (Romieu & Trenga, 2001; Farchi et al., 2003).
- **Consumption of flavinoids** in red wine and apples has been shown to be negatively associated with asthma severity (OR = 0.89). This may suggest a protective effect of flavonoids (Shaheen et al., 2001).
- **Everyday foods not measured**: Unless specifically accounted for, the addition of large sources of antioxidants to everyday foods, such as juices and cereals, will be missed by the FFQ.

7. Power and Sample Size

Several methods are available for the evaluation of the antioxidant hypothesis of asthma. A standard approach is to use the nested case-control design with affected cases defined by asthma phenotype and matching to select control subjects (Gauderman, 2002). An alternative would be to use the proportional hazard method of analysis, which uses all cases, and the time when the case is identified as well as the number of cases analyzed. The power and sample size of each of these methods is provided below and described in Section 10 of the NCS Research Plan.

For the nested case-control design, if we assume the initial cohort size is 100,000, the study period is 5 years, there is an annual incidence rate of 2.5 percent and a prevalence by age 6 of 13.1 percent , then we would expect about 11,300 cases. The minimum detectable odds ratio for a genetic main effect and a gene-environment effect (using 1.5 and 10.0 controls per case) is:

Disease	Genetic Main Effect		Gene-environment Interaction	
Incidence (%)	1:1.5	1:10	1:1.5	1:10
2.50	1.16	1.13	1.52	1.36

For the proportional hazard model we present power analyses with a cut-off value for "high" exposure of the predictor of interest (intake of antioxidants) based on the upper fifth percentile of NCS subjects (i.e., a proportion exposed is 0.05). A total sample size will be N = 100,000 and the incidence of asthma by age 6 will be 5 percent. A fixed interval proportional hazards model can be used to assess the association between exposure (history of respiratory viral infection) and the risk of asthma. For a two-sided test at 0.05, the minimal detectable hazard ratios associated with the exposure for power of 80 percent, 90 percent , and 95 percent are 1.199, 1.234 and 1.264, respectively. The estimates assume only a main effects model based on exposure to a single factor without consideration of interactions with other exposures (National Children's Study Interagency Coordinating Committee, 2003).

8. Other Design Issues

- Ethical considerations are associated with genetic tests, but such considerations underlie the overall NCS and would not be unique to this specific hypothesis.
- For nongenetic factors, the study will need to have a formal strategy and process for effectively communicating results of physiological and biochemical measures to the child's parents and a responsible health care provider.
- Study will need to have a formal strategy and process for effectively communicating results of environmental monitoring to the child's parents along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings. In addition, a mechanism needs to be identified to ensure that children found to have asthma through this study can receive adequate medical care for their illness.
- Repeated assessments can be burdensome, and the total respondent burden must be evaluated and be reasonable.
- Blood studies, especially fasting, in younger children will require careful attention.

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SOCIAL ENVIRONMENTAL INFLUENCES ON ASTHMA DISPARITIES

1. Meta Hypothesis

Disparities in the incidence, prevalence, severity, and effective management of asthma by race and socioeconomic status are significantly associated and modified by social-environmental factors and processes that influence exposure to physical environmental risk factors, psychosocial stress, and health-related behaviors.

2. Specific Hypotheses

1. The relationship between socioeconomic status, race/ethnicity, and asthma incidence, morbidity, and mortality is significantly modified by socially influenced differential exposure to physical environmental risk factors (e.g., local-source air pollutants, allergens) and psychosocial stress (e.g., poverty, community disorganization, crime and violence, social incivility, noise, crowding, hazardous conditions, physical decay, and unemployment). These effects are moderated by policies and programs that buffer the effects of economic disadvantage on families.

2. Economic, cultural, and social features of the local area are significantly associated with: (1) exposure to stressful life conditions and events; (2) the availability of social ties that provide informational, emotional, and instrumental resources to individuals and families. These factors, in turn, influence outcomes, including immunological function in the child, the likelihood that the child will develop asthma, and asthma severity and management.

3. Background and Justification

3.1 Public Health Importance

Disparities

Many studies and reviews have documented systematic differences or disparities in asthma prevalence, severity, and mortality by race/ethnicity and socioeconomic status that are not fully explained by analysis of individual-level risk factors (Shanawani, 2006; Ford & McCaffrey, 2006; Gold & Wright, 2005; McDaniel, Paxson, & Waldfogel, 2006). Asthma severity and death rates have been highest in areas with higher concentrations of poor people and minority residents, particularly African Americans (Lang & Polansky, 1994; Bai, Hillemeier, & Lengerich, 2007; Lane, Newman, Edwards, & Blaisdell, 2006). In national surveys, African Americans report higher rates of asthma and a higher increase during time (McDaniel et al., 2006). Prevalence rates vary among Hispanic populations, with Mexican-American children in the Southwest experiencing a low prevalence and Puerto Rican children in the East high prevalence (National Academy of Sciences Institute of Medicine, 2000).

National Health Interview Survey (NHIS) data reveal a higher prevalence of asthma in urban areas, but evidence suggests rates can be as high in some rural areas (National Academy of Science Institute of Medicine, 2000). Asthma mortality and hospitalization vary significantly among large cities and neighborhoods within cities (Wright & Fisher, 2003; Byrd & Joad, 2006). Severe asthma demonstrates a graded association with socioeconomic status in the United States, but the precise role is not clear because of complex interactions with race/ethnicity and other factors (Shanawani, 2006; McDaniel et al., 2006). One report suggested that the most significant race/ethnic differences in asthma prevalence are observed among the poor (Smith, Hatcher-Ross, Wertheimer, & Kahn, 2005). The

associations between race/ethnicity, socioeconomic status, and other factors seem to differ for different asthma outcome measures: incidence or risk, prevalence, severity, and mortality.

Economic and social burden

In 2004, the total cost of asthma was estimated at $16.1 billion, including $11.5 billion in direct health care costs and $4.6 billion in indirect costs (lost productivity) (American Lung Association Epidemiology and Statistics Unit, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. One study estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs (Malone, Lawson, & Smith, 2000). Asthma also poses a substantial and increasing public health burden from school absence and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Multiple aspects of the social environment influence asthma etiology and management. Although asthma is common, elucidating the complex pathways that contribute to asthma will require a large sample because interactions will be common. For example, we anticipate interactions between family economic disadvantage and social policy variables, such as the generosity of welfare programs and the design and enforcement of public housing programs and codes.

A large prospective study is needed to adequately represent a range of geographic, environmental, economic, cultural, and policy variability among neighborhoods and communities to select enough cases within neighborhoods and communities to permit estimation of neighborhood effects and to model social and physical environmental influences.

Residential mobility is a critical problem in studies of neighborhoods and neighborhood effects. A prospective longitudinal study will be able to follow respondents through time and monitor residential mobility in order to assess residential change or mobility and the role it plays in health disparities by socioeconomic status, race, and geography.

Collection of retrospective data on variables such as family stress, parenting, and cultural beliefs is susceptible to various types of information bias. These factors must be observed prospectively.

Landrigan et al. (2006) discuss the rationale and strengths for such a study, which is expected to direct the development of prevention strategies for both diseases of childhood and chronic diseases of adulthood whose developmental origins likely begin in childhood.

3.3 Scientific Merit

Current scientific understanding

The existence of social environmental determinants of asthma etiology, severity, and management is evident from the strong differentials in asthma and asthma mortality noted above. The social factors and mechanisms involved are only beginning to be studied. Wright and Fisher (2003) suggest that differentials are likely to occur via four pathways: environmental exposures, stress, health behaviors and psychological factors, and access to health care. Other investigators have emphasized similar factors that contribute to disparities in asthma, including systematic differences in outdoor and indoor pollution, traffic, allergen exposure, violence, segregation, culturally influenced health behaviors, psychosocial stress, asthma knowledge, and access to care (Shanawani, 2006; Byrd & Joad, 2006; Morello-Frosch, & Lopez, 2006; Ford & McCaffrey, 2006).

Minorities and economically disadvantaged populations are more likely to experience unhealthy environmental exposures, including antigens and pollutants that are linked to asthma. This occurs in part because social, economic, and political disadvantage increases the probability that such groups will reside in impoverished urban environments where the levels are high; and in part because the same disadvantages reduce their ability to escape such areas or improve their physical environment through collective action (Massey & Denton, 1993; Byrd & Joad, 2006; Gold & Wright, 2005). Public policies and programs may differentially buffer these effects. The concentration of minority populations in unhealthy environments is exacerbated by discriminatory housing practices (Massey & Denton, 1993) and segregation (Morello-Frosch & Lopez, 2006), theoretically governed by public policies. Studies have clearly documented linkages between low income, substandard housing, and exposure to asthma-triggering factors such as smoke, dust mites, cockroaches, rodents, dry heat, and lack of heat (Boston Medical Center and Children's Hospital Doc4Kids Project, 1998). In the Moving to Opportunity study, a housing-mobility experiment in which a random sample of families in public housing were offered the opportunity to move to low-poverty neighborhoods, the incidence of asthma attacks was reduced among the group that moved compared to those not given the opportunity to move (Katz, Kling & Liebman, 2001). It is plausible that public policies influencing housing conditions as well as housing location may also influence exposure to asthma-causing antigens. Exposure to environmental tobacco smoke also occurs disproportionately in disadvantaged populations because of the higher prevalence of smoking in low-status groups. Physical exposures such as these affect asthma etiology prenatally and throughout childhood.

Research has linked stress to both the etiology and management of asthma (Wright, Rodriguez, & Cohen, 1998; Wright, Cohen, Carey, Weiss, & Gold, 2002). Stress contributes to asthma etiology directly by impairing immune function and indirectly through its effect on smoking. This occurs both prenatally (via maternal stress and smoking) and during childhood. Stress complicates effective asthma management by reducing the psychological resources families coping with asthma have to seek care and comply with preventive measures. Few studies have documented those aspects of the social environment that create harmful levels of stress could underlie socioeconomic and racial disparities in asthma. As conceptualized here, stress is inherently a property of an individual, the result of exposure to experiences and events with which the individual cannot readily cope (Herbert & Cohen, 1993). The social environment may contribute to stress by affecting the individual's exposure to experiences and events or by affecting the individual's ability to cope. Furthermore, the cumulative balance during development between chronic and acute stress and stress-buffering supports is consequential for health (Singer & Ryff, 1997).

Economic disadvantage is associated with greater levels of psychosocial stress (Baum, Garofalo, & Yali, 1997). Children growing up in poverty show early signs of elevated allostatic load, including elevated secretion of cortisol and epinephrine and higher resting blood pressure. Children who live in sustained poverty and those who become poor are more likely to be exposed to family violence and inadequate parenting (Repetti, Taylor, & Seeman, 2002). In turn, parenting problems and family stress have been linked to both wheeze and asthma in children (Wright et al., 2002). Social service programs that alleviate poverty may influence stress levels experienced by families and children, as well as the resources available to families for coping with stress. Relevant programs include income transfer programs and programs that deliver in-kind resources and services (e.g., school lunch, housing, and home-visiting programs). Specific impacts of such programs on asthma etiology have not been assessed.

Health behaviors and psychological factors are closely associated with stress pathways. Mental disorders may be influenced by genetic risk and triggered by stressful events produced by the mechanisms elaborated above. Perceptions of control over the environment and related factors, such as self-efficacy, etc., are in part a function of the experience of unpredictable and stressful environments (e.g., violence) (Carter, Perzanowksi, Raymond, & Platts-Mills, 2001; Wright, 2006). Thus, as noted

above, individual factors and stressful environments reinforce each other in the etiology of asthma. Health behaviors may be in part a response to stress (e.g., substance use). Such behaviors are also, however, influenced by health information and community norms.

Many environmental conditions have been hypothesized to contribute to the experience of stress by the residents of urban communities. These include poverty, community disorganization, crime and violence, social incivility, noise, crowding, hazardous conditions, physical decay, and unemployment (Wright & Steinbach, 2001; Ewart & Suchday, 2002). Aspects of the social environment hypothesized to influence individuals' ability to cope with stressful events include community economic resources, social cohesion, and social support networks. The presence and integration of community institutions that provide resources and services and reinforce social ties also contribute to resiliency in the face of stress. Suggestive evidence of the value of social support comes from a study of asthma management in inner-city children. In this study, two groups received home visits but in only one were asthma prevention measures actually implemented. In both groups acute visits for asthma were reduced relative to the control group that received no home visits, but no difference existed between the two home visit groups (Carter et al., 2001).

Access to health care is relevant to both asthma etiology and management. Although asthma is more prevalent in geographic areas with a high density of health care providers (Sly & O'Donnell, 1992), access depends as much on social, cultural, and economic constraints as on geographic ones. Access issues may affect the etiology of asthma via the timeliness of prenatal care and health care during infancy and childhood. Asthma management practices are strongly related to asthma mortality and hospitalization; poor management of asthma (delays in seeking care, under use of anti-inflammatory medications) is in turn associated with a variety of social, cultural, and economic barriers to care (Wright & Fisher, 2003).

Research that identifies the nature of the social processes that contribute to these pathways is not well developed, but the pathways are likely to be multiple and complex. These processes also must be considered at multiple levels, including individual, family, and neighborhood. Contextual-level stress may be conceptualized as a neighborhood disadvantage that is characterized by factors including poverty, limited social capital, segregation, and high crime/violence rates) (Attar, Guerra, & Tolan, 1994; Goldin & Katz, 1998; Kawachi, Kennedy, Lochner, & Prothrow-Stith, 1997; Kawachi & Kennedy, 1997; Kawachi, 1999; Wilson, 1987; Woolcock, 1998; Morello-Frosch & Lopez, 2006; Ford & McCaffrey, 2006). Research has focused on individual-level risks and limits our understanding of disease causation (O'Neill et al., 2003; Strunk, Ford, & Taggart, 2002). Drawing from economics, epidemiology, sociology, and urban studies (Brock, 2001; Brooks-Gunn, Duncan, & Aber, 1997; Galster, 2001a; Galster, 2001b; Galster & Killen, 1995), asthma researchers have brought the concept of contextual (neighborhood) effects to recent study designs. There is a need to understand how both the physical and psychological demands of living in a deprived neighborhood may increase an individual's susceptibility, including genetic predisposition (Wright et al., 2004; Wright, 2006; Sandel & Wright, 2006; Wright & Fisher, 2003).

These economic, cultural, and social conditions are interrelated and feed on each another. For example, violent crime is reduced in neighborhoods with high levels of collective efficacy (Wright & Fisher, 2003). These interrelationships and pathways are better understood in the context of urban environments. Very little research has examined pathways between the social environment and health in suburban or rural areas.

3.4 Potential for Innovative Research

Research that links the characteristics and dynamics of the social environment to biological pathways resulting in the development and severity of asthma is in its infancy. A study of the scope of the National Children's Study is necessary to adequately model the contributions of various social environmental domains and factors to the behavioral, psychological, and biological processes that lead to asthma and complicate its management. Opportunities for innovation include:

- The opportunity to further develop research regarding the environmental determinants of stress-induced changes in physiological functioning. Current theory points to a variety of environmental factors but does not adequately integrate existing social science theory on the social, economic, political, and cultural processes that shape the environments that individuals occupy.

- The opportunity to extend social science and examine the relevance of existing and potentially new models of the dynamics of disadvantaged urban and rural neighborhoods. These models will be able to incorporate specific health processes and outcomes using biomarker data from mothers and infants as well as direct measurement of social environmental processes.

3.5 Feasibility

To accomplish objectives laid out within this hypothesis, the study sample should include a diversity of living units (e.g. an urban neighborhood or rural community) that are broadly representative of the United States and that are widely varied with regard to race/ethnic composition, socioeconomic status, rural/suburban/urban location, proximity to health services, public policy environment, and physical environmental exposures. Within the selected communities, it is recommended that in-depth studies be conducted to provide richer community-level data on the social dynamics, cultural norms affecting asthma management, and the impact of public policy.

The study must also make an effort to track families (focal child plus primary caretaker) to new residences and must also track the circumstances prompting such moves. Also it is necessary to get adequate samples within all areas in order to obtain the desired multilevel modeling of community effects. This is necessary because of the need to have measures of all potential exposures during pregnancy, early infancy of the child, and every two or three years during childhood.

The primary focus of measuring asthma symptoms in the participating child must be the severity and also the biological pathways underlying the etiology of disease, as described in other NCS hypotheses that focus on asthma as an outcome. It is also important to look at the social aspects of the asthma, with respect to the mother's attitudes towards symptoms, health care contacts, medication (specific prescription, dose, and frequency of administration), and compliance with recommended treatment.

Since the current hypothesis is analyzing the association between asthma and social influences such as socioeconomic status and race/ethnicity, there are many factors at the community-level that are necessary to examine for possible mediating or confounding effects. The level of neighborhood poverty, racial/ethnic composition of the area, racial or ethnic segregation, and job market characteristics are all aspects of the child's social and community environment that may play a mediating role in overall asthma risk. In addition, chemical exposures, neighborhood pollutants, and physical housing characteristics, along with the proximity of and access to health care systems also play a significant role.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Exposure to community and household factors that have the potential to influence asthma symptoms in offspring (e.g., violence, stress); access to health care; mother's attitudes/actions towards symptoms and treatment

Secondary/maternal

- Socioeconomic factors (e.g., social networks and support for mother; enrollment in welfare)

Primary/child

- Exposure to community and household factors with potential to influence asthma symptoms (e.g., violence, stress, environmental pollutants); access to health care

4.2 Methods

Primary/maternal

- Administrative record reviews
- Interviews/questionnaires

Secondary/maternal

- Interviews/questionnaires; secondary data collection on social environment

Primary/child

- Administrative record reviews
- Household environmental samples
- Interviews/questionnaires

4.3 Life Stage

Primary/maternal

- Periodic, prenatal through early childhood

Secondary/maternal

- Periodic, prenatal through early childhood

Primary/child

- Periodic, birth through year 21

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

- Asthma incidence, prevalence and severity:
    - Airway hyperreactivity
    - Lung function
    - Airway inflammation (exhaled NO)
    - Immune system function (e.g., lymphocyte subsets, cytokines, total IgE)
    - Allergic sensitization (specific IgE or allergy skin prick testing)
    - Symptom history and medication use
    - Respiratory infection history
- Asthma management
    - Number of trips to hospital
    - Health care contacts, quality, medications, compliance
    - Number of attacks

5.2 Methods

- Questionnaire of mother
- Examination, interview and testing by medical professional (e.g., history of asthma symptoms, lung function tests, specific IgE, exhaled breath for NO (eNO can be measured prior to age 5)
- Cord and blood samples of IgE and IgA and cytokines related to the allergic/asthma cascade
- Medical record reviews

5.3 Life Stage

Primary/child

- Age 2 to 21 (specific measures depend on age)

6. Important Confounders, Mediators, and Effect Modifiers

Social isolation: Social isolation, or lack thereof, has the ability to modify the effect of physical environment risk factors on asthma-related outcomes in children. The impact of physical environment risk factors may be reduced in a child that has a strong family and social network. For example, one investigation revealed African Americans in education programs had stronger views that asthma could be managed (partial correlation = 0.27) (Fisher, Strunk, Sussman, Sykes, & Walker, 2004; Schmaling, McKnight, & Afari, 2002).

Parental health attitudes: The parental care received by children has the potential to be both a confounder or effect modifier within the association between physical environmental risk factors, psychosocial stress, and health related outcomes and risk of asthma in children. Research shows that parents did a poorer job of caring for a child with asthma if they had feelings of uncertainty, helplessness, and guilt (Annett, Bender, DuHamel, & Lapidus, 2003; Trollvik & Severinsson, 2004).

Health care access: Poor health care access in inner cities, especially among African Americans and Hispanics, exacerbates the risk of asthma attacks and deaths (Lara et al., 2003; Sin, Bell, & Man, 2004; Wallace, Scott, Klinnert, & Anderson, 2004). This and other disparity factors could be behind the greater asthma prevalence, especially among Blacks. These issues are more thoroughly discussed in the disparities in asthma hypothesis and in the review by Gold and Wright (2005).

Lower socioeconomic status and some minority groups such as Blacks and Hispanics are likely to live in areas with increased air pollution, such as near freeways or industrial sites. Careful assessments of these characteristics will be important to properly control for their influence on outcomes.

Diet, physical activity, exposure to bioaerosols, stress, and infections, which will be the topic of other hypotheses, could interact with or confound associations of asthma onset. Though the following is not exhaustive, some important factors associated with atopy and asthma include: demographic group (age, race, income or education), familial characteristics (number of siblings, parent and sibling phenotypes for asthma), daycare attendance, pet ownership, living on a farm, body mass index, immunizations, dietary factors, respiratory infections, and diabetes.

Gene-environment interactions: Polymorphisms in genes involved in oxidative stress responses to air pollutant exposures and candidate asthma and allergy genes will be included. Ultimately gene-environment interactions may dictate the effect of any given factor has on disease outcome.

7. Power and Sample Size

In this example, power analyses with a cutoff value for high-exposure of the predictor is based on a conservative cutoff value for a key determinant of disparity, low income, based on the lower fifth percentile of NCS subjects (i.e., a proportion exposed is 0.05). A total sample size will be N = 100,000 and the incidence of asthma by age 6 will be 5 percent. A fixed interval proportional hazards model was used to assess the association of low income and the risk of asthma. The minimal detectable hazard ratios associated with the disparity, for power points of 80 percent, 90 percent, and 95 percent for a level 0.05 two-sided test, are 1.199, 1.234, and 1.264, respectively. The estimates assume only a main effects model based on exposure to a single factor (low income) without consideration of interactions with other exposures, genetics, family history, etc. (National Children's Study Interagency Coordinating Committee, 2003).

Effect modification of the relationship between measures of social disparity, such as income and asthma incidence, can result from environmental exposures, such as presence of a high level of psychosocial stress, living within 100 meters of a freeway or major highway, high indoor levels of allergens such as cockroach antigens, or the presence of policies and programs that buffer the effects of economic disadvantage on families. Assessment of effect modification will be carried out by including multiplicative interaction terms for the potential effect modifier and low income into each regression model. For brevity, we present power analyses for a generic effect modifier with varying prevalences and assume as above that the cutoff value for low income is based on the conservative lower fifth percentile of NCS subjects. Again, a total sample size is assumed to be N = 100,000 and the incidence of asthma by age 6 will be 5 percent. A fixed interval proportional hazards model assesses the association between low income and the risk of asthma. In this case, minimum detectable hazard ratios associated with the interaction term (multiplicative increase in the effect of low income attributable to the effect modifier) corresponding to a specified power point that can be estimated via a modification of the usual two-sample proportional hazards formula given by Schmoor, Sauerbrei, & Schumacher, 2000:

$$\lambda = \exp\left\{ \left(z_{1-\alpha/2} + z_{1-\beta}\right)\left(\sum_{i,j} 1/p_{ij}\right)^{1/2} \Big/ \left[n \Pr(\delta = 1)\right]^{1/2} \right\}.$$

In this case, λ denotes the minimum detectable hazard ratio associated with the interaction term under the alternative hypothesis, n denotes the number of subjects available in the study, $\Pr(\delta = 1)$ denotes the probability of observing an event for a typical subject in the sample over the course of follow-up (assumed to be 5 percent), p_{ij}, i,j = 0,1 denotes the probability of being in level i of the exposure (0 = low, 1 = high) and level j of the effect modifier (0 = not present, 1 = present), α denotes the type I error, 1-β denotes the power and $z_{1-\gamma}$ denotes the 1-γ quantile of the standard normal distribution. We assume that the effect modifier and low income are independent so that $p_{ij} = p_i \times p_j$, that is, the joint probability of falling into one of the four groups is equal to the product of the conditional probabilities for each covariate. Based upon this formula, Table 1 presents minimal detectable hazard ratios associated with the interaction term corresponding to power points of 80 percent, 90 percent, and 95 percent for a level 0.05 two-sided test.

Table 1. Minimum detectable hazard ratios associated with interactions. Estimates correspond to the 80%, 90%, and 95% power points of a level 0.05 two sided test

Prevalence of effect modifier	Minimum detectable hazard ratio associated with interaction term for selected power points		
	80%	90%	95%
2%	3.66	4.49	5.32
5%	2.30	2.62	2.92
10%	1.83	2.02	2.18
20%	1.58	1.69	1.79
30%	1.49	1.58	1.67
40%	1.45	1.54	1.61

8. Other Design Issues

- **Ethical/burden considerations:** General issues include the need to protect privacy of individuals and communities and when to intervene in families to protect children's health.

- **Need for community involvement:** Daycare and school cooperation would be required for some of the intended measures. Other social environmental factors may be tied to neighborhoods or communities.

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EARLY EXPOSURE TO STRUCTURAL COMPONENTS AND PRODUCTS OF MICROORGANISMS DECREASES THE RISK OF ASTHMA

1. Meta Hypothesis

Early exposure to heterologous structural components and products of biologics (microorganisms, e.g., viruses, bacteria, fungi, parasites, and common indoor aeroallergens) significantly decreases or increases the risk of asthma and other atopic diseases (e.g., eczema, allergic rhinoconjunctivitis), and/or this will be mediated by genetic and other risk factors.

2. Specific Hypotheses

1. Exposure to microbial structural components or products, indoor or outdoor allergens, viral and/or bacterial infections in utero (maternal exposure) or in early childhood decreases the risk of atopy (atopic sensitization with or without symptoms), asthma, eczema, and allergic rhinitis later in childhood.

2. Immunologic outcomes measured in those children demonstrating reduced or enhanced risk of developing asthma or atopy will reflect varying profiles of upregulation or downregulation of Th1 and Th2 lymphocytes as well as other significant immunologic consequence(s), such as the development of regulatory T lymphocytes (e.g., antigen specific suppressor T-regulatory cells).

3. Significant interactions between the host, environmental exposures (including in utero), and genetics will determine the magnitude and direction of associations in hypotheses 1-2.

3. Background and Justification

The human innate and adaptive immune systems can mount responses to heterologous structural components and metabolic products of various species of living organisms (biologics) during exposure in an environment rich in these immunoreactive factors. These microscopic and macroscopic and ogranisms include bacteria, viruses, fungi, parasites, animals, insects, and plants. The determinants of the exact nature of a particular response in a given circumstance are complex and not well understood. Almost two decades ago, Strachan (1989) proposed that reduced exposure to microbial contaminants early in life as a result of improved "hygiene" and reduced infection rates in developed countries increased the prevalence of atopy, asthma, and other allergic diseases. This hygiene hypothesis has steadily garnered scientific support (Gehring et al., 2001; Braun-Fahrlander, 2003; Liu, 2004; De Meer, Janssen, & Brunekeef, 2005; Douwes et al., 2006; Ege et al., 2006; Perzanowski et al., 2006). Some investigations produced conflicting results (Platts-Mills, Erwin, Heymann, & Woodfolk, 2005; Liu & Leung, 2006; Schaub, Lauener, & von Mutius, 2006; Vercelli, 2006). The meta hypothesis proposed above seeks to clarify the validity of the broad hygiene hypothesis and, wherever possible, elucidate the underlying causes and susceptibility factors.

3.1 Public Health Importance

Prevalence/incidence

Among children, asthma is the most common chronic illness (National Academy of Sciences Institute of Medicine, 2000). Asthma prevalence in the United States, estimated from the National Health Interview Survey (NHIS) by the American Lung Association (American Lung Association Epidemiology

and Statistics Unit, 2006), shows the prevalence of current asthma increased 85 percent from 1982 through 1996 to an estimated 14.6 million persons (55.2 per 1,000 persons). This increase was 76 percent in children younger than 18 years or 4.43 million in 1996 (62.0 per 1,000 persons). This trend paralleled increasing asthma hospitalizations and death rates in children (Akinbami, 2006; American Lung Association Epidemiology and Statistics Unit, 2006). Similar or greater increases in the prevalence of asthma during the second half of the 20th century were reported from other countries (Eder et al., 2006). Asthma prevalence increased until 2001 and plateaued in most groups except African-American children and adolescents, who have continued to experience increased prevalence annually (American Lung Association Epidemiology and Statistics Unit, 2006). Hospitalization and death rates in children have also reached a plateau or declined (Akinbami, 2006). Despite the plateau in prevalence, nonambulatory visits for asthma to physician offices by children increased after 2000 (Akinbami, 2006). Explanations could include increased public and health provider recognition of this disorder and earlier and more effective treatment. For example, it is likely the main factor mitigating increases in hospitalization, and mortality has been the dramatic increase in the use of preventive (controller) medications, chiefly inhaled corticosteroids (Suissa, Ernst, Benayoun, Baltzan, & Cai, 2000; Suissa, Ernst, & Kezouh, 2002; Stafford, Ma., Finkelstein, Haver, & Cockburn, 2003).

In 2004, the prevalence of doctor-diagnosed asthma reached 30.2 million Americans (104.7 per 1,000 persons), including 6.5 million children younger than 18. An estimated 4 million children younger than 18 experienced an asthma attack in 2004. Prevalence data in the United States both from the 12-month prevalence (before 1997) and 12-month attack prevalence of asthma (since 1997) were highest among those ages 5-14, Blacks compared with whites, and females (Akinbami, 2006; American Lung Association Epidemiology and Statistics Unit, 2006). Approximately 38 percent of the asthma hospital discharges in 2004 were children younger than 15, although only 21 percent of the U.S. population was younger than 15 years old.

Eder et al. (2006) report the prevalence of atopy (from skin tests or specific IgE) has increased in some populations, whereas, in others there has been a decrease or a plateau in prevalence since 1990. However, there is little data prior to 1990. Other epidemiologic data show the prevalence of atopy-related illnesses such as allergic rhinitis have increased in many industrialized nations during the 20th century (Diaz-Sanchez, Proietti, & Polosa, 2003).

Economic and/or social burden

In 2004, the direct health care costs of asthma were estimated at $11.5 billion while indirect costs (lost productivity) were estimated at $4.6 billion for a total of $16.1 billion (American Lung Association Epidemiology and Statistics Unit, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. One study estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs (Malone, Lawson, & Smith, 2000). Asthma also poses a substantial, increasing public health burden from school absenteeism and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Currently available databases, although useful for the study of acute exacerbations of existing asthma, are not able to directly answer questions about the effects of chronic microorganism exposure on asthma incidence. These questions require the collection of new data. They can best be studied in a large prospective cohort study design incorporating data from preconception into adulthood. A large sample size and prospective approach is needed to accurately assess the effect of timing and dose of biologic exposures, particularly during critical windows of vulnerability (e.g., specific trimester of pregnancy, early vs. later postnatal periods, etc.), on the development of childhood asthma. Identification

of children at risk for developing the severe forms of asthma would have clear public health impact. A large prospective study can provide sufficient numbers to identify risk factors and determine which children with asthma develop the severe, persistent variety.

Distinguishing the effects and interactions of biologics with other potential causative factors (e.g., genetic, air pollution, lifestyle factors, addressed by the other asthma hypotheses) requires a large sample. Accurate exposure and phenotypic data are needed to assess the significance of various asthma and allergy genotype-complex exposure interactions occurring during the development of the child that result in several different asthma phenotypes (Taussig et al., 2003; Bel, 2004). A large sample will be required to evaluate the susceptibility to the adverse effects of certain pollutants and their sources in specific genotype-phenotype clusters. Also, data are needed from children of different ethnicities living in varied housing, socioeconomic, and geographic conditions. These factors may be correlated with variations in exposures and possibly genotypes. A large sample size will be necessary to allow adjustments to account for this.

3.3 Scientific Merit

Current scientific understanding

Biologics can be parts of living organisms (e.g., animal dander, cell wall lipoproteins) or may refer to the entire organism (e.g., viruses, bacteria). Exposure to specific biologics can be episodic with periodic exposure through vector transmission, immunization, or contact with these substances in our everyday environment. Exposure also may be continuous (such as bacteria colonizing the skin and alimentary tract). These biologics or their components can induce immunologic responses which are protective, though some misdirected responses become pathologic. These responses can be identified through changes in immunologic status measured in the laboratory or as phenotypic expression.

Pathologic perturbations of the immune system control the development of atopy and atopic diseases. The primary function of this organ system is to prevent injury from foreign organisms. A complex armamentarium of cells (e.g., neutrophils and mononuclear cells); receptors (e.g., pattern recognition receptors [PRR] such as toll-like receptors [TLRs]); and soluble agents (e.g., complement, soluble cluster of differentiation molecule 14 [sCD14]), provide immediate recognition and response to proteins, lipoproteins, lipopolysaccharides, and other substances recognized as nonself via the innate immune system. Previously programmed adaptive immune system components can also initiate and execute reactions to neutralize and remove foreign substances from the body (e.g., mononuclear cells and lymphocytes [memory cells], intracellular and extracellular receptors, and soluble molecules, such as specific antibodies, interleukins, and interferons). Other environmental exposures, such as airborne pollutants, can induce or augment immunological reactions. These responses by the immune system are modulated by factors such as genetic makeup, presence of stimulatory or inhibitory cofactors, prior immunologic conditioning, and current state and level of maturity of the immune system. Not all immunologic reactions are healthy. For example, heterologous substances such as endotoxin have the potential to induce or inhibit various pathways of immunological defense thereby augmenting or diminishing a host's effective response (Vercelli, 2006). In experimental models, higher doses of endotoxin can induce immunologic tolerance, whereas low doses may augment the development of atopic sensitization. Introduced before allergen exposure, endotoxins can inhibit sensitization. However, endotoxin exposure some time after induction of sensitization can enhance the hyperreactive airway response to antigen exposure. These dose-dependent immunologic consequences depend upon different pathways. In one study, low-dose endotoxin induction of allergen sensitization and Th2 skewing was found to be independent of myeloid differentiation primary response protein 88 (MyD88), while high-dose endotoxin induction toward a Th1 profile required the presence MyD88 (Eisenbarth et al., 2002). In a murine model, however, the Th2 adjuvant activity of house dust extract was dependant upon the

presence of MyD88, indicating the requirement of TLRs for this activity (Ng, Lam, Paulus, & Horner, 2006).

Effects on immunologic function can be quantified by measurements of immunologic expression, such as the relative proportions or quantities of lymphocyte subsets, usually defined by their receptor expression or function; the type of cellular recruitment; and the cytokine profile. Th1 induction generally results in the recruitment of lymphocytes and other mononuclear cells, upregulation of TLRs, and the secretion of cytokines such as IL6, IL12, and IFN-γ, and specific antibody of the IgG class, especially of the IgG4 subclass. On the other hand, Th2 induction usually is associated with the recruitment of eosinophils, downregulation of TLRs, the secretion of IL4, IL5, IL9, IL13, and IFN-α, and by specific antibody production of IgG2 (Jabara & Vercelli, 1994; Martinez & Holt, 1999; Woodfolk, 2006). Immunologic consequences also may be assessed by measuring relevant physiologic reactions affected by regional inflammatory or immune response, such as airway hyperreactivity or obstruction and in vivo (skin testing) and in vitro (ELISA) assays for specific IgE.

The immune system of the fetus or newborn is not fully mature and demonstrates a Th2-dominance. Over the ensuing months, the infant's immune system acquires Th1-dominance, unless this natural process is interrupted by genetic, environmental, or other factors. The development of asthma and atopy is thought to result from the persistence of, or return to, a Th2-dominant phenotype from a Th1 dominant phenotype. As noted, biologics such as endotoxin induce a Th1 response under one set of conditions, a Th2 response under another, and inhibit both classes of response in other circumstances (Braun-Fahrlander, 2003). This has led to the concept of T regulatory lymphocytes ultimately controlling Th1 and Th2 activity, thereby connecting the innate with the adaptive immune system (Eder & von Mutius, 2004). Consequently, factors other than the nature of the heterologous substance to which we are exposed contribute to the ultimate effects of the response. These factors include genetic differences (e.g., single nucleotide polymorphisms, inherited atopic/asthmatic genotype); exposure characteristics (e.g., critical windows in time, temporal sequence of exposure with a second factor such as allergen, dose); epigenetic changes (e.g., DNA methylation); in utero maternal exposures (smoking, stable/barn animals); frequency, type and duration of infection, colonization, or exposure (e.g., bacterial, parasites, viruses, antibiotics, probiotics, immunizations, hygiene); air pollution exposure (e.g., ozone, particulate matter); and presence of stimulatory cofactors.

Several studies have suggested in utero or perinatal influences determine allergic disease outcomes. Jones et al. (2002) found reduced levels of sCD14 in the fetal and neonatal gastrointestinal tract (demonstrated in amniotic fluid and breast milk colostrums) were associated with an increased prevalence of atopic sensitization and eczema, although the presence of eczema was independent of atopic sensitization. TLR2 and TLR4 transcripts were identified in extracts of fetal gut and skin of these fetuses at all ages of gestation examined. The authors concluded this regulatory effect of sCD14 likely requires the interaction and binding of TLR4 and its ligand, sCD14, both of which are present in utero, and that this immunologic effect is endotoxin independent as previously described for inhibition of IgE production by sCD14 (Arias et al., 2000). However, they did not measure endotoxin in breast milk. Ege et al. (2006) discovered an inverse dose-dependent relationship between maternal exposure to farm animals and a risk of atopy in offspring, accompanied by upregulation of Th1 genetic markers (TLR2, TLR4, and CD14) in the children.

Fetuses demonstrate peripheral blood mononuclear cell proliferative responses from 22 weeks gestation, with increased responses at birth to specific allergens to which the mother was exposed during gestation (Warner, Jones, Jones, & Warner, 2000). Immunologic memory appears to begin developing in utero as a result of the transplacental transfer of small amounts of antigens, with subsequent induction of T regulatory cells that determine whether tolerance or allergic sensitization to these antigens will occur (Holt & Macaubas, 1997). This early activation of both innate and adaptive immunity could

explain the presence of critical windows of exposure to biologics early in life, even in the prenatal period, that help determine the type and predominance of immune responses that will characterize each human being, at least for infancy and childhood. The immunologic status of the newborn may, similarly, already be headed in a particular direction at birth (e.g., Th1 or Th2 predominance) with further influence by other postnatal factors that can impact the direction of immune system maturation.

Of all of the biologics, endotoxin has received the most attention. Endotoxin, a lipopolysaccharide component of gram negative bacteria cell walls, is found routinely in house dust, and, in increasing amounts, in the presence of animals. In a prospective birth cohort study, Simpson et al. (2006) reported a marked reduction in atopic sensitization and eczema with increasing endotoxin exposure (measured in living room dust), and an increased risk of nonatopic wheeze, but not atopic wheeze, in children at 5 years of age. However, this protective effect for atopy was seen only in children homozygous for the CC genotype in a promoter region of the CD14 gene (CD14/−159). Similar findings have been described elsewhere, particularly for children in the first 2-5 years of life (Gehring et al., 2001; Perzanowski et al., 2006). Diametrically opposite effects have been reported in studies under different conditions, with possession of the T allele of CD14/−159 serving to reduce, not enhance, the IgE response (Vercelli, 2003). These opposing effects prompted Kabesch (2006) to question the validity of a causative role for endotoxin, raising the possibility that endotoxin is actually a surrogate for some other, as yet, undefined immunomodulator. Vercelli, however, suggests the response to endotoxin may be bimodal or even trimodal with Th2 responses at low and very high concentrations of endotoxin exposure and Th1 responses at high concentrations. Polymorphisms in other regions of the CD14 gene can create contradictory effects on total and specific IgE production in children exposed to varying levels of endotoxin and different animal exposures, i.e., pet versus farm animals (Eder et al., 2005). Lau et al. (2005) found cat allergen exposure in infancy to be a risk factor for childhood asthma. However, very high allergen exposures early in life was associated with a reduced rate of sensitization and wheezing and increased levels of cat-specific IgG (Lau et al., 2005). A reduced prevalence of infantile wheezing has been reported with exposures to high levels of endotoxin in the presence of multiple dogs (Campo et al., 2006). Several other previously established risk factors, such as maternal smoking and daycare attendance, were also identified, however, no reduction in specific allergen sensitization was noted at 1 year of age.

Some (Braun-Fahrlander, 2003; Eder & von Mutius, 2004; Liu & Leung, 2006; Schaub et al., 2006; Vercelli, 2006) but not all (Platts-Mills et al., 2005; Racila & Kline, 2005) recent reviews have argued for the probable validity of the hygiene hypothesis construct, although not necessarily in its original form. Some studies have demonstrated an inverse relationship between early-life farm animal exposure as a surrogate for microbial biocontaminant exposure (likely to represent, at least, endotoxin) and the development of atopy, asthma, and allergic rhinitis, including some in a dose-dependent manner (Von Ehrenstein et al., 2000; Downs et al., 2001; Riedler et al., 2001). Leynaert et al. (2006) found the greatest protection in children with both early farm exposure and possession of the CD14-159TT genotype. Lauener et al. (2002) demonstrated higher amounts of CD14 and TLR2 (a receptor for peptidoglycans in gram-positive bacterial cell walls) in farmers' versus non-farmers' children. In a western European study, the development of atopy and asthma was negatively associated with farm stable exposure and, independently, with the consumption of farm milk in the first year of life (Riedler et al., 2001). These studies suggest a critical window of prenatal and early childhood exposure to produce a protective response. Windows of exposure have been described for other environmental elements (e.g., smoking) associated with the induction of asthma (Strachan & Cook, 1998). In a prospective birth cohort study of Dutch children followed for four years, endotoxin and EPS levels expressed as units per square meter (but not in units per gram of dust) in floor dust demonstrated a significant inverse association with doctor-diagnosed asthma, and, in addition, with persistent wheeze but only for extracellular polysaccharides of fungi (EPS) (Douwes et al., 2006). A weak correlation was found with biocontaminant levels and atopy. However, the authors point out the study design may have

impeded finding a positive relationship. EPS exposure, used as a marker for total fungal exposure, has been significantly positively associated with home dampness as well as respiratory symptoms in 6- to 12-year-old children (Douwes et al., 2006). Gehring et al. (2001) found exposure to higher levels of endotoxin in urban homes reduced the risk of developing atopic eczema in infants in the first 6 months, with an increased risk of experiencing respiratory infections with cough and wheezing that increased over the first year of life. Perzanowski et al. (2006) reported similar results. A number of studies have demonstrated reduced risks for the development of allergic diseases and atopic sensitization in children growing up in close proximity to farm animals (Alfven et al., 2006), particularly with early life exposure.

Attending day care between birth and 4 years of age and having a dog or cat indoors in the first 2 years of life have been shown to reduce the risk of atopic sensitization and atopic symptoms (De Meer et al., 2005). However, no reduced risk was seen when older siblings were present, contrary to one of the predictions of the original hygiene hypothesis. Ball et al. (2000) did find a significantly reduced risk of asthma in children with exposure to older siblings and also associated with attendance at day care in the first 6 months of life. They also reported increased wheezing in children with more exposure to other children at age 2 but a reduced risk of wheezing from ages 6-13 years. Significant dose-dependent relationships have been reported for the lack of vaccination and the risk of self-report of atopic diseases in children, especially when there was no family history of atopic disease (Enriquez et al., 2005).

Bidirectional gene-environment interactions can affect the resultant asthma phenotype. Only certain polymorphisms in TLR2, (e.g., carrying a T allele in TLR2/-16934), modulate the protective effect provided by the farm environment (Eder et al., 2004). Non-mutational epigenetic changes in gene expression caused by varying environmental exposures could also help explain the opposite effects of identical genotypes or discrepant results for similar exposures. NOD1 is an intracellular PPR with a leucine-rich domain whose ligand is the diaminopimelic acid (iE-DAP) found in PGN. iE-DAP is found on the surfaces of bacteria such as gram-negative bacilli and certain gram-positive bacilli such as *Bacillus subtilis* and *Listeria monocytogenes*. Activation of NOD1 induces NF-κB activation resulting in the release of pro-inflammatory cytokines. Certain polymorphic variants in the NOD1 gene have shown variable association with asthma and total serum IgE (independent of allergen-specific mechanisms), with one SNP in particular, ND+32656*2 indel strongly associated with both (OR = 6.30 for asthma and 18.4 for IgE above the 90th percentile) (Hysui et al., 2005). In another study of the NOD1 gene, central European children homozygous for the T allele in the caspase recruitment domain protein (CARD) 4 portion of NOD1-21596 (CARD4/-21596) and living on farms demonstrated reduced frequencies of atopic sensitization, allergic rhinitis and atopic asthma compared with children not living on farms. This protective effect was not seen, however, in children with the C allele (Eder et al., 2006). The complexities of gene-environment interactions reflect the fact that asthma is a complex disease of the lower airways variably characterized by reversible airways obstruction, airway inflammation, and airway hyperresponsiveness. Gene association studies indicate a complex inheritance pattern involving perhaps hundreds of genes governing the expression of varying asthma and atopy phenotypes (Ober & Hoffjan, 2006). Confounding factors can make the job of identifying truly causative genotypes more difficult. Associations may not be reproducible because of inter-study differences in definitions of phenotypes (e.g., criteria used to identify subjects with “asthma” or “atopy”). Some genetic effects may only be demonstrable when combined with other genes or environmental conditions, and associations with single nucleotide polymorphisms (SNPs) or various haplotypes likely differ between populations, which may conceal true causative associations. Despite this complexity, eight genes associated with the asthma phenotype, and frequently with atopy, have been identified in five or more studies (Hoffjan et al., 2003).

Bacteria and their components in the intestinal tract are another source of constant exposure to microbial products, and, thereby, the potential for induction of immunologic effects. *Bifidobacterium thermophilum*, a commensal bacterium of the gut, fed orally to mice enhanced the cytotoxic activity of their lymphocytes (Sasaki, Fukami, & Namioka, 1994). They are also an excellent source of antioxidants

(Pyo & Lee, 2005). Yurong et al. (2005) found significant enhancement of intestinal mucosal immunity in chickens fed a probiotics mixture of microbial agents, including *Lactobacillus acidophilus*, *Bacillus subtilis*, and *Candida utilis* early in life. Fecal short chain fatty acid patterns more often associated with the presence of *Clostridium difficile* were predominant in allergic Swedish infants (defined by at least one positive prick skin test and symptoms of asthma or eczema). Nonallergic infants demonstrated fecal patterns resembling those seen with normal intestinal bacterial flora (Bottcher, Nordin, Sandin, Midtvedt, & Bjorksten, 2000). Similar findings have been reported by Sepp in Swedish infants who demonstrated significantly more allergy and the presence of *C. difficile* vs. Estonian infants showing much less allergy and more often the normal gastrointestinal commensals *Lactobacilli* and *Eubacteria* (Sepp et al., 1997). This has also been shown to be true for 2-year-olds in these two countries (Bjorksten, Naaber, Sepp, & Mikelsaar, 1999). Certain species of *Lactobacilli* may inhibit allergen-induced IgE production by murine splenocytes, suggesting the GI bacterial flora may play a role in modulating Th1/Th2 balance (Shida et al., 1998). Other studies have shown immunomodulatory effects in animals exposed to common commensal GI bacteria (e.g., *B. subtilis*) (Panigrahi et al., 2007) or their metabolic products (Sung et al., 2005). Finally, feeding *Lactobacillus GG* to mothers prenatally and to infants in their first 6 months reduced the development of atopic diseases through the first 2 years of life (Kalliomaki et al., 2001).

We are constantly exposed to biologics and their immunoreactive components before birth, and these are only one class of many types of contaminants and other environmental exposures we encounter daily. The dual human immune system is relentlessly reacting to and protecting us from the potential harmful effects of these biologics as they come in contact with our bodies. Some of these immunologic reactions result in pathologic effects. Among these are the development of atopy, asthma, and other allergic disorders. Investigative studies directed toward elucidating the causal factors in the development of asthma continue to provide support for an updated hygiene hypothesis that explains how relatively recent changes in hygiene and reductions in biologics exposure account for the increase in asthma and atopy in developed and developing countries during the past three decades.

3.4 Potential for Innovative Research

The NCS provides the opportunity to collect longitudinally a broad range of data thought to affect the development of atopy and asthma. This includes genotyping; serologic reflections of immunologic status (specific and total and specific IgE, cytokines, lymphocytes); and environmental exposures (e.g., animal dander, dust mite, cockroach). This also allows us to examine the relationships of multiple potential causes and precipitating factors of asthma, both allergic and nonallergic, using standardized definitions for disease, atopy, and environmental exposures. By collecting data in a standardized manner and using nurses or doctors to evaluate study-defined parameters, investigators can compare and combine data across the study centers in a scientifically valid manner, contrary to what has often been done in reviews of the literature. As the study progresses, one will be able to observe the effectiveness of both proactive and reactive modalities for promoting good health, (e.g., effectiveness of smoking cessation for relevant house members; or regular vs. intermittent vs. no care by a primary care physician vs. a specialist in allergy/immunology or pulmonary medicine).

3.5 Feasibility

The probability sampling design of the NCS will allow for variability in birth order, number of siblings, family size, time spent in day care, and opportunity for contact with sources of infection and endotoxin. Sampling should include exposure to farm animals (urban/rural gradient), household pets, other sources of fecal material (such as soiled diapers or compost in the home), and socioeconomic status. The collection of specimens for genotyping or epigenetic changes are possible. Obtaining blood for serum total and specific IgE is feasible, but assays are expensive but necessary to determine atopic status and other immunological markers. Spirometry is practical after 4-5 years of age. Repeated household

measurements of allergens and other biocontaminants from dust and air are feasible and can be collected during home visits and by study participants. Analyses of these samples are expensive, yet are needed to provide evidence of exposures at specified windows of vulnerability. The study needs to track respiratory infections clearly associated with increased incidence of early wheezing illness. However, confirmation of the type of respiratory infection will be difficult and limited to a subset of confirmed viral titers, such as Vericella. Therefore, data regarding episodes of infections will be largely based on parental recall of the frequency and severity of infection. High-throughput genotyping methods and genome-wide scans will characterize and potentially discover asthma and atopy genes that interact with the exposures of interest.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Prenatal exposure to bacteria and microbial products through CBC of blood samples and Immunoassay
- Prenatal and postnatal ETS exposure

Primary/child

- Exposure to bacteria and microbial products via indoor measurements and other infection measures
- Day care history
- Family member number and history (e.g., concomitant upper respiratory infections)

4.2 Methods

Primary/maternal

- Interview/questionnaire
- Blood samples
- Urine samples
- Other physical sampling
- Household environmental survey and endotoxin and allergen sampling prenatally

Primary/child

- Household environmental endotoxin and allergen sampling
- Blood samples
- Urine samples

- Other physical sampling
- Spirometry and exhaled NO
- Interview/questionnaire to assess health history, diet, daycare attendance, exposure to pets, etc.

4.3 Life Stage

Primary/maternal

- Repeated, birth through year 5

Primary/child

- Repeated, birth through year 5

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Decreased risk of asthma measured via allergy, asthma in index child, airway reactivity

5.2 Methods

- Direct observation by medical professional
- Interview/questionnaire
- Blood samples
- Urine samples
- Other physical sampling

5.3 Life Stage

- Repeated, birth to year 21

6. Important Confounders, Mediators, and Effect Modifiers

- **Infection:** Site, type of prior infection (e.g., respiratory, gastrointestinal) does not change likelihood of protective effect.
- **Medication use:** Antibiotic or paracetamol use may increase the risk of asthma.
- **Living conditions:** Living in uncrowded conditions and in higher socioeconomic status reduces risk of asthma.
- **Location of contaminant collection:** (e.g., living room vs. bedroom, floor vs. mattress, carpeted vs. non-carpeted); units of contaminant (e.g., units/area vs. units/gram of dust); previous efforts to reduce exposure (e.g., bedding encasings) (Dillon et al., 1999; Douwes et al., 2006).
- **Use of air conditioners**
- **Obesity**
- **Lifestyle:** Diet, antioxidants, breast milk; smoking.
- **Timing of exposure:** Critical windows; before, with, or after allergen or other potential factor.
- **Lung growth:** (e.g., low-birth weight).
- **Genetics of asthma and atopy:** (Ober & Hoffjan, 2006), see NCS environmental exposures and genetic variation interactions and asthma hypothesis.

7. Power and Sample Size

The smallest detectable relative risk is approximately 1.2. This power estimate assumes a sample size of 100,000 at age of diagnosis, an asthma incidence of 5 percent, and a cutoff value for "high" exposure based on the upper fifth percentile of NCS subjects (i.e., a proportion exposed of 0.05). It assumes only a main effects model based on exposure to a single factor (e.g., early exposure to bacterial and microbial products) without consideration of interactions with other exposures, genetics, family history, etc.

8. Other Design Issues

The multifactoral determination of immunologic responses contributes to the difficulty in identifying causative factors of asthma and atopy. Although the risk of developing asthma is significantly greater in atopic children, the majority of children with asthma in the first 5 years of life is not atopic. What conditions determine whether the child develops atopic sensitization, asthma, or other allergic diseases, or both? What data must be collected to discover risk factors for each? The study design must control for non-measured potentially causative factors while assuring accurate assessment of dependent variables and accurate measurement of independent variables. For instance, the locations of sampling, previous or ongoing anti-allergy measures in affected children's homes, and assays utilized to evaluate atopy or biocontaminant concentrations can influence study results and lead to misleading conclusions. Additionally, operational definitions of disease states (e.g., asthma) must be similar to allow for

comparison of data from comparative studies. Study design should differentiate incident asthma from prevalent asthma, which can be difficult with older children.

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ENVIRONMENTAL EXPOSURES INTERACT WITH GENES TO INCREASE THE RISK OF ASTHMA AND WHEEZING IN CHILDREN

1. Meta Hypothesis

There will be a significant association with gene-environment, gene-gene, and genotype-phenotype relationships that contribute to wheezing and asthma in children.

General approaches have been proposed for evaluating gene-environment interactions (Weiss, 2001; Martinez, 2007), but groundbreaking gene-environment asthma studies in the literature also have many limitations. Some of these limitations can be addressed in the National Children's Study. Three specific hypotheses have been formulated to demonstrate how the National Children's Study can address critical questions still left unanswered.

2. Specific Hypotheses

In addition to this hypothesis on gene-environment interaction, the outcome domain of asthma has three specific hypotheses provided based on potential exposure related to air pollution, environmental tobacco smoke, viral and microbial agents, and more general "environmental" exposures such as stress and diet.

1. Environmental exposures to such agents as air pollution and environmental tobacco smoke (ETS) will increase risk for wheezing and asthma, and this effect will be moderated by certain oxidative stress genetic phenotypes such as GSTM1, GSTP1, NAT1 and NAT2.

2. Exposure to viral agents or microbial products will be significantly associated with wheezing and asthma outcomes and this effect will be dependent on certain innate immunity genes such as CD14, TLR2, TLR4, iNOS and/or, adaptive immunity genes such as IL4, IL13, IL4RA.

3. Other prenatal or postnatal environmental exposures such as stress and diet will significantly increase the risk for wheezing and/or asthma, and this effect will be moderated by specific genotype-phenotype relationships.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Among children, asthma is the most common chronic illness (National Academy of Sciences, 2000). Data on asthma prevalence from the United States from the National Health Interview Survey (NHIS), CDC's National Center for Health Statistics show the prevalence of current asthma increased 85 percent from 1982 through 1996 to an estimated 14.6 million persons (55.2 per 1,000 persons), according to reports by the American Lung Association (2006). This increase was 76 percent in children younger than 18 years to 4.43 million children in 1996 (62.0 per 1,000 persons). This trend paralleled increasing asthma hospitalization and death rates in children (American Lung Association, 2006; Akinbami, 2006). Similar or greater increases in the prevalence of asthma during the second half of the 20th century were reported from other countries (Eder, Ege, & von Mutius, 2006) and increases may still be occurring (e.g., see Lodrup Carlsen et al., 2006, for recent information from a birth cohort study in Oslo, Denmark, where now every fifth child is affected by asthma).

Between 1997 and 2000, the asthma questions in NHIS used a different measure of current asthma prevalence, which suggested a decrease. However, this difference made it impossible to appropriately compare the data during this period to past trends. In 2001, another change was made (a question was restored to restate the prevalence of current asthma regardless of attack occurrence in the past 12 months) making comparisons possible again. It then became apparent that current asthma prevalence had continued to increase until 2001, later reaching a plateau in most groups except black children and adolescents, who have continued to experience increased prevalence annually (American Lung Association, 2006).

Hospitalization and death rates in children have also reached a plateau or decreased (Akinbami, 2006). Despite a plateau in prevalence in 2001, nonambulatory visits for asthma to physician offices by children have continued to increase (Akinbami, 2006), possibly due to increased public and health provider awareness, which improved treatment of asthma. For example, there was a dramatic increase in the use of preventive (controller) medications, chief among them being inhaled corticosteroids (Stafford et al., 2003; Suissa et al., 2000; Suissa, Ernst, & Kezouh, 2002), which may have mitigated increases in hospitalization and mortality.

In 2004, 30.2 million Americans (104.7 per 1,000 persons) at some time during their lifetime had been diagnosed with asthma by a health professional. This estimate included 6.5 million children younger than 18 years. Almost four million children younger than 18 years were estimated to have experienced an asthma attack in 2004. Prevalence data in the United States from both the 12-month prevalence (before 1997) and 12-month attack prevalence of asthma (since 1997) were highest among children aged 5-14 years, blacks compared with whites, and females (American Lung Association, 2006; Akinbami, 2006). In the U.S. population, approximately 38 percent of the asthma hospital discharges in 2004 were children younger than 15 years, even though only 21 percent of the U.S. population was younger than 15 years.

Economic and social burden

In 2004, the direct health care cost of asthma was estimated to be $11.5 billion. Indirect cost (lost productivity) was estimated to be $4.6 billion. Total cost (direct plus indirect) was estimated to be $16.1 billion (American Lung Association, 2006). The more severe forms of asthma account for a disproportionate amount of the total direct costs. Malone, Lawson, & Smith (2000) estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs. Asthma also poses a substantial and increasing public health burden from school absence and restriction of usual physical and social activities (Newacheck & Halfon, 2000).

3.2 Justification for a Large Prospective Longitudinal Study

Opinions differ on the types of studies to use to address complex common disorders. In a recent debate in the literature, Willett et al. (2007) supported the combination and use of available databases to study common disorders such as asthma, while Collins and Manolio (2007) supported a new large cohort study mainly of adults (Manolio, Bailey-Wilson, & Collins, 2006). This debate covered the strengths and weaknesses of both of these approaches, but both Willet et al. and Collins and Manolio praised the strengths of a design based on a large birth cohort but questioned the feasibility of a large prospective cohort study design that incorporates in utero and early postnatal data as well as data from stages of development from infancy through childhood and into adulthood. However, the National Children's Study will provide valuable data that could be used to accurately assess the timing of exposures, particularly during critical windows of vulnerability (e.g., specific trimester of pregnancy, and

early vs. later postnatal periods, and later in childhood) on the onset of asthma in childhood and persistence into adulthood.

Accurate exposure and phenotypic data are needed to assess the importance of many asthma and allergy genotypes interacting with many exposures in relation to complex and variable asthma phenotypes. Interactions of maternal and fetal genotypes contribute to the requirement for a large sample. A large sample will be required to evaluate the susceptibility to the adverse effects of certain exposures and their sources in specific genotype-phenotype clusters.

Also, data are needed from children of different ethnicities living across the United States under varied housing, socioeconomic, and geographic conditions. These factors may account for some of the ethnic disparities related to asthma, which may be correlated with variations in exposures. A large sample size will be necessary to allow adjustments to account for this. Landrigan et al. (2006) discuss in more detail the rationale and strengths for a large prospective study, which is expected to direct the development of prevention strategies for both diseases of childhood and chronic diseases of adulthood whose developmental origins likely begin in childhood.

3.3 Scientific Merit

A recent review by Ober and Hoffjan (2006) identified a group of 10 genes that have been associated with asthma in 10 or more studies; 15 genes that have been associated with an asthma or atopy phenotype in six to 10 studies; and 54 genes that have been associated in two to five populations. The genes associated with asthma in more than 10 studies are IL4, IL13, ADRB2, TNF, HLA-DRB1, FCER1B, IL4RA, CD14, HLA-DQB1 and ADAM33. On the basis of their review, Ober and Hoffjan suggest the total number of genes associated with asthma will likely exceed 100, and the individual effect of any one of these genes on disease risk will be small. However, this review did not discuss gene-environment interaction, which was addressed in a separate article (Ober & Thompson, 2005). Both articles to which Ober contributed state there is no single "asthma gene" in all populations, and it is therefore critical to consider environmental context or gene-environment interactions to understand genetic susceptibility to asthma. They emphasized that asthma is a complex common disease with genetic susceptibility that is context-dependent due to gene-environment interactions resulting in effects apparent only in specific environments. To address these factors, studies of asthma must take into account environmental context (e.g., gender, parental asthma status, parent-of-origin effects) and use large samples, careful ascertainment of asthma phenotypes, comprehensive genetic surveys of targeted genes or genome-wide variation, and rigorous statistical approaches that account for multiple comparisons. Thus, the National Children's Study will provide data to test gene-environment effects hypothesized to be important now as well as associations that may be proposed in the future.

Examples of gene-environment interactions

One straightforward example of a gene-environment hypothesis is based on the CD14 gene and exposure to endotoxin (see Vercelli, 2004, and Vercelli & Martinez, 2006, for a review and discussion). CD14 is one of the groups of genes with evidence of association with asthma in more than 10 studies (Ober & Hoffjan, 2006). The literature suggested that a functional promoter polymorphism (CD14/-260C>T) in this gene influences IgE and asthma in opposite ways depending on level of exposure to endotoxin. Recent examples are based on studies of children exposed to small animals or large animals (Eder et al., 2006) and to low or high levels of house dust (Zambelli-Weiner et al., 2005; Simpson et al., 2006). Another example of a gene-environment hypothesis is based on the effect of ETS on asthma (Cook & Strachan, 1997). In the Children's Health Study of 6,000 children in the Los Angeles region (Peters et al., 1999), in utero ETS increased asthma and wheezing in children and current ETS increased wheezing. Pathway analysis of xenobiotic metabolism and antioxidant defenses suggested candidate genes in the

GST superfamily (GSTP1, GSTM1, and GSTP1), which are expressed in the respiratory tract and function as an enzymatic antioxidants and hydroperoxidases. Cross-sectional studies have supported the hypothesis of gene-environment interaction between GST genes and ETS. In a subset of the Children's Health Study, Gilliland et al. (2002) showed a common genotype that results in the complete lack of the GSTM1 enzyme (GSTM1 null) interacts with in utero exposure to ETS to increase the prevalence of asthma and wheezing in children.

Crossover interactions

One type of gene-environment interaction will mask main effects of genes. The crossover interaction (see Ober and Thompson, 2005, Figure 1a) has been documented for several genes (CD14, HLA-G, TLR4, and IR4RA genes). Two of these (TLR4 and HLA-G) are not in the elite group of genes with more than 10 replications but fall in the middle class with two to five replications (Ober & Hoffjan, 2006). The underlying concept of the crossover gene-environment interaction has been discussed by Vercelli and Martinez (2006). They point out that, in the nuances of human subjects, variation in regulatory processes may create variation in gene expression that is difficult to understand without consideration of appropriate exposures. The studies by Eder et al. (2005) and Zambelli-Weiner et al. (2005) were cited as examples of the same genetic polymorphism (CD14-260C>T) being associated with risk or protection depending on environmental exposures (in these two instances, to farm animals or house dust). Thus, including individuals with different environmental exposures in the same analysis may dilute a genetic main effect to the point of erasing it. Vercelli and Martinez point out that, contrary to the suggestions of some (Hall & Blakely, 2005), Nevertheless, the NCS provides the best opportunity to compensate for heterogeneity of environmental exposures do date.

Therefore, the search for gene-environment interactions in the elite group of genes with multiple replications of association with asthma (10 genes in more than 10 studies and 15 genes in six to 10 studies) may not be the best strategy, at least not for the class of crossover gene-environment interactions that dilute the main effect of genes. NCS researchers intend to evaluate gene-environment interactions for multiple exposures across all of the genes identified by Ober and Hoffjan (2006) with particular emphasis on those with few replications (i.e., the set of 54 genes with two to five replications and the set of 39 genes with a single study documenting association).

3.4 Potential for Innovative Research

Vulnerability to particular risk factors is determined not only by the genome acquired at conception but by the nature of the environment influences during critical periods of development, so a longitudinal assessment of the environment from before conception through pregnancy, fetal life, birth, and infancy could provide an opportunity to understand time-dependent susceptibility to asthma. For example, the review by Saglani and Bush (2007) emphasized the importance of the first 3 years of life and concluded that fetal and maternal genotype interact with environmental exposures to establish a risk phenotype, which interacts with antenatal exposures to set by age 3 the lifelong course of chronic asthma and lung impairment.

Also, the interplay between genes and environment is characterized by dynamic modifications to the genome, such as epigenetic modifications to nuclear and mitochondrial DNA that have been hypothesized as likely mechanisms for gene-environment interactions. For example, Valle (2004) reviews the contributions of epigenetic mechanism to genetic individuality, compares these stable alterations in gene expression that arise during development to variation in expression due to promoter allelic variation and alternative splicing, and points out that environmental experiences can have profound effects on epigenetics and individual variation in gene expression. Sanders (2006) described epigenetic regulation of Th1 and Th2 cell development that is important in asthma. Hanson and Gluckman (2005)

propose the primary mechanism for fetal adaptations and development of "thrifty phenotypes" is by epigenetic modification. An investigation of a cohort over time and under various environmental conditions provides an opportunity to collect repeated specimens for genetic analysis (see Callinan and Feinberg, 2006, for a description of this process), which could be used to assess some likely candidate epigenetic mechanisms involved in gene-environment interactions and susceptibility to asthma.

3.5 Feasibility

The National Children's Study protocol specifies the collection of biological samples that could be the source of DNA for traditional genetic analysis of candidate genes and for gene discovery based on genome-wide association scans. The anticipation of chip-based genotyping of all participants based on current technology or complete sequencing of all individuals at some point in the future will provide extraordinary detail about genetic variation of nuclear DNA. Along with the planned definition of cases based on asthma phenotypes, this provides an opportunity to use the efficient nested-case control design for subsets of the sample or the proportional hazard design for the entire sample to evaluate effects of genes, environments, and their interaction.

In addition, the Study protocol specifies collection of biological samples at multiple points over time, which provides the opportunity to evaluate epigenetic changes proposed to be the molecular mechanisms of some gene-environment effects (Hanson & Gluckman, 2005). The epigenetic assays for environmental effects of methylation and chromatin status are rapidly evolving, and current methods are surely to improve rapidly as a result of extensive current work (Callinan & Feinberg, 2006) and anticipated future work (NIH Epigenetics Workshop, 2007).

Callinan and Feinberg (2006) describe the two major modifications of DNA or chromatin (DNA methylation, the covalent modification of cytosine; post-translational modification of histones including methylation, acetylation, phosphorylation, and sumoylation) that act to regulate gene expression and review the current status of high-throughput microarray technologies for assessing DNA methylation and chromatin modification and the status of the Human Epigenome Project. Bjornsson, Fallin, and Feinberg (2004) describe an integrated framework to evaluate, in addition to direct or independent effects of DNA genotype (g_{ind}), the epigenotype (epg) and how it influences disease phenotype.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/child

- Genetics: Family history of allergy, asthma, and respiratory illness; family immune history
- Environmental exposures (home, school and daycare):
  - Environmental samples (e.g., mold, endotoxins, allergens, environmental tobacco smoke)
  - Other exposure information (housing characteristics, product usage in home, parental occupational/hobby data, food/diet questionnaire, child time activity patterns [questionnaire, diary, or GPS])

4.2 Methods

Primary/maternal

- Interview/questionnaire
- Medical record review
- Blood for genotype
- Physical sampling (air, dust) of home, school, and daycare

Primary/child

- Medical record review
- Blood for genotype
- Physical sampling (air, dust) of home, school, and daycare

4.3 Life Stage

- Periodic, birth through year 20

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Asthma, wheezing, pulmonary function/dysfunction
  - Allergic sensitization
  - Airway reactivity
  - Immune system function (e.g., lymphocytes, cytokines, IgE, interleukins)

5.2 Methods

- Examination and interview by medical professional
- Medical record review
- Blood samples
- Pulmonary function: Pulmonary function tests and spirometry

5.3 Life Stage

- Periodic, birth through year 20

6. Important Confounders, Mediators, and Effect Modifiers

- **Health care access:** Poor health care access in inner cities, especially among African Americans and Hispanics, exacerbates the risk of asthma attacks and deaths (Lara et al., 2003; Sin, Bell, & Man, 2004; Wallace, Scott, Klinnert, & Anderson, 2004). This and other disparity factors could be behind the greater asthma prevalence, especially among African Americans. These issues are more thoroughly discussed in hypothesis 4 and in the review by Gold and Wright (2005).

- **Diet, physical activity, bioaerosols, stress, and infections:** These exposures will be the topic of other hypotheses and could interact with or confound associations of asthma onset with air pollutants.

- **Polymorphisms in genes:** For the analysis of gene-environment interactions, it will be important to include polymorphisms in genes involved in oxidative stress responses to air pollutant exposures and candidate genes.

- **Socioeconomic status:** Lower socioeconomic status and some minority groups, such as Blacks and Hispanics, are likely to live in areas with increased air pollution, such as near freeways or industrial sites. Careful assessments of these characteristics will be important to properly control for their influence on outcomes.

- **Environmental exposures:** Diet, physical activity, bioaerosols, stress, and infections, which will be the topic of other hypotheses, could interact with or confound associations of asthma onset with air pollutants (specific hypothesis 1). Though the following list is not exhaustive, some important factors associated with atopy and asthma include demographic group (age, race, income, or education), familial characteristics (number of siblings, parent and sibling phenotypes for asthma), daycare attendance, pet ownership, living on a farm, body mass index, immunizations, several dietary factors, respiratory infections, and diabetes.

7. Power and Sample Size

Several methods are available for the evaluation of gene-environment hypotheses stated above. A standard approach is to use the nested case-control design, with affected cases defined by asthma phenotype and matching to select control subjects. When samples are stored and measures of exposure are expensive, the nested-case control design is preferred since it is efficient and minimizes the number and cost of measures. This approach described in Gauderman (2002) and Gauderman and Morrison (2007) provides the computer program QUANTO to estimate power and sample size.

Schwartz (2006) emphasizes the difference between the use of additive and multiplicative scales of measurement. The multiplicative scale of measurement is typically adopted, which dictates the use of ratio measures such as relative risk or odds ratio to assess disease risk based on the assumption that risk is multiplied in individuals with a risk factor compared to those without. When the additive scale is adopted, the risk difference is used based on the assumption that disease risk is added in individuals with the risk compared to those without.

Interactions in the additive model will be easier to detect than in the multiplicative model. Although the traditional approaches are used in the examples below, the use of an additive model (which would increase statistical power with all other factors held constant) could be considered.

Nested case-control example

The power calculations presented below were performed by James Gauderman using the QUANTO 1.2 computer program. The following assumptions were made:

- Initial cohort size: N
- Three percent per year loss to follow-up
- Incidence rates of 0.01, 0.05, 0.10, 0.20, 0.50, and 3.00 percent per year
- Study period of 5 years

In addition, an incidence rate of 2.5 percent (which is the approximate average annual incidence rate observed in the Children's Health Study) is assumed. Specifically, as reported in McConnell et al. (2006), 14 percent of children aged 5-7 reported physician-diagnosed asthma and 13 percent reported prevalent asthma at that age. An annual incidence rate of 2.5 percent would yield a prevalence by age 6 of 13.1 percent. From the above incidence rates, the expected number (n) of cases that will accrue during the 5-year period for each incidence rate can be derived. Based on the initial cohort size of N = 100,000 projected for the National Children's Study, these are as follows:

Disease incidence (%)	Cases expected (n)
0.01	47
0.05	235
0.10	470
0.20	938
0.50	2,331
2.50	11,191
3.00	13,304

From these, the following minimum detectable odds ratio for a genetic main effect relative odds ratio for a gene-environment effect is calculated:

- Gene with 10 percent allele frequency and dominant inheritance model
- Environmental exposure with 10 percent prevalence
- Marginal genetic and environmental effects of 1.5 in gene-environment calculations
- Eighty percent power
- 0.0001 Type I error rate, two-sided alternative hypothesis

With disease incidence rates less than 0.1 percent per year and the above assumed prevalences of genetic susceptibility and environmental prevalence, gene-environment interactions will be nearly impossible to detect. For asthma (with assumed annual incidence rate of 2.5 percent), interactions of modest size will be detectable.

The above calculations assumed environmental factor prevalence of 10 percent. An increased prevalence may be present, and this would increase the ability to detect gene-environment

interactions. The following table shows the change in minimum detectable relative odds ration for gene-environment interaction effect with increased environmental factor prevalence (20 percent and 30 percent), for a few values of incidence/year and 1.5 controls per case.

	Environmental factor prevalence		
Disease incidence (%)	10%	20%	30%
0.01	>>10.00	>>10.00	>>10.00
0.05	>10.00	9.00	7.80
0.10	7.10	4.70	4.20
0.20	4.00	3.00	2.70
0.50	2.50	2.00	1.90
2.50	1.52	1.35	1.34
3.00	1.47	1.39	1.31

The prevalence of the environmental factor has a large impact on the minimum detectable interaction effect size when the incidence rate is small (e.g., for 0.20, a decrease from 4.0 to 2.7, about 33 percent). The improvement is less when the incidence rate is high (e.g., for 2.50, a decrease from 1.52 to 1.34, about 12 percent). The same patterns would be observed as a function of the prevalence of genetic susceptibility.

8. Other Design Issues

There may be ethical considerations associated with genetic tests, but such considerations are relevant to the NCS as a whole and would not be unique to this specific hypothesis. For nongenetic factors, the study will need to have a formal strategy and process for effective communication of clinically relevant and actionable physiological and biochemical results to the child's parents. The study also will need to have a formal strategy and process for effective communication of clinically relevant and actionable results of environmental monitoring to the child's parents along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings. Daycare and school cooperation would be required for some of the intended environmental exposure measures. In addition, a mechanism needs to be identified to ensure that children found to have asthma through this study can receive adequate medical care for their illness. Repeated assessments can be burdensome, and the total respondent burden must be evaluated and be reasonable.

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OBESITY AND INSULIN RESISTANCE FROM IMPAIRED MATERNAL GLUCOSE METABOLISM

1. Meta Hypothesis

Impaired maternal glucose metabolism during pregnancy is directly related to the risk of obesity and insulin resistance in offspring.

2. Specific Hypotheses

1. Fetal macrosomia and increased risk for obesity later in life is associated with an increased maternal supply of glucose during gestation.

2. The association between fetal macrosomia and an increased maternal supply of glucose during gestation varies by gestational period during which hyperglycemia occurs.

3. The combined interaction of maternal hyperlipidemia and hyperglycemia during gestation is more strongly associated with fetal macrosomia and increased risk for obesity later in life than either predictor alone. Recent evidence suggests the addition of hyperlipidemia to the presence of hyperglycemia improves the ability to predict the risk of large-for-gestational-age babies (Bo et al., 2004). Data analysis should consider regression models with both maternal lipid and glucose concentrations as variables.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

The prevalence of overweight among children is greater than 16 percent among those 6 or older, and this prevalence has increased during the past 40 years (Hedley et al., 2004; Kuczmarski, Flegal, Campbell & Johnson, 1994). Overweight children are at increased risk for being overweight in adulthood (Serdula et al., 1993), which is associated with increased risk of type 2 diabetes, hypertension, and coronary artery disease (Freedman, Khan, Dietz, Srinivasan, & Berenson, 2001). Furthermore, being overweight as a child increases the risk of developing type 2 diabetes before age 21 (Sinha et al., 2002). The total prevalence of diabetes in women ages 20-39 is 1.7 percent and 6 percent among those ages 40-49 (Harris et al, 1998, as cited in Beckles & Thompson-Reid, 2001). Among reproductive-aged women with diabetes, about one-third (35.4 percent) of women younger than 40 and about one-quarter (26.7 percent) of those 40 or older did not know that they had the disease (Harris et al, 1998, as cited in Beckles & Thompson-Reid, 2001). About 4-7 percent of pregnancies are complicated by gestational diabetes (Kjos & Buchanan, 1999).

Impaired glucose tolerance is categorized into either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) depending upon the test used to diagnose it. In a cross-section of U.S. adults ages 40-74 tested from 1988 to 1994, 33.8 percent had IFG, 15.4 percent had IGT, and 40.1 percent had pre-diabetes (IGT, IFG, or both). Applying these percentages to the 2000 U.S. population, about 35 million adults ages 40-74 would have IFG, 16 million would have IGT, and 41 million would have pre-diabetes (National Institute of Diabetes and Digestive and Kidney Diseases, 2005). Based on data from the 1999-2000 National Health and Nutrition Examination Survey, approximately 2 percent of U.S. adults between 20-39 are affected by IFG (Centers for Disease Control and Prevention [CDC], 2003).

Economic and/or social burden

Because child obesity is a risk factor for adult obesity, child obesity contributes to the more than $40 billion annual cost of obesity in the United States (Colditz, 1992). Obese people suffering from diabetes make up about half of all such persons suffering from diabetes but are responsible for nearly 60 percent of the cost (Finkelstein, Brown, Trogdon, Segel, & Ben Joseph, 2007). Estimates from the Medical Expenditure Panel Survey, the National Health Interview Survey, and National Health Accounts estimated costs at $50 billion to $80 billion in 1998 for overweight and obesity combined (Finkelstein, Fiebelkorn, & Wang, 2003). State Behavioral Risk Factor Surveillance Surveys estimate combined expenditures at $75 billion, from $87 million in Wyoming to $7.7 billion in California. The number of hospital discharges associated with obesity-related diseases in the United States has increased during the past three decades. Childhood and adolescent obesity-associated annual hospital costs, adjusted for inflation, increased more than threefold from $35 million during 1979-1981 to $127 million during 1997-1999. The percentage of total hospital costs that these numbers represent increased from 0.43 percent to 1.70 percent, respectively (Wang & Dietz, 2002).

3.2 Justification for a Large Prospective Longitudinal Study

Findings from the National Children's Study (NCS) are becoming more important as the incidence of obesity increases. A 2006 review of the increased incidence of obesity revealed approximately one-third of all pregnant women in the United States are obese (King, 2006).

A longitudinal study with national representation across the United States is essential to assess the development of obesity in the offspring. A small study would likely not be sufficient to answer the question because many variables affect the development of obesity. As opposed to a small cohort investigation, the components of the NCS focus on childhood obesity, as it is related to maternal gestational glucose tolerance, will be more likely to identify factors associated with childhood obesity. In addition, the longitudinal nature of the study data will provide stronger statistical confidence in identifying specific variables on the causal pathway of childhood obesity.

3.3 Scientific Merit

Current scientific understanding

Determining the strength of the association between impaired maternal glucose metabolism during pregnancy and overweight in children is a timely issue because the prevalence of overweight among women of childbearing age has increased (Mokdad et al., 1999) and being overweight is a risk factor for impaired maternal glucose metabolism (Kjos & Buchanan, 1999). The offspring of mothers who have type 1 diabetes are at increased risk of being overweight, and this effect is often evident as young as age 4 (Whitaker & Dietz, 1998). Studies that grouped mothers with gestational diabetes with type 1 and type 2 diabetics have shown an increased risk of overweight offspring (Whitaker & Dietz, 1998). On the basis of these observations, offspring of mothers with gestational diabetes or lesser degrees of impaired glucose metabolism are suspected of being at increased risk of obesity, but there are little data available to address the issue, and those data that are available have extremely limited statistical power (Whitaker & Dietz, 1998; Dabelea & Pettit, 2001).

3.4 Potential for Innovative Research

The currently proposed study is innovative and timely because of the increased incidence of obesity in the United States. The design of the NCS provides the unique opportunity to perform a natural history study of childhood obesity, which can be more precisely followed in a prospective manner to

further identify growth trajectories that lead to obesity in adulthood. Additionally, information about maternal glucose levels during gestation can be used to identify significant risks to childhood and therefore adult obesity.

The Study will recruit a large number of women prior to pregnancy. This will allow researchers to collect observational data on both mother and the developing fetus soon after conception. By observing early fetal development, reactions of the fetus to maternal hyperlidemia and hyperglycemia during gestation at various points in childhood can be described. The design of the Study will use the contributions of body composition at birth, growth trajectory of lean and fat body mass, and fat/lean body mass ratio to adult insulin resistance can be parsed. Variation in the occurrence of insulin sensitivity due to status at birth, childhood and adolescence growth trajectory, and dietary intake may be determined.

3.5 Feasibility

The data collection is feasible. Data required to test this hypothesis and suggested specific hypotheses are included in the currently proposed study protocol. The feasibility of obtaining serial information from birth and throughout childhood adequate to characterize childhood growth, body composition, and regional body fat (in particular, omental fat) remains to be settled. Characterizing growth trajectory throughout childhood will require more frequent measurements than currently planned.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

Physician report or preexisting or gestational diabetes

- Blood glucose and HgbA1C
- Serum lipid profiles
- Serum insulin or related samples (e.g., C-peptide)
- Genetic samples (for exploration of potentially relevant genes such as VNTR insulin, glucokinase)

4.2 Methods

Primary/maternal

Blood samples

- Medical records review
- Interviews
- Physical exams-anthropometry

4.3 Life Stage

Primary/maternal

- Prenatal (first and third trimesters) and birth

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Insulin resistance:
  - Serum insulin levels
  - Glucose levels
  - HgbA1C
- Obesity:
  - IGF body size
  - Habitus body composition

5.2 Methods

- Blood samples, physical exams
- Anthropometry, body composition

5.3 Life stage

- All measures: Birth and periodic

6. Important Confounders, Mediators, and Effect Modifiers

- **Lipid profile:** Increased lipid levels are associated with an increased risk of insulin resistance (McGarry, 2002).
- **Glucokinase mutation:** Glucokinase mutation is associated with increased risk of maturity onset diabetes of the young (Kahn, Vicent, & Doria, 1996).
- **Hormone levels, such as cortisol, growth hormones, insulin-like growth factors:** Elevated levels of these and other hormones are associated with obesity and insulin resistance in children (Reinehr & Andler, 2004).
- **Genetic markers for obesity:** Certain genetic markers increase the risk of obesity.
- **Parent's body mass indices:** Body mass index and obesity are associated with certain genetic markers (Maes, Neale, & Eaves, 1997).

- **Family history of diabetes and obesity:** A family history of diabetes and obesity increases a child's risk (Centers for Disease Control and Prevention [CDC], 2005; Maes et al., 1997).

- **Lifestyle factors:** Less active lifestyles would increase the risk of obesity and insulin resistance (Sinha et al., 2002).

- **Nutrition:** Poor nutritional and high caloric diet would increase the risk of obesity and insulin resistance (Ogden, Flegal, Carroll, & Johnson, 2002).

- **Socioeconomic status and demographics:** Children of lower economic status and racial groups (particularly Native Americans, Hispanics, African Americans, and Asians) are at higher risk for obesity and insulin resistance (CDC, 2005).

7. Power and Sample Size

About 4-7 percent of pregnancies are complicated by gestational diabetes (Kjos & Buchanan, 1999). Assuming 100,000 children and an exposure prevalence of 5 percent:

- Smallest detectable relative risk for obesity (prevalence 10 percent, using 30 kg/m2 definition) is approximately 1.2

- Smallest detectable relative risk for metabolic syndrome (assumed prevalence 0.4 percent) is approximately 1.7

8. Other Design Issues

- Addressing this hypothesis based on obesity and insulin resistance measures at later life stages may be adversely impacted by attrition of study subjects.

- Blood studies, especially fasting, in younger children will require attention.

- Accurate characterization of growth trajectories are best when based on study measurements and not reports. This would require in-person visits more frequently during childhood than is currently planned and would introduce additional costs.

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OBESITY AND INSULIN RESISTANCE FROM INTRAUTERINE GROWTH RESTRICTION

1. Meta Hypothesis

Intrauterine growth restriction (IUGR) is associated with subsequent risk of central-body obesity and insulin resistance in offspring independent of subsequent body mass index.

2. Specific Hypotheses

1. IUGR is associated with a higher ratio of fat to lean body mass at the same body mass index beginning in childhood and persisting into adulthood and increased insulin resistance in offspring.

2. Adult insulin resistance is more likely in infants born with IUGR who:

    2.1 Demonstrate catchup growth during infancy or childhood.

    2.2 Demonstrate catchup in fat mass that exceeds catchup in linear dimensions or head size.

    2.3 Demonstrate early adiposity rebound.

    2.4 Have growth trajectories associated with insulin resistance which differ for males and females.

    2.5 Have growth trajectories leading to increased fat/lean body mass ratio which are associated with higher per kilogram or centimeter dietary intake.

3. Children born with IUGR who experience growth/body composition trajectories (see above) will also have significantly different timing of other developmental events (e.g., adrenarche, pubarche).

4. Children with IUGR, regardless of their postnatal growth trajectory, will demonstrate elevated cortisol, IGF 1, and IGF 2 levels at birth and into adulthood.

5. Measures of linear and body composition status/trajectories during fetal life will more precisely predict adult insulin resistance than size at birth.

6. Matrilineal birth size history (i.e., birth size of mother, grandmother) interacts with current intrauterine environment to affect birth size.

7. Late gestation and birth differences in body composition among IUGR infants predispose to:

    - 7.1 Differing body composition and growth trajectories throughout childhood, and

    - 7.2 Differences in the percentage demonstrating insulin resistance as adults.

8. The relationship of IUGR mediated by postnatal growth trajectory differs by ethnicity.

9. Childhood adiponectin levels are increased in IUGR children showing evidence of insulin resistance (measured by HOMA-IR).

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Intrauterine growth retardation: IUGR occurred in 7.6 percent of births in 2000 based on birth records of more than four million births (Boulet, Alexander, Salihu, Kirby, & Carlo, 2006). Prevalence of low birth weight (less than 2,500 grams) has increased in most ethnic groups between 1995 and present. The Pregnancy Nutrition Surveillance System reported a 2004 low birth weight rate of 7.5 percent for whites, 11.7 percent for African Americans, and 6.3 percent for Hispanics (Centers for Disease Control and Prevention [CDC], 2006). Some infants are not growth retarded at this weight and some high gestational age infants are growth retarded at higher weights (Boulet et al, 2006).

Insulin resistance: About 12.6 percent of the National Health and Nutrition Examination Survey (NHANES) sample had high fasting glucose or medication use indicating treatment for abnormal glucose metabolism. Figures were highest in Mexican Americans and higher in African Americans than whites from the NHANES 1988-94 (Ford, Giles, & Dietz, 2002). About half the variation in insulin resistance is explained by body mass indices (McLaughlin, Allison, Abbasi, Lamendola, & Reaven, 2004). Prevalence of the metabolic syndrome was about 23 percent, increasing from 7 percent in early adulthood to 42 percent in 60- to 69-year-olds. The rate was about the same in men and women in whites, but higher in Mexican American and African American women. Insulin resistance by various definitions occurred in 2-20 percent of normal weight, 12-60 percent of overweight, and 48 to 80 percent of obese children in the NHANES sample 1999-2002 (Lee, Okumura, Davis, Herman, & Gurney, 2006). The prevalence of diagnosed diabetes in 2001 on BRFSS was 7.9 percent, up from 7.3 percent in 2000, and was highest in African Americans (11.2 percent).

Obesity and abdominal obesity: In the NHANES sample (Ogden et al., 2006), 13 percent of adults were overweight and 16 percent were obese. Compared with the 1999-2000 NHANES wave, in the 2003-2004 NHANES wave 17 percent of all children 2-19 and 32 percent of adults 20 and older were overweight, with significant increases for children, adolescents, and men; rates were higher in ethnic minorities (58 percent of African American women ages 40-59 are obese compared to 38 percent of white women).

Abdominal obesity (defined as waist circumference greater than 102 centimeters in men and greater than 88 centimeters in women), was 39 percent (31, 23, and 31 percent for white, African American, and Mexican American men, respectively, and 44, 62, and 63 percent in white, African American, and Mexican American women, respectively) (Gillum, 1999).

Economic and/or social burden

Obese persons suffering from diabetes comprise about half of all such persons but are responsible for nearly 60 percent of health care costs (Finkelstein, Brown, Trogdon, Segel, & Ben Joseph, 2007). Estimates from the Medical Expenditure Panel Survey, the National Health Interview Survey, and National Health Accounts estimated costs at $50 billion to $80 billion in 1998 for overweight and obesity combined (Finkelstein, Fiebelkorn, & Wang, 2003). State Behavioral Risk Factor Surveillance Surveys estimate combined expenditures at $75 billion, with states ranging from $87 million for Wyoming to $7.7 billion for California. Among hospital discharges in the United States, the number of discharges associated with obesity-related diseases has increased during the past three decades. Childhood and adolescent obesity-associated annual hospital costs, adjusted for inflation, increased more than three fold from $35 million during 1979-1981 to $127 million during 1997-1999. The percentage of total hospital

costs these numbers represent increased from 0.43 percent to 1.70 percent, respectively (Wang & Dietz, 2002).

3.2 Justification for a Large Prospective Longitudinal Study

Most studies of the relationship of IUGR and adult insulin resistance are based on retrospective data and limited to information about size at birth and adult outcomes with no information available about different periods during prenatal development. Results of studies linking IUGR growth trajectory to adult outcomes have been contradictory because of differing definitions of key dependent and independent variables, use of different measurements, and limitations on follow-up. Many of the apparent confounders for this phenomenon (e.g., levels of such hormones as cortisol and insulin-like growth factors) are likely embedded in the same causal framework with IUGR that underlies the fetal origins of later life phenomena. Moreover, other confounders (such as genetic/familial factors, ethnicity, childhood and early adult body composition not associated with a history of IUGR) have not been sufficiently controlled.

Only a large prospective study will be able to adequately model this framework and determine the roles of the various factors embedded in it. The National Children's Study (NCS) design will provide prospective measurements of:

- Maternal nutritional deprivation or other stressors at different periods during prenatal development,
- Serial fetal growth throughout gestation (or minimally in the second and third trimesters),
- Serial fetal body composition including regional adiposity,
- Size and body composition at birth and then throughout childhood, adolescence and early adulthood,
- Dietary intake of mother during pregnancy and the offspring postnatally, and
- Key hormonal levels in
    - The mother during pregnancy, and
    - The infant at birth and then throughout childhood and adulthood.

Family factors (e.g., sibling birth size, body composition of other family members, maternal history of birth size [through report or via access to medical records]) will better control confounding.

3.3 Scientific Merit

This hypothesis addresses one of the fetal-origins hypotheses advanced by Barker (Barker & Osmond, 1987, 1988; Barker, Osmond, Golding, Kuh, & Wadsworth, 1989; Barker, 1990), which postulates an association between IUGR and features of the metabolic syndrome (including insulin resistance, dyslipidemia, hypertension, and coronary heart disease) in adulthood. Studies and commentary from many disciplines have contributed to the literature on this hypothesis (McMillen & Robinson, 2005; Armitage, Khan, Taylor, Nathanielsz, & Poston, 2004), and it has been expanded to include other adult outcomes besides metabolic syndrome, including rapid onset hypothyroidism (Kajantie et al., 2006),

immune dysfunction (McDade, 2005), chronic lung disease (Lucas et al., 2004; Gluckman, Hanson, & Pinal, 2005), maturational timing (Ibanez & d Zegher, 2006; Ibanez, Jimenez, & de Zegher, 2006), lean body mass (Li, Stein, Barnhart, Ramakrishnan, & Martorell, 2003), male reproductive dysfunction (Main, Jensen, Asklund, Hoi-Hansen, & Skakkebaek, 2006), and puberty and adrenarche (Van Weissenbruch & Delemarre-van de Waal, 2006). The preponderance of evidence supports the theory though several investigators have been unable to support it (Huxley & Neil, 2004; Desai, Gayle, Babu, & Ross, 2005; Tilling et al., 2004; Huxley, Neil, & Collins, 2002).

Initially, Barker hypothesized that the observed association was due to the "thrifty phenotype (Hales & Barker, 1992)," also called predictive adaptive response (Gluckman & Hanson, 2004) or phenotypic plasticity (Kuzawa, 2005), which is the ability of the fetus to adapt its growth to maternal constraints due to nutrient or oxygen limitation or another stressor. The observed adult outcomes were putatively associated with a mismatch between the limited prenatal environment and a postnatal environment with plentiful resources. The mechanism linking the adult outcome with fetal growth restriction was postulated to be fetal metabolic programming (the "setting" of a physiological system by an insult during a sensitive period resulting in long-term consequences). This phenomenon is observed in many animal and plant species (Kuzawa, 2005) and relates to highly conserved metabolic networks regulated by cell-level, genetically based processes (Lampl, 2005). An alternative to this hypothesis is that IUGR and the adult metabolic syndrome are secondary to genetically determined insulin resistance that results in impaired insulin mediated growth in the fetus as well as insulin resistance in adulthood (Hattersley & Tooke, 1999).

Studies during the last decade have led to a more detailed and broadened understanding of this hypothesis. The important observations documented:

- Adult outcomes vary depending upon body composition at birth among IUGR infants and differ by gender and in a different way by ethnicity (Levy-Marchal & Czernichow, 2006; Eriksson, Forsen, et al., 1999; Jaquet et al., 2005; Landmann et al., 2006; Modi et al., 2006; Forsen, Eriksson, Tuomilehto, Osmond & Barker, 1999; Hack et al., 2003).

- The relationship between lean body and fat mass in adulthood better predicts insulin resistance (Landmann et al., 2006; Hediger et al., 1998; Ong, 2006; Gale, Martyn, Kellingray, Estell, & Cooper, 2001; Levitt, Lambert, Woods, Seckl, & Hales, 2005; Loos et al., 2001; Phillips, 1995; Kensara et al., 2005) and is likely related to the limitation on postnatal muscle cell hyperplasia (Eriksson, Forsen, Osmond, & Barker, 2003).

- Growth trajectories (catchup in linear growth and fatness) mediate observed adult outcomes (Ong, 2006; Barker, Osmond, Forsen, Kajantie, & Eriksson, 2005; Dulloo, 2006).

- Maternal causes of IUGR are one subset of fetal "stressors" and metabolic programming is likely driven by early effects on the hypothalamic-pituitary axis that result in excess glucocorticoid secretion (by fetus, mother and placenta) that upregulates production of cortisol during postnatal life (Dulloo, 2006; Seckl & Meaney, 2006; Vaag et al., 2006; Ward, Syddall, Wood, Chrousos, & Phillips, 2004; Lesage, Blondeau, Grino, Breant, & Dupouy, 2001; Worthman & Kuzara, 2005).

- The hypothalamic-pituitary-adrenal axis reprogramming is due to altered pancreatic development, which is the metabolic shift that allows the brain to be preferentially

- This may specifically affect development of organ systems in the fetus that in turn affect subsequent metabolism during postnatal life (Armitage, Khan, Taylor, Nathanielsz, & Poston, 2004).

- The combined effects of fetal programming and postnatal growth (linear and body composition trajectory) lead to metabolic alteration in adipose tissue hormonal secretions (e.g., adiponectin and leptin), which also affects the development of insulin resistance in adulthood or earlier.

- The presence or magnitude of the fetal programming effect differs depending upon polymorphic genes (Eriksson, Lindi, et al., 2003; Heude, Petry, Pembrey, Dunger & Ong, 2006; Ong et al., 1999) and in different population groups (Freedman, Khan, Serdula, Ogden, & Dietz, 2006) and may be regulated at least in part by gene imprinting (Dunger, Petry, & Ong, 2006; Fowden, Sibley, Reik, & Constancia, 2006; Young, 2001; Reik et al, 2003).

- Transgenerational effects may affect this adaptive process, with the fetus assessing the quality of the maternal environment relative to information about past intrauterine environments (Heude et al., 2006).

3.4 Potential for Innovative Research

The design of NCS provides the unique opportunity to perform a natural history study of IUGR, which can be more precisely characterized (using ultrasound measurements of size and body composition) prospectively related to the occurrence of IUGR and, in turn, to adult outcomes. Information about family disposition to birth size (based on maternal birth size history and birth size of siblings) can be incorporated into the definition of IUGR to develop a more precise definition of the disorder. Similarly, detailed information about growth during childhood in relation to expected growth (based on anthropometric and body composition information about family members) will allow more precise determination of expected status and trajectory.

The Study will recruit a large number of women prior to pregnancy which will allow researchers to collect observational data on both mother and the developing fetus soon after conception. By observing early fetal development, reactions of the fetus (in terms of whole body growth, segmental and organ specific growth, and body composition) to intrauterine environment at various points in gestation can be described. The design of the study will use the contributions of body composition at birth, growth trajectory of lean and fat body mass, and fat/lean body mass ratio to adult insulin resistance can be parsed. Variation in the occurrence of insulin sensitivity due to status at birth, childhood and adolescence growth trajectory, and dietary intake may be determined.

3.5 Feasibility

Data that would be required to test this meta hypothesis and suggested specific hypotheses are included in the current protocol. The feasibility of obtaining serial information throughout fetal life (by ultrasounds completed in each trimester) adequate to characterize fetal growth, body composition, and regional body fat (in particular, omental fat) remains to be settled. Characterizing growth trajectory

throughout childhood will require more frequent measurements than are currently planned. Though of lesser quality, abstraction of primary care provider records for anthropometric measurements might be substituted. Body composition using a more direct method than anthropometry (e.g., DEXA) would increase the testability of the body composition related hypotheses. Body mass index does not adequately separate lean and fat body mass (Kensara et al., 2005). Hormonal measurements at birth using cord blood can be completed. While the inclusion of this measure may be prohibitively expensive for inclusion within the entire study population, it may be possible to include more precise body composition examinations, such as DEXA scans, within a subsection of the study population. Doing so could also be used to test hypotheses related to the adequacy of using approximate body composition estimates such as body mass index. Making use of cortisol measurements in the mother and child, included as key measures within other study hypotheses, is also planned.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal/family

- Fetal size measures (e.g., long bones, head and organ sizes)
- Cortisol
- Maternal body composition measures
- Sibling birth and fetal sizes, where available
- Maternal birth size history
- Nutrient (micro and macro) intake
- Placental hormonal levels
- Documentation of maternal insulin resistance

Primary/child

- Birth
  - Cord blood hormonal levels (cortisol, insulin-like growth factors)
  - Birth anthropometry and body composition (direct or anthropometry)
  - Observations and examination documenting IUGR
- Childhood
  - Anthropometry and body composition measures
  - Dietary intake

4.2 Methods

Maternal

- Anthropometry
- Ultrasounds
- Interviews
- Placental analysis

Child

- Anthropometry
- Direct body composition measurements
- Neonatal examinations

4.3 Life Stage

Primary/maternal

- Prenatal, second and third trimester

Primary/child

- Birth
- Childhood

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Measures of obesity
- Regional body fat
- Insulin resistance

5.2 Methods

- Blood sample (blood glucose levels, fasting blood glucose levels, and fasting challenge blood glucose levels), physical exam anthropometry (for body mass index), and body composition

5.3 Life Stage

- Birth, 6, 12, 18, 30, and 36 months; yearly throughout childhood

6. Important Confounders, Mediators, and Effect Modifiers

- **Glucokinase mutation:** Glucokinase mutation is associated with an increased risk of maturity onset diabetes of the young (Hattersley et al., 1998); however, evidence suggests this mutation does not result in programming in later life (Velho, Hattersley, & Froguel, 2000).

- **Hormone levels such as cortisol, growth hormone, and insulin-like growth factors:** Elevated levels of these and other hormones are associated with obesity and insulin resistance in children (Reinher & Andler, 2004).

- **Genetic markers for obesity:** Certain genetic markers increase the risk of obesity, such as the insulin variable number of tandem repeats polymorphism, located in the insulin gene promoter and variants of the gene ENPP1, known to inhibit insulin receptors.

- **Parent's body mass indices:** Body mass index and obesity are associated with certain genetic markers (Maes et al., 1997).

- **Family history of diabetes and obesity:** A family history of diabetes and obesity increases a child's risk (Maes et al., 1997, CDC, 2005).

- **Lifestyle factors:** Less active lifestyles would increase the risk of obesity and insulin resistance (Kuczmarski, Flegal, Campbell, & Johnson, 1994).

- **Nutrition:** Poor nutritional and high caloric diet would increase the risk of obesity and insulin resistance.

- **Socioeconomic status and demographics:** Children of lower economic status, and particular ethnic and racial groups (especially Native Americans, Hispanics, African Americans, and Asians), are at a higher risk of obesity and insulin resistance (CDC, 2005).

7. Power and Sample Size

The prevalence of high fasting glucose tolerance or the history of diabetes or glycemic medication use for adults age 20 years and older (Ford et al., 2002) are indicators for the outcome variable, insulin resistance (Table 1), and prevalence of infants born weighing fewer than 2,500 grams, according to data from the Pediatric and Pregnancy Nutrition Surveillance System (CDC, 2006) (Table 2), is used as an indicator for the risk factor, intrauterine growth retardation in calculation of sample size.

Table 1. Prevalence of NHANES adults with high fasting glucose or diabetes or glycemic medication use by gender and ethnicity

Ethnicity	Males	Females
Whites	15.6	8.5
African Americans	14.5	15.5
Mexican American	21.4	18.5

Table 2. Percent of infants born < 2,500 gm from the Pediatric and Pregnancy Nutrition Surveillance System by ethnicity (genders combined)

Ethnicity	Percent
Whites	7.5%
African Americans	11.7%
Mexican American	6.3%

Of births in the United States in 2005, 55 percent were non-Hispanic white, 14 percent were non-Hispanic black. Data for Mexican Americans comes from 2004: 16.5 percent were Mexican American. Using these proportions and correcting for sample attrition over 20 years (yearly retention rate of 98 percent assumed), in an analysis restricted to specific races, the smallest odds-ratios that can be reliably detected (with a power of 80 percent and a two-sided 95 percent confidence interval) by race and gender are presented in Table 3.

Table 3. Smallest odds ratios for insulin resistance (as indicated by high fasting glucose levels or medication use) given a birth weight < 2,500 gm by gender for three ethnicities

Ethnicity	Males	Females
Non-Hispanic Whites	1.30	1.58
Non-Hispanic Blacks	1.49	1.47
Mexican American	1.52	1.55

8. Other Design Issues

- Blood studies, especially fasting, in younger children will require careful attention. Obtaining consent for the use of DNA may be an issue.
- Addressing this hypothesis based on central obesity and insulin resistance measures at later life stages may be adversely impacted by attrition of study subjects.
- While one ultrasound may be included as part of routine prenatal care, additional ultrasounds may introduce added cost.
- Accurate characterization of growth trajectories should be based on study measurements and not reports. This would require in-person visits more frequently during childhood than is currently planned and would introduce additional costs.
- Because of problems using body mass index, direct body composition measurements are necessary for accurate characterization of fat and lean body mass. Direct body composition measures (with the exception of bioelectrical impedance) are costly.

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BREAST-FEEDING ASSOCIATED WITH LOWER RATES OF OBESITY AND LOWER RISK OF INSULIN RESISTANCE

1. Meta Hypothesis

Breast milk feeding compared with infant formula feeding is associated with lower rates of obesity and lower risk of insulin resistance.

2. Specific Hypotheses

1. Breast-feeding is associated with lower rates of childhood obesity in a dose response relationship in the United States.

2. The protective effect of breast-feeding on the risk of overweight is evident from infancy through adolescence and persists after controlling for confounding effects of other environmental factors.

3. Lower amounts of protein early in life ("the early protein hypothesis") (Koletzko, 2006) is positively associated with childhood obesity.

4. Protein and energy intake are different for infants who receive exclusive breast-feeding, breast-feeding plus complementation (formula feeding), and exclusive formula feeding. These differences are associated with obesity risk with higher cumulative protein and energy intake in infancy being associated with childhood obesity.

5. Serum leptin and adiponectin concentrations are higher in breast-fed than formula-fed infants and are associated with less rapid weight gain and lower risk of childhood obesity and insulin resistance.

6. Termination of breast-feeding is associated with a decrease in serum leptin concentration in the child and subsequent increase in weight gain.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

In 2005, 73 percent of all U.S. born infants were breast fed (either exclusively or in combination with formula feeding) before initial hospital discharge. By 6 months of age, only 39 percent of infants were breast fed. There are marked differences by race in breast-feeding initiation, exclusivity, and continuation at 6 months of age (Centers for Disease Control and Prevention [CDC], 2005b). The prevalence of overweight among children is greater than 16 percent among children aged 6 years or older, and this prevalence has increased during the past 40 years (Hedley et al., 2004; Kuczmarski, Flegal, Campbell, & Johnson, 1994). While the obesity epidemic is occurring in boys and girls in all 50 states, specific subgroups (e.g., African American, Hispanic, American Indian) are disproportionately affected (Koplan, Liverman, Kraak, & Committee on Prevention of Obesity in Children and Youth, 2005; Strauss & Pollock, 2001). Being overweight as a child is a risk factor for other childhood diseases such as asthma (Oddy et al., 2004) and type 2 diabetes (Sinha et al., 2002). Childhood obesity is associated with adult obesity (Serdula et al., 1993; Parsons, Power, & Manor, 2003), type 2 diabetes, hypertension, and coronary artery disease (Freedman, Khan, Dietz, Srinivasan, & Berenson, 2001).

Economic and/or social burden

Because child obesity is a risk factor for adult obesity, child obesity contributes to the more than $132 billion annual cost of obesity in the United States (American Diabetes Association, 2003). Obese persons suffering from diabetes make up about half of all such persons but are responsible for nearly 60 percent of the cost (Finkelstein, Brown, Trogdon, Segel, & Ben Joseph, 2007). Estimates from the Medical Expenditure Panel Survey, the National Health Interview Survey, and National Health Accounts estimated costs at $50 billion to $80 billion in 1998 for overweight and obesity combined (Finkelstein, Fiebelkorn, & Wang, 2003). State Behavioral Risk Factor Surveillance Surveys estimate combined expenditures at $75 billion, with states ranging from $87 million for Wyoming to $7.7 billion for California. Among hospital discharges in the United States, the number of discharges associated with obesity-related diseases has increased dramatically during the past three decades. Childhood and adolescent obesity-associated annual hospital costs, adjusted for inflation, increased more than threefold from $35 million during 1979-1981 to $127 million during 1997-1999. Even more interesting, the percentage of total hospital costs that these numbers represent increased from 0.43 percent to 1.70 percent, respectively (Wang & Dietz, 2002).

3.2 Justification for a Large Prospective Longitudinal Study

Prior studies have either not included samples representative of the diverse U.S. population or have been underpowered to examine and compare the relationship between breast-feeding and childhood obesity across racial groups. Studies have been confounded by comparisons using exclusive breast-feeding or ever breast fed as the definition of breast-feeding, leading to samples with mixed breast/bottle feeding in one of the comparison groups. The relationship of breast-feeding to the development of childhood obesity has been studied primarily in cross-sectional or retrospective study designs with differing definitions of breast-feeding, overweight, and obesity. A prospective longitudinal study will investigate the complex relationship between infant feeding patterns and growth trajectories from infancy through adolescence while controlling for relevant environmental and genetic factors. The longitudinal data collection planned for the National Children's Study (NCS) should allow for clearer differentiation of the composition of early infant feeding and its relationship to obesity during childhood.

3.3 Scientific Merit

Current scientific understanding

The relationship between infant breast-feeding and subsequent overweight has been extensively examined. The results of meta-analyses and systematic reviews support a small protective dose response relationship between breast-feeding and childhood obesity (Harder, Bergmann, Kallischnigg, & Plagemann, 2005; Owen, Martin, Whincup, Davey Smith, et al., 2005; Arenz, Ruckerl, Koletzko, & van Kries, 2004) and this association appears to persist from infancy through adulthood (Dewey, 2003; Gillman et al., 2001). Whether this association is causal is not clear. The effect size is small and may be confounded by factors such as socioeconomic status, ethnicity, diet, and physical activity (Dewey, 2003).

Questions remain whether the protective effect, if any, is due to constituents of breast milk, metabolic programming, regulation/control of intake by mother and/or infant, or family lifestyle/environmental programming that are different for breast-fed and formula-fed infants (Dewey, 2003; Bergmann et al., 2003). Existing studies that relied on retrospectively reported breast-feeding histories that define breast-feeding as exclusive breast-feeding or any breast-feeding have not adequately addressed the impact of cumulative breast milk intake (duration plus breast milk as a percentage of

intake). Bogen, Hanusa, & Whitaker (2004) found exclusive breast-feeding for at least 16 weeks or 26 weeks with formula supplementation was associated with lower obesity at age 4 than formula feeding.

If breast-feeding does help prevent subsequent obesity, this would be one of the few protective preventive measures available (Dietz, 2001). The relationship of racial and ethnic differences in infant feeding practices (e.g., approximately 41 percent of white non-Hispanic infants are breast fed at age 6 months [15 percent exclusive breastfeeding], compared with 25 percent of black children [9 percent exclusive breast-feeding] and 42 percent of Hispanic children [14 percent exclusive breast-feeding]) (CDC, 2005b) to subsequent differences in childhood obesity requires more exploration (Grummer-Strawn, Mei, & CDC Nutrition Surveillance, 2004).

There is conflicting evidence concerning the relationship between breast-feeding and insulin resistance. Breast-feeding has been reported as an independent protective factor against type 1 diabetes (Sadauskaite-Kuehne, Ludvigsson, Padaiga, Jasinskiene, & Samuelsson, 2004), but no relationship was found between breast-feeding and HOMA scores (homeostasis model assessment [Matthews et al., 1985]) as a measure of insulin resistance (Lawlor et al., 2005). In a systematic review of breast-feeding influence on the risk of type 2 diabetes, breast-feeding was associated with lower blood glucose and serum insulin concentrations in infancy and the decreased risk of type 2 diabetes in later life (Owen, Martin, Whincup, Smith, et al., 2005). It has been hypothesized that the beneficial effects of breast-feeding may be attributed to the long-chain polyunsaturated fatty acids in breast milk that suppress the production of proinflammatory cytokines, enhance the number of insulin receptors, and decrease insulin resistance (Das, 2002) or to the beneficial effects of adipokines on immune response (Weyermann et al., 2006).

Recent studies suggest the role of leptin found in breast milk is a bioactive substance involved in the regulation of energy intake positively affecting satiety and regulation of feeding frequency and amount. It may prime or set the neuroendocrine regulation of later feeding appetite and metabolism. Leptin has additional effects on immunomodulation, growth, development, and endocrine hormonal regulation (e.g., insulin). Human milk is a source of many nutrient and biological compounds (e.g., IGF-1, insulin, leptin) that regulate food intake, metabolism, and body composition (Locke, 2002; Savino, Costamagna, Prino, Oggero, & Silvestro, 2002). Leptin concentrations and leptin-to-weight ratios are higher in breast-fed than formula-fed infants. Serum leptin concentrations in infants are also positively correlated with maternal body mass indices (BMI) (Savino, Liguori, Oggero, Silvestro, & Miniero, 2006), which is correlated with obesity development (Dubois & Girard, 2006). The role of leptin in the complex nature of regulation of energy homeostatis and body weight requires continued investigation.

Breast-feeding has been associated with reduced systolic blood pressure in children (Lawlor et al., 2005; Martin, Gunnell, & Davey Smith, 2005) with lower cholesterol levels in adolescence (Owen et al., 2002), and insulin resistance in adulthood (Ravelli et al., 2000). It has been hypothesized that the advantages of breast-feeding for cardiovascular health may be due to slower growth in breast-fed than formula-fed babies. “The growth acceleration hypothesis” (Singhal & Lucas, 2004) suggests rapid weight gain in infancy adversely programs the metabolic syndrome, including childhood obesity (Ong et al., 2006; Dennison, Edmunds, Stratton, & Pruzek, 2006).

3.4 Potential for Innovative Research

Assessment of cumulative breast milk intake relative to total intake in the early months of life will help clarify the dose response relationship with childhood growth trajectories. Detailed analysis of feeding records and adiposity measures will examine differences in feeding and growth patterns for race/ethnicity groups. Analysis of sibling pairs will support investigation of gene-environment influences. Complementary analyses of feeding patterns, environmental factors, and biochemical markers will help uncover the complex interaction of variables associated with development of obesity in children.

Evaluation of the significance of early rapid growth for children of different gestational ages and weights will provide evidence for development of guidelines regarding postnatal weight management (Monteiro & Victora, 2005). Biochemical analysis of breast milk and child blood samples will extend the emerging science related to breast milk biochemistry and metabolic/endocrinologic effects on the growing child. The NCS offers the opportunity to distinguish the contributions of genes, gestation, and postnatal nutrition to childhood obesity (Jeffery et al., 2006).

3.5 Feasibility

Data collected through feeding diaries, maternal report, anthropometric assessments, and biologic samples will be used as data sources. Collection of data at multiple intervals during the first year of life will provide data needed to address the study hypotheses.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Breast milk feeding and duration, including exclusive and partial breast-feeding (Labbock & Krasovec, 1990), length of time of breast-feeding (as a surrogate for amount and a measure of self-regulation)
- Composition of breast milk, including leptin and adiponectin concentrations, and fatty acid composition (Ailhaud & Guesnet, 2004)

Primary/child

- Cumulative breast milk exposure in the first year of life relative to total milk/protein intake
- Timing of introduction of non-milk/solid foods
- Total energy intake

4.2 Methods

Primary/maternal

- Interview(s)
- Feeding diaries

Primary/child

- Breast milk sample(s)

4.3 Life Stage

Primary/maternal

- Birth and periodically through age 1

Primary/child

- Birth and periodically through age 1

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Insulin resistance
- Growth and obesity

5.2 Methods

- Insulin resistance: HgbA1C, lipoproteins, fasting glucose and insulin may be obtained in late childhood and adolescence. A glucose challenge test may be implemented in adolescence, though the timing and nature of that test has not been determined. The development of noninvasive measures of blood glucose, such as those based on infrared spectroscopy, measurement of interstitial fluid glucose, or the accumulation of advanced glycation end products in skin may provide additional serial measures of glucose levels as the study progresses.
- Growth and obesity: IGF, adiposity (skin fold thickness and waist circumference), weight, weight for height, and head circumference. Instrumental measures of body composition, such as bioelectric impedance analysis, dual X-ray absorptiometry, or air displacement plethysmography (available as BodPod or PeaPod) may be implemented later in childhood or adolescence but not in early childhood.
- Other: Medical record reviews may provide information regarding clinical diagnoses of diabetes or obesity.

5.3 Life Stage

- Measures of growth and obesity: Starting at birth (or even before, if prenatal ultrasounds are considered) and periodically during the study.
- Measures of glucose metabolism: Blood draw starting at 36-month visit and periodically during the study.

6. Important Confounders, Mediators, and Effect Modifiers

- **Lipid profile:** Increased lipid levels are associated with an increased risk of insulin resistance (McGarry, 2002).
- **Glucokinase mutation:** Glucokinase mutation is associated with increased risk of type 2 diabetes, formerly known as adult-onset diabetes, among children (Kahn, Vicent, & Doria, 1996).
- **Hormone levels such as cortisol, growth hormone, insulin-like growth factors:** Elevated levels of these and other hormones are associated with obesity and insulin resistance in children (Reinehr & Andler, 2004).

- **Parent's body mass indices:** BMI and obesity are associated with certain genetic markers (Maes, Neale, Eaves, & Lindon, 1997). Maternal obesity is associated with obesity in offspring (Hedley et al., 2004) and an additive interaction between maternal prepregnancy BMI and lack of breast-feeding on childhood obesity risk has been reported (Li et al., 2005). In breast-fed infants, serum leptin concentration is positively associated with maternal BMI (Savino et al., 2006). Maternal weight gain in pregnancy should also be evaluated as a potential confounder. Paternal obesity is associated with childhood obesity and may reflect familial patterns of energy intake and expenditure (Scaglioni et al., 2000).

- **Family history of diabetes and obesity:** A family history of diabetes and obesity increases a child's risk of obesity (Maes et al., 1997; U.S. Department of Health and Human Services [HHS], 2005).

- **Lifestyle factors:** Less active lifestyles would increase the risk of obesity and insulin resistance (Hedley et al., 2004).

- **Nutrition:** Poor nutritional and high caloric diet would increase the risk of obesity and insulin resistance (Ogden et al., 2002). Protein intake is higher in bottle-fed babies and may increase the risk of obesity (Scaglioni et al., 2000; Haisma et al., 2003). Early introduction of complementary food is associated with greater infant weight gain (Baker, Michaelsen, Rasmussen, & Sorensen, 2004). Breastfeeding in the first year of life is associated with less restrictive maternal control of feeding and lower energy intake after infancy (Fisher, Birch, Smicilas-Wright, & Picciano, 2000; Taveras et al., 2004).

- **Socioeconomic status and demographics:** Children of lower economic status, ethnic and racial groups (particularly Native Americans, Hispanics, African Americans, and Asians) are at higher risk of obesity and insulin resistance (Liese et al., 2001).

- **Infant weight gain:** Amount (Dubois & Girard, 2006) and rapidity of weight gain in the first 6 months of life (Ravelli et al., 2000) are associated with the risk of childhood obesity.

- **Smoking during pregnancy:** This is associated with childhood obesity (Bergmann et al., 2003; Dubois & Girard, 2006; Liese et al., 2001; Adams, Harvey, & Prince, 2005; Hediger, Overpeck, Kuczmarski, & Ruan, 2001).

7. Power and Sample Size

Based on NHANES 2003-2004 data, 18.8 percent of children ages 6-11 are overweight (i.e., BMI for age at 95th percentile or higher based on CDC growth charts) (Ogden et al., 2006). For the outcome where 18.8 percent of the population is expected to be overweight at age 8 (approximate midpoint of the 6-11 age range) and 61 percent of the population are not breastfed at 6 months (CDC, 2005b), the smallest detectable relative risk for overweight (BMI greather than or equal to the 95th percentile) at age 8 would be 1.08.

The rate of breastfeeding differs by race. The percentage of infants who are breastfed at 6 months is approximately 41 percent for white non-Hispanic infants, 25 percent for black infants, and 42 percent for Hispanic infants (CDC, 2005b). Based on preliminary NCHS data for 2005 (Hamilton, Martin, & Ventura, 2007), 24 percent of births were classified as Hispanic, 14 percent as black non-

Hispanic, and 62 percent as white or other races. The smallest detectable relative risk for overweight at age 8 would be 1.10 for white non-Hispanic children, 1.14 for Hispanic children, and 1.20 for black children in the NCS.

Using 4 percent of 15-year-olds estimated to have metabolic syndrome (based on NHANES III data indicating 4-10 percent of 12-19-year-olds [Cook, Weitzman, Auinger, Nguyen, & Dietz, 2003; DeFerranti et al., 2004]), the smallest odds ratio that can be reliably detected is 1.19 for the entire study sample and 1.21, 1.32, and 1.50 for white non-Hispanic, Hispanic, and black subsamples, respectively.

Assuming a breast-feeding prevalence of 50 percent (in line with estimates from NHANES III) (Fisher et al., 2000), and using the power assumptions noted above, the smallest detectable relative risk for obesity would be 1.05 and for metabolic syndrome would be 1.3.

These sample power calculations were based on simple bivariate comparisons without consideration of interactions, multiple measures, or the clustered survey design, all of which will decrease the power associated with each example.

8. Other Design Issues

- Blood studies, especially fasting, in younger children, will require attention. Obtaining consent for the use of DNA may be an issue.
- Addressing this hypothesis based on obesity and insulin resistance measures at later life stages may be adversely impacted by attrition of study subjects.

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FIBER, WHOLE GRAINS, HIGH GLYCEMIC INDEX AND OBESITY AND INSULIN RESISTANCE

1. Meta Hypothesis

During childhood, consumption of a high glycemic load diet is associated with obesity and subsequent insulin resistance.

2. Specific Hypotheses

1. Consumption of diets with high glycemic load is associated with childhood overweight and obesity independent of total energy consumption and expenditure.

2. In addition to its contribution to a diet's glycemic index, dietary soluble fiber modifies any relationship between glycemic load and obesity or inflammation.

3. Genetically determined underexpression of the GLUT4 gene, in either the GLUT4 gene itself or other genes influencing GLUT4 expression (e.g., D2, CASQ1), strengthens any association between glycemic load and subsequent obesity or insulin resistance.

4. Consumption of a high glycemic load diet is associated with elevated systemic markers of oxidative stress (e.g., isoprostanes) and inflammation (e.g., CRP, IL-6).

5. Polymorphisms in the GST family of genes resulting in decreased glutathione-S-transferase activity strengthen the association between glycemic load and oxidative stress and inflammation.

6. Elevated systemic markers of oxidative stress and inflammation associated with dietary glycemic load in childhood are also associated with risk of insulin resistance in adolescence, independent of overweight or obesity.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

In the United States, obesity among adults and overweight among children has steadily increased during the past three decades (Ogden et al., 2006; Ogden et al., 2004; Flegal & Troiano, 2000). Approximately 4 percent of children ages 6-11 were overweight in the early 1970s compared with 19 percent in 2003-2004. Being overweight during childhood is related to obesity in adulthood (Serdula et al., 1993) and the attendant health risks, including type 2 diabetes mellitus (Freedman, Khan, Dietz, Srinivasan, & Berenson, 2001; Sinha et al., 2002). Prevalence estimates of pediatric type 2 diabetes in the United States are limited and varied due to the diverse populations and methods used in the studies and the limitations of small sample sizes (Fagot-Campagna, 2000; Fagot-Campagna, Saadine, Flegal, & Beckles, 2001). However, among young U.S. adults age 20-39 in 1999-2002, the combined incidence of diagnosed and undiagnosed (based on a fasting blood glucose) diabetes was 2.3 percent. This represented an increase in diabetes of approximately 50 percent since 1988-1994 (Cowie et al., 2006).

3.2 Justification for a Large Prospective Longitudinal Study

The National Children's Study (NCS) is positioned to advance the understanding of the relationship between dietary glycemic load, obesity, and type 2 diabetes. The majority of studies referenced above were based on an adult population and were either cross-sectional or had relatively limited follow-up. The potential influence of childhood diets on both contemporary and subsequent pathophysiology is unknown. This is true for elucidation of the potential etiology of type 2 diabetes. A longitudinal study is necessary to capture the temporal sequence of glycemic load, overweight or obesity, oxidative stress and inflammation, and insulin resistance or diabetes. Understanding those relationships will enable targeting of interventions early in life that will help prevent the development of obesity, type 2 diabetes, and associated cardiovascular pathologies later in life.

3.3 Scientific Merit

Understanding the underlying causes driving the national trends in childhood obesity, diabetes, and related pathology is crucial to guide prevention and treatment. An obvious explanation for the increase in obesity is an increase in the imbalance between energy intake and energy expenditure. Other factors associated with diet may contribute to the increase in obesity and diabetes in important, though more subtle, ways. One of these factors may be the increasing consumption of processed foods and the differences in the metabolic characteristics of those foods compared with previous diets. In particular, the increased bioavailability of carbohydrates found in processed foods has the potential to contribute to obesity, to type 2 diabetes indirectly through obesity, and perhaps directly to type 2 diabetes independent of fat accumulation.

One commonly used measure of a food's glucose availability is the glycemic index, a comparison of the rise in blood glucose after consumption of a specified amount of carbohydrate-containing food to the rise of glucose after the consumption of the same amount of a "control" carbohydrate (generally white bread or white rice). A glycemic index for a mixed meal can be calculated by taking the weighted average of the glycemic index of each carbohydrate-containing food consumed during the meal. The glycemic load of a serving of food is the product of the glycemic index and the available carbohydrate in that serving, and therefore may provide a better measure of a diet's metabolic impact. Other dietary components, most notably soluble (or viscous) fiber, directly contribute to a food's glycemic load or may influence carbohydrate metabolism and subsequent effects which will be discussed in Section 6 of this hypothesis.

Foods with a relatively high glycemic index, by definition, result in relatively high blood glucose levels in the immediate postprandial period. The metabolic influence of a high glycemic index meal transcends the direct effects on postprandial blood glucose and insulin release. The exaggerated insulin response to the initial glucose surge results in an overcorrection and leads to a relative hypoglycemia shortly after the meal. In contrast, consumption of a low glycemic index meal invokes an attenuated insulin response and relatively stable serum glucose levels and low fatty acids (Frost & Dornhorst, 2005; Ludwig, 2002).

In rats, consumption of isocaloric high glycemic and low glycemic loads results in increased weight and increased adiposity among animals fed a high glycemic index diet (Pawlak, Kushner, & Ludwig, 2004). In addition, evidence suggests that, compared to an isocaloric low glycemic index meal, consumption of a high glycemic index meal results in less satiety and leads to increased caloric intake following that meal, at least in obese adolescents (Ludwig et al., 1999). Thus, in comparison to diets with a low glycemic load, high glycemic load diets seem to result in increased weight gain and fat mass on a calorie-for-calorie basis and may stimulate increased caloric intake and poses a double menace.

In addition to the relationships with weight and adiposity, high glycemic load diets have been associated with increased insulin resistance and, possibly, type 2 diabetes. The results of large-scale epidemiologic studies examining the association between glycemic index or load and diabetes are inconsistent (Liese et al., 2005; Schulze et al., 2004, for instance). However, experimental evidence in animals and humans suggests a relationship between high glycemic load, insulin resistance, and (among rats) disruption of pancreatic islet structure (Frost & Dornhorst, 2005; Ludwig, 2002). A number of potential mechanisms that may underlay a relationship between glycemic index and insulin resistance have been proposed, such as the direct effect of fatty acids on insulin metabolism or the down-regulation of insulin receptors in a high-insulin environment. Another possible explanation that may be related to the etiology of insulin resistance and the subsequent pathophysiology associated with diabetes is the association of high glycemic load with evidence of oxidative stress and inflammation.

A body of literature has examined the relationship between diabetes and systemic markers of both oxidative stress and inflammation. The underlying rationale for most of those studies is that hyperglycemia results in oxidative damage and stress. The physiologic response to that stress is inflammation, which is implicated in the numerous vascular pathologies associated with diabetes (Hu et al., 2006; Monnier, 2006; Qi & Hu, 2007; Sorenson, Raben, Stender, & Astrup, 2005; Esposito et al., 2002). However, some authors have suggested the oxidative stress and inflammation associated with diabetes is not a result of the condition but a potential precursor and cause of diabetes or insulin resistance (Duncan & Ines Schmidt, 2006; Browning & Jebb, 2006).

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/child

- Dietary intake
- Glycemic index and load

4.2 Methods

Assessment of glycemic index and glycemic load in the NCS will be dependent on three factors: accurate measurement of dietary intake, translation of foods into a glycemic index and load, and a sufficient number of dietary assessments throughout childhood to capture temporal changes in diet. The third factor is the simplest to assure. Dietary assessments, in the form of food frequency questionnaires and dietary recalls, will occur repeatedly throughout infancy and childhood. Early in life, they will be completed as proxy questionnaires by the mother or other caregiver. As the children get older, they will report on their own diet. Exact tools have not been specified for those collections, and their content and mode of administration will likely evolve between now and the time they are instituted.

Limitations in the accuracy of dietary intake by interview measures, whether food frequency, recall, or diary, are well-recognized (Trabulsi & Schoeller, 2001; Livingstone et al., 1992). There are systematic errors in the reporting of total energy intake and specific macro- and micro-nutrient intake. For the purposes of this hypothesis, the reporting of consumption of specific foods will enable calculation of a glycemic index, even if the misreporting of amounts of foods compromises accurate calculation of glycemic load (Hui & Nelson, 2006). Additional methods of dietary assessment in the NCS, such as the collection of duplicate diets of the children's meals or the digital photography of certain meals, is under consideration.

Calculation of glycemic index and glycemic load from the reported dietary intake involves merging the dietary data with a published database containing the glycemic measures (Foster-Powell, Holt, & Brand-Miller, 2002). While not all foods may be found within a specific database, databases can be combined to achieve reasonable coverage. An inherent difficulty in assigning glycemic index or load to an individual's diet is the variability between people and within a person in response to a carbohydrate-containing food. The glycemic indices in published databases are generally based on average response to a particular food in a study population. However, not all people will have the same metabolic response to the same food, and an individual's response to a certain food may not be consistent through time (Frost & Dornhorst, 2005).

Overall, while the ability to calculate a precise glycemic index for each individual's diet is limited due to difficulties in accurately assessing diet and then assigning glycemic values, the longitudinal nature and frequent assessments included in the NCS should allow for sufficient discrimination to enable accurate categorization for epidemiologic analysis.

4.3 Life Stage

- From birth and periodically throughout course of Study

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

5.2 Methods

- Physical growth (height, weight, circumferential measures, etc.) and body composition
- Assessment of glucose and insulin metabolism (biological/blood samples, e.g., HgbA1c, blood glucose levels, and fasting blood glucose levels in older children, when appropriate)

5.3 Life Stage

- Measures of growth and adiposity: Starting at birth (or even before, if prenatal ultrasounds are available to analyze for such data) and periodically during the Study.
- Measures of glucose metabolism: Blood draw starting at 36-month visit and periodically throughout the Study.

6. Important Confounders, Mediators, and Effect Modifiers

- **Dietary fiber:** Reduced dietary fiber intake, particularly soluble (or viscous) fiber, has, like high glycemic index, been associated with obesity and type 2 diabetes (Johnson, 2005). Some of this association is likely due to the relationship between soluble fiber and glycemic index, a carbohydrate-containing food with a relatively large amount of soluble fiber will have a lower glycemic index than a food with similar carbohydrate composition but less fiber. However, there is also evidence that soluble fiber may have additional influence on both lipid and glucose metabolism through inhibition of reabsorption of bile salts and the fermentation of undigested

carbohydrate in the colon. Assessment of dietary fiber intake is subject to the same limitations as glycemic index, as discussed in Section 4. The specification of soluble fiber content, rather than total fiber content, of specific foods is difficult given currently available databases.

- **Genetic influences and gene-environment interactions:** The genetic contributions to obesity and diabetes are manifold. Two potential areas of interest for this hypothesis may be the influence of the glucose transporter family of genes on the development of obesity and insulin resistance, and the effect of reduced activity of the Glutathione-S-Transferase (GST) family of genes on glycemia-related inflammation.

- **Glucose transport:** Cellular access to and metabolism of glucose is largely regulated by the GLUT family of genes. Decreased activity of GLUT4, the insulin-regulated glucose transporter, is associated with insulin resistance in animal models. The regulation of GLUT4 is complex, depending on glucose and insulin levels, as well the activity of other genes. To date, no specific polymorphisms of the GLUT4 gene have been identified that are directly associated with decreased function (Friedel et al., 2002). However, polymorphisms in other genes, such as those related to thyroid hormone or calcium metabolism, have been identified that result in decreased expression of GLUT4 and thus are associated with insulin resistance (Canani et al., 2005; Fu et al., 2004).

- **GST:** The GST family of genes is responsible for the conjugation of glutathione to potentially toxic compounds including reactive oxidative byproducts. Decreased activity of the GST enzymes is thus associated with an increase in oxidative stress-related inflammation. Well-described polymorphisms resulting in decreased activity of GST have been related to asthma and some cancers. Those polymorphisms also may be associated with an increased risk of glycemia-induced inflammation and subsequent diabetes or insulin resistance (Bolt & Thier, 2006).

- Other potential confounders:

  - Parental body habitus.

  - Parental or family history of insulin resistance or type 2 diabetes.

  - Exercise or energy expenditure, and

  - Demographic factors such as socio-economic status, race, and ethnicity.

7. Power and Sample Size

The prevalence of overweight children among children in the United States is approximately 15 percent, providing an opportunity to explore subpopulation differences in the relationship between diet and body composition. While the prevalence of insulin resistance among U.S. children is essentially unknown, there appear to be dramatic differences in subpopulations, with estimates from 0.1 to 5 percent (Fagot-Campagna et al., 2000). In early adulthood the prevalence increases to approximately 2 percent overall with variation by racial or ethnic subgroup.

Similarly, the distribution of dietary glycemic index or glycemic load among U.S. children has not been well described. However, if one assumes a 20 percent exposure rate of high glycemic index (the upper quintile compared with the rest of the population), a prevalence of insulin resistance of 0.50 percent in the "low" glycemic index population, and an alpha error of 0.05 percent and beta error of 0.20 percent, the full study population of 100,000 can be used to uncover a relative risk of approximately 1.4 without accounting for the clustered sample or multiple measurements.

8. Other Design Issues

- Blood studies, especially fasting, in any minor or younger children will require attention. Obtaining consent for the use of DNA may be an issue.

- Addressing this hypothesis based on obesity and insulin resistance measures at later life stages may be adversely impacted by attrition of study subjects.

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GENETICS, ENVIRONMENTAL EXPOSURES, AND TYPE I DIABETES

1. Meta Hypothesis

The development of beta cell autoantibodies and subsequent type 1 diabetes is causally associated with the interaction between genetic susceptibility, early exposure to viral infections, and early exposure to cow's milk protein or other dietary components.

2. Specific Hypotheses

1. The development of beta cell autoantibodies and subsequent type 1 diabetes is causally associated with:

    1.1 Genetic susceptibility (e.g., HLA D3 or D4 haplotypes) (Concannon et al., 1998; Pugliese, 1999; Rewers et al., 1996; Caillat-Zucman et al., 1992);

    1.2 Delayed exposure to Coxsakie (enterovirus) or other viral infections until later childhood or adolescence (Viskari et al., 2004; Skarsvik et al., 2006; Laugesen & Elliott, 2003);

    1.3 Early exposure to cow's milk protein (the timing and quantity of A1 β-casein consumption) or other dietary components (Schatz, Rogers, & Brouhard, 1996); and

    1.4 Interactions between these factors (Harris, 1995; Charatan, 2002; WHO Multinational Project for Childhood Diabetes Group, 1991; Green & Patterson, 2001; Kondrashova et al., 2005).

2. Insulin-producing β-cells, in the event of inhibited interferon production, are highly susceptible to acute infection by Coxsackie virus, resulting in type 1 diabetes.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Of all chronic diseases affecting children, type 1 diabetes is one of the most common, with prevalence rates at approximately one in every 5,000 children in the United States (SEARCH for Diabetes in Youth Study Group, 2006). The incidence of the disorder is increasing worldwide at an estimated rate of 3 percent per year (Onkamo, Vaananen, Karvonen, & Tuomilehto, 1999), particularly among those younger than 5 and ethnic minorities. About 12.6 percent of the National Health and Nutrition Examination Survey (NHANES) sample had high fasting glucose or medication use indicating treatment for abnormal glucose metabolism. Figures were highest in Mexican Americans and higher in African Americans than whites, according to NHANES data from 1988-94 (Ford, Giles, & Dietz, 2002). Estimates of prevalence of impair glucose tolerance among children, by race, have been shown to be increasing, and are similar to the distribution of impaired glucose tolerance among adults. (Sinha et al, 2002)

Economic and social burden

Studies describing the economic costs of diabetes often consider the direct or medical costs of the disease, but less frequently examine the indirect costs, such as the value assigned to morbidity, disability, and premature mortality associated with type 1 diabetes.

Important medical costs relate to the daily management of the disease and the treatment of late-stage complications. The annual costs of type 1 diabetes typically range between $1,500 per person for the standard insulin regimens to nearly $6,000 for insulin pump protocols (Ford, Giles, & Dietz, 2002). Out-of-pocket health care costs for families with type 1 diabetes in the United States typically exceed $1,000 per year (Marrosu et al., 2004). These costs increase substantially after long-term complications develop. In 2002, the direct medical and indirect costs attributed to diabetes were estimated at $132 billion (Hogan, Dall, & Nikolov, 2003).

3.2 Justification for a Large Prospective Longitudinal Study

While Type 1 diabetes is a rare disease within the entire U.S. populations ages 21 years and younger with an incidence of about 20 per 100,000 per year, among chronic diseases affecting children, it is one of the most prevalent (CDC, 2005).. To study the etiology of such a rare condition, a large sample of high-risk individuals is needed. The National Children's Study (NCS) as planned with 100,000 births recruited and followed to age 21 years and storage of biological specimens, will likely include at least 100 incident cases, allowing nested case-control studies (and potentially case-cohort adjunct studies) to test the proposed hypotheses regarding gene-environment interactions in the etiology of type 1 diabetes. The database generated by the Study will provide an invaluable resource for hypothesis testing and developing new treatments and strategies for preventing type 1 diabetes. In addition, the nationally representative sample to be recruited for the Study will allow exploration of geographic and ethnic differences within the United States in the prevalence and etiology of type 1 diabetes.

3.3 Scientific Merit

Theory supporting hypothesis

The etiology of type 1 diabetes remains unclear. Epidemiologic patterns, including the dramatic geographic variations in type 1 diabetes incidence rates, variation in risk by ethnicity, the peak age at onset of puberty, and the more frequent diagnosis of the disease during the winter suggest viruses, nutrition, and socioeconomic factors are involved (Harris, 1995). Potential environmental risk factors have been investigated in numerous populations, but the studies yielded conflicting results. This has been due, in part, to a failure to account for disease susceptibility genes. Although genome screens found evidence of linkage to more than 15 potential genes, the primary locus of susceptibility (IDDM1) is located in the HLA region of chromosome 6 (Concannon et al., 1998; Mein et al., 1998). Estimated relative (odds ratios range from 10-100) and absolute risks (approximately 3-6 percent through age 20) associated with high-risk HLA genotypes have been studied in many populations (Laugesen & Elliott, 2003).

Current scientific understanding

Although lifelong insulin therapy is the only treatment for type 1 diabetes, there are currently several large clinical trials designed to evaluate a variety of approaches for primary (i.e., avoidance of cow's milk formula) and secondary disease prevention (i.e., high doses of nicotinamide, oral/nasal insulin) (Schatz et al., 1996). Newborns, children, and young adults who have a first-degree relative with type 1 diabetes are being screened for early preclinical markers (i.e., beta cell autoantibodies) and high-

risk HLA susceptibility alleles. Those who are positive for these preclinical markers are eligible for randomization. However, approximately 90 percent of individuals who develop type 1 diabetes have no family history of the disease and are not eligible for these trials (Rewers et al., 1996). For the public health impact of any of these interventions to be realized, they must be based on the general population and not high-risk family members. As a result, several natural history studies are following newborns who have high-risk HLA susceptibility alleles (Rewers et al., 1996; Charatan, 2002). Only half of the children who eventually develop type 1 diabetes carry these genes. Thus, approximately half of the remaining future incident cases will occur among those who screen negative for high-risk HLA genotypes. These individuals are being excluded from follow-up.

Since 2000, a group of European investigators have explored the hypothesis that type 1 diabetes has an epidemiology similar to polio because the populations most severely impacted by the chronic disease are children. There has been an increase in the incidence of type 1 diabetes throughout Europe in recent decades of a magnitude that can only be explained by environmental changes or gene-environment interaction (Green & Patterson, 2001). The multinational group has shown there is an inverse relationship between the background rate of enterovirus infections in a population and the incidence of type 1 diabetes (Viskari et al., 2004) and a six-fold gradient in the incidence of type 1 diabetes between Finland and the immediately adjacent Karelia area of Russia. The Finland and Karelia populations have a similar frequency of chromosome 6 high-risk HLA haplotypes (Kondrashova et al., 2005). The same group has also demonstrated there is no difference in the frequency of beta-cell auto-antibodies between Finnish and Karelian school children (Kondrashova et al., 2007).

Findings reported by the group are what would be predicted by the polio model -populations with the same genetic background are more or less at risk for type 1 diabetes depending on the age at which Coxsackie B (enterovirus) infections occur.

The studies to date have used ecological or case-control designs. The opportunity to carry out longitudinal studies would provide real promise for further exploration of this hypothesis, which if supported, would suggest opportunities for prevention.

3.4 Potential for Innovative Research

The study offers an unprecedented opportunity to complement the investigations of high-risk children by evaluating different disease susceptibility genes and environmental exposures. For example, there is evidence to suggest the contribution of HLA susceptibility alleles varies by age (Kondrashova et al., 2007). The largest relative and absolute risk estimates for children who carry high-risk genotypes are strongest for children younger than 5. The contribution of the same genotypes is much less dramatic among children with an older age at onset (older than 10). This suggests that around the time of puberty, loci other than those in the HLA region and/or environmental exposures may play a more important etiologic role. Thus, the evaluation of gene-environmental interactions in the development of type 1 diabetes must be evaluated among children with and without high-risk HLA susceptibility alleles.

3.5 Feasibility

Testing of the proposed hypotheses will require systematic collection and storage of information on family history of type 1 diabetes, child and family genetics, infant and child nutritional histories, longitudinal medical history and biomarkers of viral infections, and biomarkers of immune responses and onset of insulin resistance and diabetes mellitus. Data required to test this hypothesis and suggested specific hypotheses are included in the currently proposed Study protocol.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/child

- Measures of diabetes susceptibility:
  - Genetic markers (e.g., insulin gene VNTR-insulin-dependent diabetes mellitus gene)
  - Family history of type 1 diabetes and other autoimmune disorders
- Environmental exposures:
  - Viruses (particularly Coxsackie virus B4)
  - Infant/childhood nutrition (particularly cow's milk and A1 β-casein content)
  - Pesticides or other chemical exposures

4.2 Methods

Primary/child

- Blood test
- Questionnaire
- Periodic environmental sampling
- Medical record review

4.3 Life Stage

Primary/child

- Prenatal and years 1, 2, 5, 10, 15, and 20

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary/child

- Beta cell and other autoantibodies
- Manifestation of type 1 diabetes (e.g., average blood sugar level [HgbA1C], lipid profile, serum insulin levels, cortisol)

5.2 Methods

- Blood sample
- Physical exam
- Medical record review
- Questionnaire

5.3 Life Stage

- Periodically throughout participation

6. Important Confounders, Mediators, and Effect Modifiers

- **Unknown environmental factors:** Increase in risk associated with type 1 diabetes not sufficiently explained by genetic susceptibilities; most likely significant contribution by environmental factors (Hogan et al., 2003).
- **Family, medical history factors:** Several genetic loci implicated in risk for T1D, e.g., CTLA4 exon 1 A49G polymorphism; genotype higher in patients (12.6 percent) than controls (4.2 percent); may be family association ($p = 0.0229$) (Levin et al., 2004; Pitkaniemi, Onkama, Tuomilehto, & Arjas, 2004; Ziegler & Ziegler, 1989; Murao et al., 2004).
- **Obesity:** Lower risk for transient ischemic dilation (TID) associated with obesity even among genetically susceptible children (Zalloua et al., 2004).

7. Power and Sample Size

With an incidence of about 20 per 100,000 per year in U.S. populations ages 21 and younger, the study sample will likely yield at least 100 incident cases of type 1 diabetes. This number would yield more than 90 percent power, using a nested case-control design, to detect odds ratios of 2.5 or greater for exposures that affect approximately 10 percent of the general population. Less frequent exposures, such as the HLA haplotypes associated with type 1 diabetes, which may occur in only 2 percent of the population, may not be identified as significant predictors of type 1 diabetes unless their association is at least four- or five-fold.

The sample power calculations are based on simple bivariate comparisons without consideration of interactions, multiple measures, or the clustered survey design, all of which will decrease the power associated with each example. Most especially, the interaction between specific genotypic factors and multiple environmental exposures will need to be considered when estimating the lowest detectable odds ratio.

8. Other Design Issues

- **Ethical/burden considerations:** There may be ethical considerations associated with genetic tests, but such considerations underlie the overall study and would not be unique to this specific hypothesis. Collecting blood samples to obtain genetic information on parents and grandparents would make the study design complex.

For nongenetic factors, the Study will need to have a formal strategy and process to effectively communicate the results of physiological and biochemical measures to the child's parents and to a responsible health care provider. The Study also will need to have a formal strategy and process to effectively communicate the results of environmental monitoring to the child's parents along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings, as state and/or federal laws require.

- **Cost/complexity of data collection:** Case/parent trios may be considered as a possible sampling unit.

- **Cost of sample analysis:** The use of exposure- and outcome-dependent sampling from stored or archived samples would provide efficiency (Green, Casabonne, & Newton, 2004).

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REPEATED MILD TRAUMATIC BRAIN INJURY AND NEUROCOGNITIVE DEVELOPMENT

1. Meta Hypothesis

Repeated mild traumatic brain injury (rMTBI) has a cumulative adverse effect on neurocognitive development.

2. Specific Hypotheses

1. Although most patients with a single mild traumatic brain injury (MTBI) appear to recover completely and have no detectible brain abnormalities, they are susceptible to worse outcomes with subsequent rMTBIs. Specifically, with rMTBI there is dysfunction in neurocognitive development associated with brain pathology that progresses through time. These changes are more pronounced with each successive MTBI.

2. Those participants with a single MTBI are at greater risk for a subsequent or recurrent MTBI than those children who have yet to experience one (Swaine et al., 2007).

3. Background and Justification

MTBIs, also known as "concussions," are a silent epidemic, affecting 1.1 million patients each year in the United States (Centers for Disease Control and Prevention [CDC], National Center for Injury Prevention and Control, n.d.; Centers for Disease Control and Prevention [CDC], 2003; Langlois, Rutland-Brown, & Tomas, 2004). In current practice, a patient with a MTBI is defined as a person who has had a traumatically induced disruption of brain function as manifested by either a short loss of consciousness, memory dysfunction, or a Glasgow Coma Score (GCS) of 13-15 (Carroll, Cassidy, Holm, et al., 2004; Teasdale & Jennett, 1974). MTBI patients evaluated in hospital emergency departments are typically discharged to home after an unremarkable computerized tomography. However, approximately 15 percent of these patients are at risk of suffering persisting neurocognitive dysfunction such as memory dysfunction or difficulties with concentration (Anderson et al., 2001; Belanger et al., 2005; Carroll et al., 2004; Hawley et al., 2002; Karaus & McArthur, 1996; Langlois, Rutland-Brown, & Tomas, 2004; McAlllister et al., 1999). Furthermore, all MTBI patients may be at risk for a disproportionate degree of morbidity with a repeated MTBI in close temporal sequence. Notably, it is thought that a large percentage of these patients suffer persisting cognitive dysfunction (Cantu, 1998; Guskiewicz et al., 2003; Kelly & Rosenberg, 1998). Though the mechanisms behind "second impact syndrome" (Cantu, 1998) are not well understood, it raises questions about cumulative effects of rMTBIs, especially among children. The classic setting for a repeat TBI is athletic competition. For example, an estimated 20 percent of high school football players sustain at least one concussion each season (Cantu, 1998). College football players with more than one concussion have persisting cognitive dysfunction (Guskiewicz et al., 2003). Outside athletic competition, it is likely that some children sustain rMTBIs during normal activities of childhood. Indeed, although the cause is not clear, a history of TBIs is a risk factor for incurring a subsequent TBI (CDC, National Center for Injury Prevention and Control, n.d.; Centers for Disease Control and Prevention [CDC], 2000, 2003; Ashwal, Holshouser, & Tong, 2006; Taylor et al., 2002).

There is little research on the outcomes of rMTBIs in children. Most of the literature that addresses TBIs in the pediatric population focuses on the sequelae of an isolated moderate or severe TBI (Gordon, 2006; Salcido & Costich, 1992).

Characterization of the long-term outcome of children with rMTBIs would provide a platform to pursue preventive interventions, help guide patient management, and potentially reveal therapeutic targets.

3.1 Public Health Importance

Prevalence/incidence

According to the Centers for Disease Control (http://www.cdc.gov/ncipc/tbi/TBI.htm), there are 1.4 million patients of all ages who sustain TBIs in the United States each year. Approximately 1.1 million of these patients are MTBI patients who are typically treated and released from an emergency department (Kraus & McArthur, 1996; Langlois, Rutland-Brown, & Tomas, 2004; CDC, 2003; Thurman et al., 1999). Among children ages 0-14 years, it is estimated that TBIs result in 2,685 deaths, 37,000 hospitalizations, and 435,000 emergency department visits annually (CDC, National Center for Injury Prevention and Control, n.d.; CDC, 2003; Kraus & McArthur, 1996; Langlois, Rutland-Brown, & Tomas, 2004) Overall, these numbers are thought to vastly underestimate the prevalence of pediatric TBIs because many cases of MTBIs are not seen in an emergency department. The actual incidence of rMTBIs among children is not known. However, children and young adults make up the majority of TBI patients (CDC, 2003; Schneier et al., 2006) and patients with at least one TBI are at increased risk of subsequent injury (Anderson et al., 2000, 2001; Bijur, Haslum, & Golding, 1996; Cantu, 1998; Guskiewicz et al., 2003).

Economic and/or social burden

Direct medical costs and indirect costs such as lost productivity due to a TBI totaled an estimated $60 billion in the United States in 2000 (CDC, National Center for Injury Prevention and Control., n.d.). This figure is an underestimate because it does not include non-hospitalized persons, the costs of lost productivity, diminished quality of life, and the indirect costs borne by family members and friends who care for persons with a MTBI (CDC, 2003). For example, more than a third of prospective MTBI patients had not returned to work within three months after being injured (Boake et al., 2005; Levin et al., 1987, 2001, 2005; McAllister et al., 1999). No data exists for the impact of rMTBIs in children on society. Nonetheless, persistently reduced cognitive abilities and developmental problems in these patients would obviously represent a substantial emotional and financial burden.

3.2 Justification for a Large Prospective Longitudinal Study

The fact that little is known about the impact of rMTBIs in children is a compelling justification for this study. As above, many patients with a MTBI suffer persisting neurocognitive dysfunction and an initial MTBI predisposes patients to a worsened outcome with repeat exposures. For characterization of the epidemiology and impact of rMTBI, a large prospective longitudinal study is imperative. Success in this effort would guide preventive interventions, such as new guidelines for participation in sporting events. In addition, an enhanced understanding of rMTBI would help guide patient management such as diagnostic evaluations and rehabilitation. Finally, identification of pathologies associated with rMTBI could reveal therapeutic targets.

3.3 Scientific Merit

Numerous studies have documented the long term and often devastating consequences of severe TBIs (Anderson et al, 2000). More recent attention has focused on potential long-term sequelae of moderate and mild traumatic brain injuries and the effects of repetitive insults. Among the outcomes of MTBIs reported in adults are postconcussal syndrome (dizziness, nervousness, irritability, difficulty

concentrating) and information processing abilities (Carlsson, Svardsudd, & Welin, 1987). Some signs and symptoms may not appear until days to weeks following the concussion. At least two studies have suggested that the effects of multiple mild injuries in adults are cumulative (Jordan & Zimmerman, 1990; Gronwall, Wrightson, 1975). Furthermore, studies have documented a period of increased vulnerability following an initial insult. This "second impact syndrome" describes the catastrophic or even fatal outcomes that can occur when repeat brain injuries are sustained in a short time and may be related to metabolic changes in the brain and changes in brain blood flow that can persist for some time following an initial impact (Bergsneider et al., 2001). Some authors believe that the second impact syndrome is due to an un-diagnosed brain injury, which is then followed by another blow to the head. However, the effects of MTBIs on recurring head injuries in young children are not known. One of the reasons for this lack of information is that the signs of postconcussal syndrome may be difficult to detect in young children. In addition, few controlled studies have been done on MTBIs in children, and none have been done in children younger than age 5.

This lack of knowledge is of particular concern because mild head injuries are more common in children than in adults (Luerssen, 1988). Additionally, very young children may be more susceptible to persistent deficits secondary to TBIs than older children. Relative to their older counterparts, younger children had a poorer recovery of both IQ (Anderson et al., 2000) and of discourse function (Chapman & McKinnon, 2000) following a TBI. However, some animal studies have suggested that the immature brain may actually confer some protection (Bergsneider et al., 2001). What is clear is that outcomes depend both on the severity of the injury and on the maturational state of the brain (Bergsneider et al., 2001).

Studies have shown that persons who have had at least one TBI are at increased risk for a subsequent TBI (Salcido & Costich, 1992; Annegers, Grabow, Kurland, & Laws, 1980). Only a limited number of studies have examined the cumulative effects of mild or moderate head injuries in children (Bijur et al., 1996). Results of the study performed by Bijur et al. indicated that children who had sustained multiple mild head injuries did not differ in cognitive outcomes from matched children who had sustained multiple non-head injuries. In this study subjects were identified after the injury occurred, so pre-morbid assessment of their neurocognitive ability was unavailable for comparison. Instead, peers were used as controls for neuro-cognitive assessment. Further, the study had a small number (278) of children with more than one head injury, and the study also did not focus on very young children.

3.4 Potential for Innovative Research

It is anticipated that as the study progresses, the use of repeated standard neurocognitive outcome measures (including the potential use of surrogate biomarkers of injury) will allow elucidation of unknown features of rMTBI and the impact of rMTBI on trajectories of development. Elucidation of neuropathological mechanisms of brain injury from mild head trauma thorough neuroimaging techniques would enhance understanding of the pathophysiology of this exposure. Such measure are not feasible for the entire cohort but may be possible by internal adjunct studies on a sub-sample of individuals. In addition, these findings may reveal potential mechanisms that lead to a lowered threshold for morbidity with repeated injuries. Experimental confirmation of these mechanisms could lead to the development of novel therapeutic strategies to mitigate the exacerbated effects of rMTBI.

3.5 Feasibility

A standard battery of neuropsychological measures already exists that can detect neurocognitive dysfunction in single TBI and rMTBI. Collectively, several previous studies and a recent expert working group has provided a report to the CDC with detailed methodology for assessing

outcomes in children with TBI (CDC, 2000; Bijur et al., 1996; Gale et al., 2005; Gordon, 2006; Guskiewiz et al., 2003; Hawley et al., 2002; Inglese et al., 2005; Taylor et al., 1999, 2002).

Conventional brain imaging fails to identify changes in MTBI that are otherwise identified with histopathology (Adams et al., 1982; Ashwal et al., 2006; Bigler, 1999; Wallesch et al., 2001). New neuroimaging techniques have identified brain signal changes after MTBI suggestive of pathology, such as diffuse axonal injury and change in the volume of the brain (Adams et al., 1982; Arfanakis et al., 2002; Ashwal et al., 2006; Inglese et al., 2005; Wilde et al., 2005, 2006). It is anticipated that these changes will become more overt with repeated injuries. These advanced neuroimaging techniques include magnetization transfer imaging, diffusion-tensor imaging, susceptibility weighted imaging and brain volumetry (Adams et al., 1982; Arfanakis et al., 2002; Gale et al., 2005; Inglese et al., 2005; Levin et al., 2000; Tong et al., 2004; Wilde, Chu et al., 2006; Wilde, Hunter et al., 2005). Notably, most major pediatric centers have capabilities to perform these advanced neuroimaging techniques, and could be applied to an exposed sub-group as an internal adjunct study.

As a first diagnostic step, it would be ideal to have a simple blood test to rapidly identify brain pathology in rMTBI (Begaz et al., 2006; Qureshi, 2002; Rosen et al., 1998). Although there are no current conventional tests for surrogate markers of TBI in the serum, many promising approaches are currently in development and are anticipated to be available well within the study period (Begaz et al., 2006; Oureshi, 2002; Rosen et al., 1998).

Collectively, these measures can noninvasively detect the progression of neurocognitive and neuropathologic changes after rMTBI.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/child

- Repeated TBI (to be compared to patients with a single TBI and patients with no history of TBI).

4.2 Methods

Primary/child

- Interviews/questionnaires
- Medical record review
- Advanced neuroimaging for a targeted sub-group as an adjunct study if possible
- Evaluation of serum surrogate markers of brain pathology
- Evaluation of behavioral, neurological and developmental surrogate markers

4.3 Life Stage

Primary/child

- After birth through 21 years

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary

- Neurocognitive development: Behavioral, neurological and developmental outcomes.

5.2 Methods

Primary

- Examinations including neuropsychological, cognitive, and behavioral tests
- Interviews/questionnaires with parents on child behavior
- School records review (grades/performance/behavior)
- Medical record review
- Possible advanced neuroimaging for targeted subgroup as an adjunct study

5.3 Life Stage

Primary

- Periodically at all intervals after birth

6. Important Confounders, Mediators, and Effect Modifiers

- **Demographic variables:** Age, gender, and ethnicity may influence social acceptance of physical aggression of children or parental physical discipline of the child.
- **SES:** Children of disadvantaged backgrounds are at risk for poorer recovery from TBI (Taylor et al., 1999, 2002).
- **Risk-taking assessment:** Children with higher levels of risk-taking attitudes are more likely to sustain a second TBI. (Anderson et al., 2000)
- **Media influences:** Frequency and content of television viewing and video and computer use may impact a child's behavior and risk of injury.
- **Neighborhood characteristics:** Geographic area of residence as well as neighborhood characteristics and school environments put children at risk for poorer recovery from TBI. (Taylor, 2002)

- **Gene-environment interactions:** Parenting effects may be confounded with shared genetic tendencies toward risk taking and injury.

- **Parental IQ:** Parental IQ has been shown to affect parental depression, early parenting, a child's IQ, a child's behavior, and the interaction between parent and child behavior as well as home environment more globally and may have confounding effects on the prediction of head injury.

7. Power and Sample Size

All children with traumatic brain injury

According to the CDC, 7 of every 1,000 children under age 10 visit the emergency room for head trauma each year. Children with rMTBI would be expected to be more prevalent in this study in any given year. There will also be an opportunity to aggregate the subset of the study population with repeated head trauma events over the course of the study.

Power estimates are for analyses of variance including three matched groups of participants: those with repeated mild traumatic brain injuries; those with single mild traumatic brain injuries; and those with no brain injuries. Because CDC estimates that 0.7 percent of children seek medical attention for head trauma each year, the multiplicative probability of repeated head trauma prompting an emergency room visit is estimated at 0.000049 percent per year. In the National Children's Study (NCS), with a total sample of 100,000 and 20 years duration, this would result in a conservative estimate of 100 cases of repeated mild traumatic brain injury over the course of the Study. This is in fact a very conservative estimate, as the CDC statistics are based on emergency room visits and therefore do not include other forms of medical attention that might be sought for mild traumatic brain injuries such as visits to a primary care physician. If this group were matched to others with single mild traumatic brain injuries and no traumatic brain injuries, the analysis would entail three groups of 100 cases each.

Below are estimates of power for small, medium, and large effect sizes. Using the estimated sample of 100 per group, and an alpha of .05, power is sufficient for analyses with medium and large effect sizes.

Effect size	R^2	Power
Large	.14	.99+
Medium	.06	.98
Small	.01	.32

8. Other Design Issues

- Additional neuropsychological testing: Imaging for exposed sub-group if possible, and surrogate marker analysis would provide an additional burden to the participant and family but may be required for other aspects of the cohort study. More in-depth tests may be considered for the subsample of children suffering a single MTBI, and a non-injured control group, which would add additional burden to these subgroups and their families.

- Cost/complexity of data collection: Because this hypothesis will be assessed through ongoing data collection from subjects throughout the Study, response and retention rates of participants will be important.

- Ethical/burden considerations: Family privacy must be protected for the collection of sensitive information about injury and associated parenting variables. Detected instances of child abuse and/or neglect must be reported to authorities.

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BEHAVIORAL EXPOSURES, GENETICS, AND CHILDHOOD OR ADOLESCENT ONSET AGGRESSION

1. Meta Hypothesis

Biological, physical, and psychosocial components of the environment and their interactions with specific genetic variations are associated with and determine patterns of increased onset and maintenance of antisocial physical aggression.

2. Specific Hypotheses

1. Behavioral exposures of parenting styles and other psychosocial experiences (such as domestic violence, family conflict, parental criminality, and substance abuse) interact with specific genetic variations (gamma aminobutyric acid receptors, levels of monoamine oxidase, serotonin, and dopamine) to determine early onset and persistent chronic physical aggression.

2. Adolescent onset physical aggression is determined by a different pattern of interacting environmental and biological factors than for early onset persistent aggression. These exposure factors include poor parent-child relations, lack of parental/neighborhood supervision and family activities, high levels of family conflict, restricted opportunities to participate in adult-supervised conventional activities (sports, music, drama, etc.), access to weapons, exposure to antisocial peers, exposure to violence in the media (including video games), victimization by peers, and characteristics of the neighborhood environment.

3. The onset and extent of increased persistent chronic physical aggression and adolescent onset physical aggression, as distinguished from normative developmental patterns of physical aggression, can be individually predicted by the combination and pattern of interacting genetic, psychosocial, and contextual exposure factors.

3. Background and Justification

The use of physical aggression arises during childhood as a normative behavior with most individuals demonstrating some level of physical aggression at some point during childhood or adolescence (Alink et al., 2006; Brame, Nagin, & Tremblay, 2001; Cote, Vaillancourt, LeBlanc, Nagin & Tremblay, 2006). For most children, the use of physical aggression then declines with age. For some individuals, physical aggression then increases again during adolescence (Moffitt, 1993). Investigators have begun to tease out a limited number of developmental "trajectories," or longitudinal patterns, of aggressive behavior across childhood and adolescence that have been confirmed within a variety of populations (Broidy et al., 2003).

Two main developmental trajectories for aggressive antisocial behavior have been proposed: "life-course persistent" and "adolescence-onset" antisocial behavior (Moffitt, 1993). Life-course persistent, or early-onset, antisocial behavior is proposed to appear as early as preschool and to be characterized by severe persistent aggression accompanied by multiple problem behaviors including noncompliance, lying, stealing, poor academic performance, truancy, and excessive alcohol and drug use during adolescence. Adolescence-onset antisocial behavior is proposed to have little or no precursor in childhood behavior problems, and the antisocial behavior itself appears to be less severe, less frequent, and limited to adolescence.

The more serious early-onset aggression has been confirmed, particularly in boys, through research from several countries (Broidy et al., 2003; Cote et al., 2006; Eley, Lichtenstein, & Moffitt, 2003; Nagin & Tremblay, 2001; Van Lier & Crijnen, 2004). This developmental trajectory may include varying rates of physical aggression at different ages, but, those who are most aggressive as toddlers appear to be most likely to be aggressive through subsequent developmental stages (Brame et al, 2001; Cote et al., 2006; Lacourse, Nagin, Vitaro, Cote, Arseneault, & Tremblay, 2007).

Monoamine oxidase (MAOA), as a catabolic enzyme, regulates monoamine transmitter levels in the central nervous system. The activity of this enzyme is partly genetically regulated. The MAOA gene has several common polymorphisms, which Sabol et al (1998) was first to report in the promoter region of the MAOA gene. The nonpromoter MAOA gene polymorphisms have been implicated in mood disorders, aggression, and suicide. Some studies found that the certain polymorphisms of MAOA are associated with bipolar affective disorder (Rubinsztein, Leggo, Goodburn, Walsh, & Jain, 1996; Preisig et al, 2000). In addition, an association with bipolar disorder was found in one meta-analysis (Furlong et al, 1999). Current research is focusing on the possibility of MAOA influencing the risk for suicidal behavior by affecting aggressive/impulsive traits, mood disorders, or other factors. Studies that plan a developmental and longitudinal approach offer a more promising future in this area of research.

3.1 Public Health Importance

Prevalence/incidence

Mothers' reports indicate many children begin to use physical aggression before the end of their first year of life (Tremblay et al., 1999). The cumulative rate of physical aggression increases thereafter, reaching a peak between 18 and 24 months when almost 80 percent of children are reported to have been physically aggressive at some point (Tremblay et al., 2004; Keenan & Wakschlag, 2000; Hay, Castle, & Davies, 2000). About 29 percent of highly aggressive early childhood children persist in their aggressive behavior through adolescence, with 3-16 percent of children overall manifesting the life-course persistent pattern depending on the definition of aggression and the stage in childhood that is examined (Brame et al, 2001; Broidy et al., 2003; Cote et al., 2006; Nagin & Tremblay, 1999). Boys are much more likely to show the life-course persistent pattern than girls; research estimates the difference at 10:1 (Moffitt, & Caspi, 2001). Boys who demonstrate the highest levels of persistent aggressive behavior appear, from multiple studies, to be at highest risk for violent and criminal behavior as adults (Nagin & Tremblay, 1999; Fergusson & Horwood, 1995).

There is also evidence of aggression and general rule violations that emerge during adolescence. During adolescence, the prevalence of physical fighting appears to increase, with about one-third of high school students reporting being involved in a physical fight (Eaton et al., 2006). Research has estimated the prevalence of adolescent-limited aggression at 22 percent, with boys 1.5 times more likely to be in this group than girls (Moffitt, & Caspi, 2001).

Economic and social burden

It is estimated that 25 percent of the tangible costs associated with violent crime in the United States are attributable to juveniles (Miller, Fisher, & Cohen, 2001). Based on this assumption and a 1996 report estimating the tangible costs of violent crime in the United States, the estimated annual cost of juvenile violence in 2003 dollars was $24.3 billion (Miller, Cohen, & Wiersema, 1996).

Some early intervention efforts have been successful in reducing both economic and social burdens. One successful intervention was a randomized control group intervention with mothers and their

young children who were at risk for antisocial behavior. The intervention, which involved home visitation by a nurse from pregnancy to child age 2, reduced the incidence of arrests and conviction in the children in the home visitation group 15 years later, during adolescence (Olds et al., 1998). Preschool intervention has also been effective at reducing antisocial outcomes in children at risk for poor social outcomes with an estimated savings to society of $7-$16 for every dollar spent by the time the children reached age 40 (Nores, Belfield, Barnett, & Schweinhart, 2005). This indicates knowledge about causal factors can be translated into effective treatment modalities.

3.2 Justification for a Large Prospective Longitudinal Study

Although there are various prospective, longitudinal studies documenting physical aggression and violence and their risk factors, many confound physical aggression and verbal or indirect aggression, delinquency, hyperactivity, oppositional behavior, or other disruptive behaviors, lumping together disparate constructs. Some studies have small samples; none is based on a representative sample of the United States; few have prospectively collected data during pregnancy; few have collected data from birth; few have collected data on girls or examined differences among ethnic or racial groups; none has done so among Latinos; and each assessed only a subset of the potential risk factors, often singly or in small groups, not simultaneously and comprehensively.

While there are some existing longitudinal data on the trajectory of physical aggression through childhood and into adolescence, a compelling justification for the National Children's Study (NCS) to include outcomes related to physical aggression is the ability to link prenatal, genetic, familial, and environmental exposures as well as the interactions between exposures with membership in a particular trajectory group. A large longitudinal sample will be needed to identify the full range of trajectory groups. It is clear that a relatively consistent group uses physical aggression with higher-than-average frequency throughout childhood. There is variability even within this group (Brame et al., 2001; Cote et al, 2006; Tremblay et al., 2004) possibly indicating the presence of multiple subgroups. Only a large cohort study will suffice to answer questions about the etiology of aggression during childhood and its manifestations through adolescence.

Once the trajectories are identified, longitudinal observation will allow better understanding of the sequencing of influences. For example, it is not clear whether early coercive parenting leads to children who are more aggressive, whether children who are physiologically predisposed to be aggressive reinforce the use of more coercive parenting (Jaffee et al, 2004), or whether a shared genetic predisposition may predict parenting style and childhood aggressive behavior. The depth of longitudinal data from the NCS will help untangle these issues.

3.3 Scientific Merit

During the past decade, a series of cohort studies have collected longitudinal data on the frequency of use of physical aggression through childhood and adolescence. These studies identified three to five distinct developmental trajectories for physical aggression, depending on the country, sample, and size of the cohorts (Broidy et al, 2001). These trajectories include early appearance of normative aggression with a decline through childhood; life-course persistent aggression involving early-onset of high levels of aggression maintained over time; late-onset, adolescence-limited aggression; and other similar but slightly varied trajectories across studies.

Some differences in etiology of the early-onset and late-onset trajectories have been identified, but the actual pathways are still unclear. Most research has focused on etiology of the early-onset, life-course persistent pattern. Because the majority of children belong to the trajectory of early appearing low-level aggression that remits quickly, it has been hypothesized that physical aggression is a

normal part of early development, but a series of factors inhibit it (Brame et al., 2001; Nagin & Tremblay, 2001). These factors have been shown in at least one study (the gene for MAO-A in boys) to interact directly with the social environment to affect the life-course pattern of use of physical aggression (Caspi et al., 2002), although this interaction has not been replicated in more recent reports (Young et al., 2006). Research has also implicated an interaction between biological risk (e.g., perinatal and birth complications, maternal illness during pregnancy) and social risk (e.g., harsh or neglectful parenting) in the development of life-course persistent aggression (Brennan, Hall, Bor, Najman, & Williams, 2003). Increasing evidence suggests early adverse experiences such as maltreatment may affect children differently depending on their genotypes (Jaffee et al, 2004). This evidence leads to questions about the multigenic etiology of physical aggression. Other such interactions will likely be identified as more information is collected regarding general brain chemistry and its genetic associations and variations, and as the relationship of exposures to particular developmental trajectories is better elucidated (Moffitt, Caspi, & Rutter, 2005).

Late-onset, adolescence-limited physical aggression seems to be associated primarily with environmental factors, including harsh parenting, high levels of adolescent life stress, and association with deviant peers (Brennan et al., 2003; Aguilar, Sroufe, Egeland, & Carlson, 2000). The adolescent-onset trajectory also does not show the types of gender differences that life-course persistent trajectory does, with more girls engaging in adolescent physical aggression than in early-onset stable aggression (Moffitt, & Caspi, 2001). Late-onset physical aggression is not, however, benign. It is associated with higher levels of internalizing symptoms such as depression during adolescence (Aguilar et al., 2000), and with elevated mental health problems, substance use, and nonviolent crime during young adulthood (Moffitt, Caspi, Harrington, & Milne, 2002). More research can uncover the range of longitudinal correlates of this trajectory.

3.4 Potential for Innovative Research

No studies to date have had sufficient diversity and sample size to identify and analyze the range of trajectories of aggression. The NCS will provide the opportunity to not only identify the trajectories but also to develop subgroups of sufficient size to delve effectively into genetic, environmental, and social correlates and interactions among them.

The Study will also collect more information on maternal and paternal genetics and prenatal exposures than has ever been available previously. More thorough mapping of factors that influence the development of aggression should be possible. Targeted intervention for children at risk for antisocial behavior has been shown to work, so results from this research should allow for more effective targeting of those at risk.

Research has not uncovered clear exposures that differentiate adolescents with late-onset physical aggression from those who are at risk yet do not engage in physical aggression. Because this pattern is not fully transient and because even limited forays into physical aggression are costly to the individual and to society, the opportunity to elucidate the origins of this pattern will be important.

3.5 Feasibility

These studies are feasible because physical aggression has been the focus of substantial measurement at different ages. Repeated measures of aggression will be critical to permit tracking of the trajectories. Fortunately, there are valid and reliable measures of physical aggression easily administered and parsimonious, beginning in the toddler and extending well adulthood.

Such measures can be administered to a range of respondents (parents, teachers, and children) to permit the collection of a variety of perspectives and contexts. Longer, more detailed measures can be done through survey or interview, and brief measures can be administered by phone. For children, parent report is a standard measure in the field and would provide data to compare with other longitudinal studies. For school-aged children, teacher report and parent report have both been used and would be important to collect for comparability with past research. In addition, child self-report is possible among older children, and adolescents are often uniquely informative about their own, otherwise undetected, delinquent behavior.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Alcohol, drug, tobacco use
- Nutrition
- Stress
- Age at child's birth

Primary/family

- Socioeconomic status (income, parental education, employment)
- Parenting (warmth, discipline, monitoring, maltreatment)
- Family stress
- Interparental conflict
- Domestic violence
- Neighborhood characteristics

Primary/child/adolescent

- Difficult temperament
- Cognitive development
- Congenital anomalies/birth complications
- Peer relationships/affiliations
- Substance use (alcohol, drug, tobacco)

4.2 Methods

Primary/maternal

- Interview
- Questionnaire
- Clinical events and ongoing health history

Primary/family

- Interview
- Questionnaire
- Direct observation of parent-child relationship

Primary/child/adolescent

- Interview (parent, child, teacher)
- Questionnaire (parent, child, teacher)
- Direct testing of children
- Clinical events and ongoing health history

4.3 Life Stage

Maternal

- Prenatal

Family

- Infancy, childhood, adolescence

Child/adolescent

- Infancy (parent, direct testing)
- Childhood (parent, child, teacher)
- Adolescence (parent, child, teacher)

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Physical aggression
- Criminal behavior

5.2 Methods

- Questionnaires (parent, child, teacher)
- Direct observation of parent-child interaction
- Public record

5.3 Life stage

- Questionnaires: Regularly beginning in the toddler period and through age 21
- Observation: Infancy, childhood, adolescence
- Criminal behavior: Age 21

6. Important Confounders, Mediators, and Effect Modifiers

- **Demographic variables:** Age, gender, and ethnicity may influence social acceptance of physical aggression of children or parental physical discipline of the child.
- **Media influences:** Frequency and content of television viewing and video and computer use may impact a child's aggressive behavior.
- **Neighborhood characteristics:** Geographic area of residence as well as neighborhood characteristics may affect a child's aggression.
- **Gene-environment interactions:** Parenting effects may be confounded with shared genetic tendencies toward aggression between parents and children.
- **Parental IQ:** Parental IQ has been shown to affect parental depression, early parenting, a child's IQ, a child's behavior, and the interaction between parent and child behavior as well as home environment more globally and may have confounding effects on the prediction of child aggression.

7. Power and Sample Size

Starting with the birth cohort of 100,000, the minimum odds ratio between measures of the child's physical aggression and hypothesized exposures will depend on the measure of physical aggression used, the prevalence of the exposure, and the age at which the assessment is completed. For this discussion, higher levels of exposure are assumed to contribute to higher levels of the outcome. The

calculations assume a target of 80 percent power using a two-sided 95 percent confidence interval and an intraclass correlation based on the study's sample design.

For the approximately 16 percent of children with high levels of physical aggression from infancy to age 12), the smallest reliably detectable odds ratio is 1.10 with an exposure prevalence of 25 percent. If the exposure used in the analysis has a prevalence of 5 percent, the minimum detectable odds ratio goes up to 1.21. If this same analysis is restricted to a subset of 10 percent of the population (possibly corresponding to the prevalence of a genetic marker), the minimum detectable odds ratio goes up to 1.54.

Assuming an assessment at age 20 when assessing the incidence of physical aggression in adolescence and still assuming 16 percent of children with high levels of physical aggression, the smallest reliably detectable odds ratio with an exposure prevalence of 25 percent is 1.11.

Assuming 4 percent of children have particularly high levels of aggressive behavior related to an exposure with a prevalence of only 5 percent, the minimum detectable odds ratio is 1.42. If this same analysis is restricted to a subset of 10 percent of the population (possibly corresponding to the prevalence of a genetic marker), the minimum detectable odds ratio goes up to 2.17. Thus, the power for testing these hypotheses in the Study is very good.

Considering interactions with genetic factors for aggression, a hypothesized gene (MAOA) and environment (child abuse) interaction was evaluated by considering the two most common variable number tandem repeat polymorphisms, the three-repeat variant with low activity and the four-repeat variant with high activity. These alleles have a population prevalence of about 33 percent and 62 percent, respectively (Brame et al., 2001). Child abuse was cited as 8 percent severe, 28 percent probable abuse and 64 percent with no abuse. Power analyses based on a prevalence of 5 percent in the unexposed population (conservative according to Caspi et al.) result in a required sample size of 31,650.

8. Other Design Issues

- **Ethical/burden considerations**: Family privacy must be protected for the collection of sensitive information about aggression and associated parenting variables. Detected instances of child abuse and/or neglect must be reported to authorities. Regarding burden, although regular assessment of aggression is required, there are brief measures that can be used for phone contact, which will reserve more in-depth measures for in-person contact.

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ANTECEDENTS AND RESILIENCY TO TRAUMATIC LIFE EVENTS IN CHILDHOOD

1. Meta Hypothesis

Antecedent factors such as genetic risk, family structure, neighborhood and community factors interact with traumatic life events to predict the risk of anxiety disorders.

2. Specific Hypotheses

1. The cumulative effects of repeated exposure to traumatic events (parental divorce, death of a family member) increase the risk of anxiety disorders in children.

2. A child's set of resiliency factors buffers the relationship between exposure to traumatic events and subsequent posttraumatic stress disorders.

3. Childhood intelligence is an antecedent for both risk of exposure to traumatic events and subsequent development of anxiety disorders.

4. A child's sensitivity to traumatic events has a strong genetic component, which interacts with the timing and magnitude of traumatic events to predict the risk for anxiety disorders.

5. The severity of a child's posttraumatic stress symptoms in reaction to potentially traumatic events increases the risk of negative physical health and functional outcomes.

6. Genetic variants implicated in neurobiological mechanisms central to the "fight-or-flight" response (dysregulation in the HPA axis, locus coeruleus/noradrenergic system, and limbic-frontal brain systems) influence the vulnerability to anxiety disorders after exposure to traumatic events.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Anxiety disorders in children are a public health burden due to their economic costs, comorbid mental disorders, chronicity through life, and association with general medical conditions. As a general class, anxiety disorders are the most prevalent class of mental disorders (Kessler et al., 2005), with a cumulative prevalence of 9.9 percent by age 16 (Costello et al., 2003) lifetime prevalence of 28.8 percent (Kessler et al., 2005). Frequently occurring anxiety disorders include generalized anxiety disorder (GAD), phobias, panic disorders (PD), obsessive-compulsive disorder (OCD), posttraumatic stress disorder (PTSD), and separation anxiety disorder (SAD). The median age of onset for the class of anxiety disorders using retrospective recall is age 11 (Kessler et al., 2005), making this especially relevant for the young participants of the Study. Specific and social phobias have a median age of onset of 7 to 13 years, respectively. SAD and PTSD have a median age of onset of 7 to 23 years, respectively.

Mortality/morbidity/quality

Anxiety disorder subtypes are linked. Each subtype has a potentially different underlying multifactorial etiology; however, anxiety disorder subtypes frequently co-occur. As an example, as many as 83 percent of individuals with GAD also have another active anxiety disorder (Dyck et al., 2001). The presence of a pre-existing anxiety disorder also increases the subsequent risk of developing another anxiety disorder subtype. A four-year longitudinal study found that adolescents with SAD had an increased risk of developing subsequent PD with agoraphobia (hazards ratio [HR] = 18.1), specific phobia (HR = 2.7), GAD (HR = 9.4), and OCD (HR = 10.7) (Bruckl et al., 2007). Anxiety disorders also have an extremely high comorbidity with major depression, which is among the top five leading causes of disability and disease burden in the world (Murray et al. 1997; Wang et al., 2003; Sartorius 2001). This comorbidity is estimated to be as high at 70 percent (Kessler et al., 1999), which provides further evidence that anxiety disorders are extremely costly for society.

Individuals with anxiety disorders are at higher risk, compared to nonaffected individuals, for adverse cardiovascular events (Kubzansky et al., 1997) and suicide attempts or ideations (Allgulander, 1994; Sareen et al., 2005). Medical illnesses are frequently associated with anxiety as in the case of diabetes for which GAD occurs in 14 percent of cases (Grigsby et al., 2002). Anxiety symptoms, and specifically GAD symptoms, both predicted greater risk for coronary heart disease, which was independent and in excess of the risk associated with major depression (Barger & Sydeman, 2005).

Economic and/or social burden

Child data on economic costs are limited. In the adult population, the economic burden of anxiety disorders is estimated to be $42.3 billion to $46.6 billion annually in the United States (Dupont et al., 1996; Greenberg et al., 1999). Health costs directly associated with treatment of anxiety disorders are substantial. Average estimated total costs for individuals diagnosed with an anxiety disorder are $6,475, and having a diagnosis of PTSD increases that cost by $3,940 (Marciniak et al., 2005). Individuals with the highest health costs are anxious patients with depression, individuals with PTSD or GAD, and patients diagnosed with anxiety and a comorbid medical condition such as diabetes (Marciniak et al., 2005).

Preventability/malleability

Whereas pharmacological treatments for anxiety disorders continue to be modified for efficacy, the prevention of anxiety symptoms has not been as commonly addressed. The focus of targeted prevention programs should aim to understand what factors increase risk of exposure to traumatic events and to reduce the incidence of anxiety disorders like PTSD, GAD, and PD in individuals who have been exposed to trauma by boosting factors related to resiliency.

3.2 Justification for a Large Prospective Longitudinal Study

Because of the dearth of longitudinal studies on early antecedents of trauma, previous research has not been successful in clearly outlining causal mechanisms underlying exposure to trauma, identifying critical periods of vulnerability during development, and targeting opportunities for intervention. The longitudinal nature of the Study would allow prospective assessment of variables that have not been incorporated in studies of childhood traumatic stress, its antecedents, and consequences. These include genetic risk factors and prebirth parental variables (such as parent history of trauma exposure and anxiety disorders). The Study will advance the current understanding of risk associated with trauma exposure by elucidating causal pathways in which exposure to trauma is influenced by antecedent factors, exposure to trauma confers risk for anxiety disorders, and associations between traumatic exposure and anxiety outcomes are buffered by resiliency.

It is also essential to acknowledge the risk of anxiety disorder subtypes differs by exposure type and differing periods of vulnerability. Whereas the majority of previously published studies focuses on the risk for a specific disorder (e.g., PTSD), the Study will be able to identify how risk factors and the chronicity (relapse and recurrence) of anxiety disorder subtypes change during development.

3.3 Scientific Merit

Traumatic events and anxiety disorders

Exposure to physical or emotional trauma is a commonly suspected risk factor for anxiety disorders such as GAD, PD, and PTSD (Brantley, Mehan, Ames, & Jones, 1999; Brown et al., 2000). Many of the larger epidemiological studies of trauma exposure and anxiety symptoms in youth have focused on abuse, violence, and victimization (Boney-McCoy & Finkelhor 1995, Hanson et al., 2006). Only recently have studies begun to focus on children or youth assessed exposure to the full range of potentially traumatic events (PTEs) both high and low in magnitude (Breslau et al., 2004; Breslau, Lucia, & Alvarado, 2006; Costello, Erkanli, Fairbank, & Angold, 2002; Giaconia et al., 1995, Storr, Ialongo, Anthony, & Breslau, 2007). Breslau et al. (2004) and Storr et al. (2007) reported on a community-based prospective study that followed a group of 2,311 urban children enrolled at ages 6-7 and into young adulthood. By age 20-23, 82 percent of this sample had experienced at least one PTE, and 7 percent had PTSD at some point in the observation period. Across child/youth community-based studies on PTSD, the conditional probability of PTSD given exposure to any PTE ranged from 8.3 percent to 14.5 percent (Breslau et al., 2004, 2006; Storr et al., 2007). Several types of PTEs (most notably sexual assault and other assaultive violence) were found to confer a much higher risk of developing PTSD.

Many studies on the direct exposure to mass traumatic events focus only on posttraumatic stress symptoms in children (Goenjian et al., 2001; Smith, Perrin, Yule, & Rabe-Hesketh, 2001). PTEs are not only associated with PTSD. PTEs have been linked with greater risk of numerous mental disorders, including GAD or generalized anxiety disorder syndrome (defined as GAD for at least 2-weeks), PD (Bandelow et al., 2002), and major depression (Blazer, Hughes, & George, 1987; Angst & Vollrath, 1991; Newman & Bland, 1994; Kendler et al., 2003). Very few studies on the effects of PTEs have incorporated the broader range of anxiety disorders. Despite increasing awareness of PTSD onset after natural disasters and war, responses to traumatic events such as these are not limited to PTSD. As an extreme example, children in New York City public schools who were interviewed six months after September 11, 2001, were screened for elevated mental disorders in addition to PTSD. The most prevalent disorders identified were probable agoraphobia (14.8 percent), SAD (12.3 percent), followed by PTSD (10.6 percent), and GAD (10.3 percent) (Hoven et al., 2005).

PTEs in the form of alterations in family structure or environment have also been implicated in the risk for anxiety disorders. In addition to family history of GAD, an adverse family environment during childhood was associated with risk of GAD but was not a shared risk factor during childhood for major depression (Moffitt et al., 2007). Therefore, although there is high comorbidity between these two disorders, this suggests they have different etiological pathways. Traumatic stressors such as parental conflict, childhood physical abuse by a father figure, and the absence of a parent or adult confidant during childhood have also been associated with an increased risk of developing social phobia (DeWit et al., 2005). Two recent longitudinal studies provide evidence that trauma in the form of injuries may be associated with anxiety disorders. Unintentional injuries were associated with subsequent childhood separation anxiety symptoms (Rowe, Simonoff, & Silberg, 2007). Injury events in which adolescents perceived a threat to their life or having lack of control over the event were also strongly associated with PTSD risk (Holbrook et al., 2005).

Neurobiology of trauma exposure

When humans are exposed to stress or trauma, a complex activation of behavioral, autonomic, sensory-motor, cognitive, and neuroendocrine systems is elicited (Sanchez, Ladd, & Plotsky, 2001). The most commonly linked system to the stress response is the limbic-hypothalamic-pituitary-adrenal (LHPA) axis. Activation of the LHPA axis results in the downstream secretion of glucocorticoids (e.g., cortisol) and is an adaptive, critical mechanism by which humans cope with exposure to challenges. Although the stress response can be a protective function (McEwen, 1998), chronic exposure to stress can be damaging to neurons. Hypersecretion or hyposecretion of glucorticoids leads to neuronal atrophy, neurotoxicity, and neuroendangerment. Neuroendangerment occurs when the vulnerability of neurons alters their capacity to deal with subsequent neurologic insults (Sapolsky, 1996). Structural imaging studies on adults with exposure to stress and subsequent PTSD support these biological findings (e.g., reduced hippocampal volumes were found in adults and combat veterans with PTSD [DeBellis et al., 1999]). The association between hippocampal volumes and childhood PTSD is not as consistent (DeBellis et al., 1999; Bremner et al., 1995; Carrion et al., 2001; Carrion, Weems, & Reiss, 2007; Tupler & DeBellis, 2006). These conflicting findings are likely to be due to developmental differences in the biological mechanisms behind stress exposure, the continued maturation of the hippocampus throughout childhood and adolescence, and possible differences in the vulnerability of the LHPA system during the developmental period (Cicchett & Walke, 2001).

Childhood resiliency

Some children appear to be resistant to tremendous trauma whereas other children struggle after a stressful event. Resiliency refers to the concept of individual variations in response to comparable experiences. Resiliency also accounts for evidence that exposures to stress can increase resistance to later stress (Rutter et al., 2006). Thus, it is important to recognize that the type, timing, and magnitude of traumatic exposures during childhood may play a role in accumulating resiliency against later stress.

The resiliency concept purports that a host of factors, both proximal and distal, contribute to the variability in stress response. Prenatal factors, genetic makeup, developmental stage, prior exposures to stress, and economic hardship may act together to determine the different ways in which humans' neural systems react to a stressful event (McEwen et al., 1994; Sapolsky et al., 1994).

Genetic risk for traumatic exposure and anxiety disorders

There is evidence for a genetic influence on the exposure to certain forms of trauma and susceptibility to trauma and stress. Twin data suggest that certain forms (e.g., violent or assaultive) of trauma and stressful events have a genetic influence (Stein, Jang, et al., 2002; Kendler et al., 1993; Foley, Neale, & Kendler, 1996). This observation may be mediated through personality traits, such as neuroticism, which predispose a person to certain traumatic events and consequent anxiety disorders (Kendler, Myers, & Prescott, 2002; Stein, Jang, et al., 2002; Hettema, Prescott, & Kendler, 2004). Additionally, research by Yehuda, Halligan, & Bierer (2001) has documented an increased susceptibility to PTSD following exposure to traumatic events in the adult offspring of Holocaust survivors with PTSD compared to offspring of survivors without PTSD. The relative and overlapping contributions of genetic susceptibility to both exposure and anxiety require further research.

Family and twin studies suggest the familial aggregation of many types of anxiety disorders is high (Hettema, Neale, & Kendler, 2001). Individuals with PD have higher rates of psychiatric disorders in their families in general, especially PD and GAD (Bandelow et al., 2002). Summary odds ratios associated with family history range from 4.0 to 6.0 for PD, GAD, phobias, and OCD (Hettema et al., 2001).

Evidence for a genetic influence on PTSD risk has come from adult twin studies (Lyons et al., 1993, True et al., 1993, Stein, Jang, et al. 2002) which demonstrate an elevated risk of PTSD in the monozygotic (MZ) co-twin of a PTSD proband, compared to dizygotic (DZ) co-twins. Studies of veteran and community twin samples suggest that genetic influences account for about one-third of the variance in PTSD risk. The heritabilities for anxiety disorders range from 30-40 percent, denoting that the vast proportion of variance in risk may be explained by environmental factors (Hettema et al., 2001).

A growing number of studies have attempted to pinpoint candidate genes for anxiety disorders. One study found that polymorphisms in the GAD1 (glutamic acid decarboxylase) region were significantly over-represented in cases with a variety of anxiety disorders (Hettema et al., 2006). Association studies on PTSD and candidate genes involving in the dopaminergic system are conflicting, but Segman et al. (2002) present the strongest evidence for an association (Koenen, 2003). Further elucidation of the mechanisms through which genetic variants might influence PTSD risk comes from recent work by Koenen et al. (2005), who have demonstrated an association between polymorphisms in FKBP5 (posited to play a role in HPA axis regulation) and peritraumatic dissociation in children with burn injury. Despite the efforts of many studies to identify genes underlying anxiety disorders, concrete and adequately replicated evidence for specific genetic loci remains elusive (Merikangas & Low, 2005).

Physical health consequences of trauma exposure and anxiety disorders

There is evidence that both exposure to PTEs and the development of traumatic stress symptoms are each associated with poorer health and functional outcomes. For example, the Adverse Childhood Experiences study (retrospective adult report of adverse childhood experiences) has documented links between the degree of exposure to adverse childhood experiences (many of which would fit the definition of potentially traumatic event) and a wide range of physical health outcomes in adulthood (Dube et al., 2003; Dong et al., 2004). Child data is more limited, but suggests a similar link between trauma exposure and/or PTSD and adverse physical health and functional outcomes. Cross-sectional evidence to date also suggests, but cannot yet establish conclusively, that PTSD may serve as a mediator for the effect of traumatic exposure and adverse physical health outcomes such as circulatory, endocrine, and musculoskeletal conditions (Seng et al., 2005). In a preschool-age Head Start sample, exposure to violence and PTSD symptoms were also each associated with greater health problems such as asthma and gastro-intestinal problems (Graham-Bermann & Seng, 2005). Few longitudinal analyses have been conducted to support PTSD and other anxiety disorders as having a mediating effect in the causal pathway from PTEs to physical health outcomes. Only one study with a 24-month follow-up interval reported that injured adolescents had an increased risk for PTSD and that prolonged PTSD symptoms were associated with lower health-related quality of life (Holbrook et al., 2005).

Advancement of scientific understanding

The prospective data available to date regarding antecedents or predictors of exposure to PTEs, antecedents of anxiety disorders, and of the impact on child health and functioning, suggest that there are likely to be complex interactions among individual, family, and community factors. None of the existing prospective studies can shed light on etiological pathways that begin in early childhood and carry forward into adolescence or adulthood, and none include factors such as genetic risk, parental trauma, or history of psychiatric disorders. The National Children's Study (NCS) offers an ideal opportunity to tease apart these interactions and understand pathways to risk and how resilience buffers against children's all-too-common exposures to stressful or difficult events.

Capturing a broader range of exposures can provide a better estimate of the public health impact of exposure to trauma. Studying traumatic events requires examination of the continuum of events from high to low magnitude. Lower magnitude events occur more frequently throughout the lifespan compared to high magnitude events. While some experiences (such as sexual assault) may lead to a higher conditional probability of anxiety disorders, others (such as motor vehicle crashes) can potentially produce more cases in the population because of their frequency (Norris, 1992). By assessing the continuum of exposures, the field can move forward in its understanding of the circumstances by which the magnitude, timing, and chronicity of events contribute to the risk of childhood anxiety disorders. Increased understanding and awareness of PTEs may ultimately lead to improved preventive and treatment options for the occurrence and impact of traumatic events in childhood.

Directly addressing the lack of research on what factors contribute to resiliency, and whether these same factors contribute to risk of exposure to PTEs, will aid future efforts to prevent exposure, potentially alter factors to boost resiliency, and identify critical periods of vulnerability to intervene against the negative consequences of traumatic exposures.

3.4 Potential for Innovative Research

The NCS offers a unique opportunity to address the limitations of existing studies and to add significantly to our understanding of the scope and impact of exposure to PTEs in childhood. There has been no nationally representative study of the prevalence or consequences of exposure to the full range of PTEs in children and adolescents.

The literature to date suggests there are antecedent factors that affect underlying neuronal processes which control the stress response. These may be genetic, prenatal, behavioral, or cognitive in origin. However, these factors may also influence the likelihood that a child will encounter stressful or traumatic events. Furthermore, the same factors could determine the extent to which a child will respond with adverse psychopathology in the form of PTSD, GAD, or PD. There are complex relationships between biological processes involving the stress response, exposure to stressors or trauma, and the development of anxiety disorders. Only sophisticated longitudinal studies employing a developmental approach will be able to disentangle these intricate processes. There has not been a study of child and youth trauma exposure or anxiety disorders that is nationally representative, covers a broad range of traumatic event types repeatedly across childhood development, and provides sufficient prospective longitudinal data to trace relationships through time among family and genetic risk factors, trauma exposure, anxiety symptoms, and physical or functional health.

3.5 Feasibility

The longitudinal nature of the NCS makes it highly feasible to address the proposed hypotheses. The NCS assessment schedule targets critical periods of development to assess exposures and outcomes. Valid and reliable measures are available to assess 1) exposures across the full range of PTEs, 2) anxiety symptoms on a continuum (symptom) and categorically (diagnosis), 3) physical and functional health outcomes, and 4) a host of antecedent factors to risk of exposures.

Addressing this hypothesis is feasible given the structure of the study. Assessment of children's and parents' exposure to PTEs will be required to tackle the significant questions and hypotheses described here. Empirical data on the impact of traumatic stress interviews on research participants indicates high degrees of acceptance of these topics in research interviews.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Exposure to potentially traumatic events
- Lifetime history of psychiatric disorders (e.g., depressive or anxiety disorders)
- Family history of psychiatric disorders (e.g., depressive or anxiety disorders)
- Genotyping for genetic risk factors
- Behavioral problems (e.g., antisocial, violent or criminal behaviors)
- Parenting style
- Parent-child interactions
- Community resources, social capital, etc.
- Socioeconomic status (income, wealth, education)

Primary/paternal

- Exposure to potentially traumatic events
- Lifetime history of psychiatric disorders (e.g., depressive or anxiety disorders)
- Family history of psychiatric disorders (e.g., depressive or anxiety disorders)
- Genotyping for genetic risk factors
- Behavioral problems (e.g., antisocial, violent, or criminal behaviors)
- Parenting style
- Parent-child interaction

Primary/child

- Exposure to potentially traumatic events (e.g., maltreatment, neglect, abuse, family conflict, serious illness, injury, witnessing/learning of trauma to a loved one, romantic break-up, or loss of a friend)
- Cognitive and intelligence measures
- Genotyping for genetic risk factors
- Behavioral problems (e.g., antisocial, violent or criminal behaviors)

- Parent-child interactions
- Cortisol levels

Secondary/maternal, teacher proxy report

- When a child is too young for self-reported exposures, a mother can report her child's exposure to potentially traumatic events and stressful life events
- Behavioral problems (e.g., disruptive, aggressive, antisocial, violent, or criminal behaviors)

4.2 Methods

- Blood samples for genotyping
- Salivary cortisol
- Interview/questionnaires for exposures to traumatic or stressful events (e.g., Life Events Interview section of the Child and Adolescent Psychiatric Assessment, alternatively, the NIMH Diagnostic Interview Schedule)
- Parent-child interaction task
- Family environment (e.g., HOME)
- Cognitive and intelligence testing (e.g., Wechsler Intelligence Scale for Children-Revised or Woodcock-Johnson subtests)
- Behavioral problems (e.g., Child Behavior Checklist, or Strengths and Difficulties Questionnaire)

4.3 Life Stage

Primary/maternal

- Repeated assessments throughout the study

Primary/paternal

- Repeated assessments throughout the study

Primary/child

- Repeated assessments from early childhood through the study. Self-reported exposures can be assessed after approximately age 7

Secondary/maternal, teacher proxy report

- Repeated assessments of exposures during early childhood until the child is age 7
- Behavioral problems can be assessed by mothers and/or teachers at the study visit that falls closest to when child enters first grade

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Anxiety symptoms or categorical (yes/no) diagnosis of an anxiety disorder
- Other comorbid psychiatric disorders or symptoms
- Physical health (e.g., chronic conditions such as diabetes, asthma, or those involving the circulatory, endocrine, gastrointestinal, or musculoskeletal systems)
- Functional health (e.g., quality of life)

5.2 Methods

- DSM-IV-based assessments for anxiety disorders (e.g., Child and Adolescent Psychiatric Assessment or the NIMH Diagnostic Interview Schedule for Children)
- Physical health outcomes via direct physical assessments or medical record reviews
- Functional outcomes via observational assessments and interviews

5.3 Life Stage

- Repeated assessments throughout the study. Relevant instruments will be administered where they are validated for specific age ranges

6. Important Confounders, Mediators, and Effect Modifiers

- **Gender:** Females are approximately twice as likely to have anxiety disorders such as GAD compared to males (Wittchen, Zhao, Kessler, & Eaton, 1994). Males are more likely to be exposed to a PTE, but females are more likely to develop PTSD given exposure. Gender may also influence the course of some anxiety disorders such as PD (Yonkers et al., 1998).
- **Age:** Age has differential effects on specific types of anxiety disorders. As one example, the prevalence rates of GAD are low in adolescence but increase substantially with age (Witttchen & Hoyer, 2001).
- **Race/ethnicity:** Anxiety disorders in general are found more commonly in racial/ethnic minorities (see Kessler & Wittchen, 2002 for review).

- **Socioeconomic status (SES):** In general, anxiety disorders occur more frequently in people with lower income and education (Wittchen et al., 1994; Brawman-Mintzer & Lydiard, 1996; see Kessler & Wittchen, 2002 for review). The extent of risk associated with SES is dependent upon the type of anxiety disorder.

- **Family history of anxiety and other psychiatric disorders:** Parental family history of major depression and anxiety disorders conveys the risk of anxiety disorders to their offspring (Biederman et al., 2001).

- **Family environment:** Costello et al. (2002) found that family environment factors increased exposure-specific risk. Having a parent with a criminal record or a disorganized family environment increased the likelihood of exposure to some PTEs (sexual abuse, traumatic events occurring to loved ones), but did not affect the likelihood of others (physical violence, serious accidents or illness).

- **Stressful life events:** Certain stressful life events (e.g. parental separation or divorce, moving, or changing schools) increase the chances of experiencing a later PTE (Costello et al. 2002).

- **Intelligence and cognitive ability:** Breslau et al.(2006) found that a lower IQ at age 6 predicted both greater trauma exposure and more PTSD symptoms by age 17. Youths who scored higher in reading readiness tests were also at lower risk for exposure to assaultive traumas (Storr et al., 2007).

- **Major depression:** Depression is often correlated with anxiety disorders and has a similar genetic component (Kendler et al., 2007). There is evidence that depression has different etiological pathways such that it is not the same underlying disorder and therefore must be considered an important confounder.

- **Pre-existing behavioral problems and other psychiatric symptoms/disorders:** Early aggressive/disruptive behavior problems have been found to predict greater exposure to assaultive violence but not other nonassaultive PTEs by late adolescence (Breslau et al., 2006, Storr et al., 2007). Additionally, anxiety and depressive problems at age 6 predicted greater conditional risk for PTSD, given exposure to a PTE, by late adolescence (Breslau et al., 2006, Storr et al., 2007). In adolescents ages 14-24, the presence of a pre-existing anxiety disorder at baseline predicted exposure to traumatic events during the follow-up period, possibly due to a tendency to report events as being particularly horrific (Stein, Hofler, et al., 2002). In adolescents with major trauma, the risk of PTSD was significantly and strongly associated with drug and alcohol abuse and other adolescent behavioral problems (Holbrook et al., 2005). In addition, the presence of illicit drug use in adolescents and young adulthood predicted later onset of traumatic events that were assaultive and sexual in nature (Stein, Hofler, et al., 2002).

- **Cigarette smoking:** Cigarette smoking is correlated with anxiety symptoms (Breslau, Kilbey, & Andreski, 1991; Amering et al., 1999). The direction of causality remains to be determined, but recent longitudinal analyses have suggested that cigarette smoking during adolescence may precede the onset of and increase risk for agoraphobia, GAD, or PD in early adulthood (Johnson et al., 2000).

7. Power and Sample Size

Given an exposure measure (such as a PTE) and a categorical outcome measure (i.e., the occurrence of an anxiety disorder), the relationship between exposure and outcome can be measured using an odds ratio. The following table shows the minimum odds ratio that can be reliably detected as a function of the age of assessment and assumptions about the prevalence of the outcome and exposure. The calculations assume a target of 80 percent power using a two-sided 95 percent confidence interval and an intraclass correlation based on the NCS sample design (0.04 for both the exposure and outcome). For the calculations, the assumed outcome is either the presence of anxiety disorder at age 16 (with a reported prevalence of 9.9 percent) and the presence of an unspecified anxiety disorder subtype as of age 7 (with as assumed prevalence of 1 percent). Three values for the exposure prevalence are assumed to illustrate how the minimum detectable odds ratio varies with exposure prevalence.

Table 1. Detectable Minimum Odds Ratios

Assumed outcome	Prevalence of the outcome	Age of assessment	Prevalence of the exposure	Minimum odds ratio that can be reliably detected
Anxiety disorder at age 16	9.9%	16	5%	1.26
			15%	1.16
			50%	1.11
Anxiety disorder subtype at age 7	1.0%	7	5%	1.82
			15%	1.49
			50%	1.37

Many of the hypothesized relationships between exposure and outcome involve interactions with other factors, such as demographic factors and the presence of a genetic risk factor. Calculating the minimum detectable odds ratio for an interaction requires additional assumptions. However, as a general rule, the minimum detectable odds ratio for an interaction involving exposure is roughly equal to or greater than the square of the minimum detectable odds ratio for the exposure. Thus, an example of the minimum detectable odds ratio for an interaction between exposure and gender, given the assumptions in the table above, ranges from roughly 1.23 (1.11 squared) to 3.31 (1.82 squared).

8. Other Design Issues

Lifetime history of psychiatric disorders is an important component of studying the risk for having an anxiety disorder(s). One method is to obtain self-reported, doctor-diagnosed psychiatric conditions, which often yields pertinent information about diagnosable conditions only for those who have available medical resources (access to psychiatric services, etc). It is beneficial to capture both continuous symptoms and/or categorical diagnoses for participants who may not have the benefit of being diagnosed by a clinician. Performing a full diagnostic interview with study participants may be time-consuming; however, several approaches exist to reduce participant burden and yield well-standardized information about psychiatric diagnoses. The NCS may choose to administer screening questions for specific psychiatric disorders. Participants who screen positive for psychiatric disorders of interest can be followed up at a later time by phone or self-administered interviews by which a full diagnosis can be obtained. This diagnostic process will be validated within the Study sample to determine the association between the diagnostic profiles after the positive screens vs. conducting a full diagnostic interview.

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HORMONALLY ACTIVE ENVIRONMENTAL AGENTS AND REPRODUCTIVE DEVELOPMENT

1. Meta Hypothesis

Prenatal and postnatal (including peripubertal) exposure to hormonally active environmental agents can alter development of the reproductive system resulting in multiple outcomes that can occur at various stages of development and may result in cumulative effects over time.

2. Specific Hypotheses

1. Prenatal and postnatal exposure to hormonally active environmental agents can alter early childhood development with direct implications for reproductive health and function (e.g., hypospadias, hypothyroidism, obesity).

    1.1 Male exposure to phthalates in the prenatal period is associated with hypospadias.

    1.2 Polybrominated diphenyl ether exposure during the prenatal period is associated with hypothyroidism that leads to altered reproductive development among highly exposed children.

    1.3 Exposure to bisphenol A increases the risk of obesity in genetically susceptible children.

2. Prenatal and postnatal exposure to endocrine-active environmental agents can alter sexual maturation and reproductive function (e.g., timing of puberty, polycystic ovarian disease) that are dependent both on earlier changes in reproductive development and on more proximal exposures and effects.

    2.1 Exposure to bisphenol A in early childhood is associated with an acceleration in age of onset of puberty in girls.

    2.2 Exposure to phthalates during early childhood is associated with the development of polycystic ovarian syndrome in adolescent females.

    2.3 Critical time windows for exposure to lead and associated delays in age of onset of puberty in girls are both in early childhood and around the time of sexual maturation.

    2.4 Genetic polymorphisms can alter sensitivity to phthalate exposures in childhood.

3. Background and Justification

Development of the reproductive system begins early in gestation and continues through infancy, childhood, adolescence, and into adulthood. A number of adverse outcomes can occur as the result of interference with development of this complex system that includes the reproductive organs, endocrine system, and hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axis that control their development and function. Exposure to environmental agents that are hormonally active agents (HAAs), which are also called endocrine disruptors (EDs), has been shown to affect the reproductive system in both animals and humans. Early outcomes include birth defects such as hypospadias and cryptorchidism in boys, as well as hormonal changes such as hypothyroidism in boys

and girls, which interfere with optimal reproductive health. Later outcomes from the same exposures may include alterations in growth, timing, and progression of puberty, and disease states such as polycystic ovary disease (PCOS) and endometriosis in females and testicular dysgenesis syndrome in males. Such changes may be cumulative; that is, adverse outcomes at early ages may predispose an individual to be at greater risk for additional adverse effects (e.g., cryptorchidism and later changes in fertility [Lee, 2005] or early menarche and breast cancer [Vihko & Apter, 1986]). Recent studies in animals suggest exposure to certain HAAs during fetal life may cause multiple abnormalities after birth, (e.g., abnormal reproductive organs, increased tumors), and once adulthood is reached, an increase in the incidence of infections. These changes appear to be transgenerational, that is, effects that are carried into subsequent generations because of changes in DNA methylation patterns that are transmitted in the male germline to the next generation (Anway, Cupp, Uzumcu, & Skinner, 2005; Chang, Anway, Rekow, & Skinner, 2006).

Increasing trends in hypospadias (Paulozzi, Erickson, & Jackson, 1997; Paulozzi, 1999) and cryptorchidism (Paulozzi, 1999) have been reported in the United States and other countries. Secular trends of decreasing age at menarche in girls have been reported in Europeans, North Americans, and Australians during the last century. Recent data suggest decreasing age for menarche as well as in measures of puberty onset (e.g., onset of breast and pubic hair development) in the United States (Herman-Giddens et al., 1997; Herman-Giddens, 2006; Kaplowitz, Slora, Wasserman, Pedlow, & Herman-Giddens, 2001; Lee, Guo, & Kulin, 2001). Data are fewer for timing of puberty in boys and some of the male pubertal markers may not be as reliable as in females, but age at genital growth and pubic hair development appears to be earlier in recent years (Herman-Giddens, 2006). These changes may result from better (and over) nutrition, greater and earlier growth, increasing incidence of obesity (Lee et al., 2007; Wang, 2002), and environmental or socioeconomic factors.

A variety of environmental chemicals have been cited in the literature as potential HAAs, including insecticides and herbicides (e.g., dichlorodiphenyltrichloroethane [DDT], atrazine); pharmaceuticals (drug estrogens); chemicals associated with consumer goods/household products (e.g., bisphenol A, phthalates, nonylphenol, polybrominated diphenyl ethers [PBDEs], perfluorinated compounds [PFOA, PFOS]); industrial chemicals (e.g., polychlorinated biphenyls [PCBs], dioxins, polycyclic aromatic hydrocarbons [PAHs]); heavy metals (e.g., arsenic, lead, mercury, and cadmium); and natural hormones such as the phytoestrogens (Gray, Ostby, Cooper, & Kelce, 1999; Howdeshell, Hotchkiss, Thayer, Vandenbergh, & Vom Saal, 1999; Rubin, Murray, Damassa, King, & Soto, 2001; Schonfelder et al., 2002; Ashby, Tinwell, Stevens, Pastoor, & Breckenbridge, 2002; Wolf, Ostby, & Gray, 1999; Fenton, Hamm, Birnbaum, & Youngblood, 2002; Kuriyama, Talsness, Grote, & Chahoud, 2005; Talsness et al., 2005; McDonald, 2005; Lilienthal, Hack, Roth-Harer, Grande, & Talsness, 2006; Ceccatelli, Faass, Schlumpf, & Lichtensteiger, 2006; Eriksson, Fischer, & Fredriksson, 2006). Recent studies of exposure to some environmental agents suggest they may accelerate (Blanck et al., 2000; Krstevska-Konstantinova et al., 2001) or delay (Den Hond et al., 2002; Wu, Buck, & Mendola, 2003; Selevan et al., 2003) pubertal development in girls. Data on the effects of HAAs on age at puberty in boys are fewer (Den Hond et al., 2002) but indicate an association between PCB and polychlorinated dibenzofuran (PCDF) exposures with delayed puberty and decreased penile length (Den Hond & Schoeters, 2006). These observations are concordant with laboratory data on the effects of HAAs (Gray et al., 1999; Yu et al., 2004). Because there are only limited data on specific critical windows for chemical exposures in relation to timing of puberty, the entire prepubertal period, including in utero growth and development and the peripubertal period, should be considered as critical times for exposures.

3.1 Public Health Importance

Prevalence/incidence

Exposure to phthalates and other HAAs is widespread in American children (Centers for Disease Control and Prevention [CDC], 2003), and animal studies increasingly suggest the potential for toxicity at current levels of exposure (Vom Saal & Hughes, 2005). Exposure to HAAs in the residential environment can occur from sources such as drinking contaminated water, breathing polluted air, ingesting food, and contacting or ingesting contaminated soil or dust, as well as using certain commercial products containing synthetic HAAs (e.g., cleaners, pesticides, cosmetics and food additives) (National Research Council [NRC], 1999). Rudel Camann, Spengler, Korn, & Brody (2003) investigated potential indoor exposures to numerous endocrine disruptors found in consumer uses. Analyses of indoor air and dust found 52 HAA compounds in air including phthalates (plasticizers, emulsifiers), o-phenylphenol (disinfectant), 4-nonylphenol (detergent metabolite), and 4-tert-butylphenol (adhesive). Sixty-six endocrine disrupting compounds were present in dust samples taken from homes, with frequent detections of penta- and tetrabrominated diphenyl ethers (flame retardants) and numerous pesticides in dust. An intermediate of a flame retardant banned in 1977 (2,3-dibromo-1-propanol), as well as the banned pesticides heptachlor, chlordane, methoxychlor, and DDT were also frequently detected in dust and air (Rudel et al., 2003).

One common congenital anomality, hypospadias affects 27-55 out of 10,000 births in the United States (Paulozzi, Erickson, & Jackson, 1997; Paulozzi 1999) or 0.8 percent of male live births (Pohl, Joyce, Wise, & Cilento, 2007). Cryptorchidism rates vary by gestational age and birth weight, affecting 3 percent of full-term male newborns (up to 7.7 percent in low birth weight males), decreasing to about 1 percent by age 1 due to spontaneous descent (Pohl et al., 2007). In utero exposure to phthalates has been associated with decreased anogenital distance. This suggests phthalate exposure may cause this structural change (Swan et al., 2005). Polycystic ovarian syndrome (PCOS) is the most common endocrine abnormality of premenopausal women, affecting 6.6 percent of a sample of 400 women (age 18-45 years) seeking a pre-employment physical (Azziz et al., 2004). Symptoms of PCOS may emerge during late puberty and shortly thereafter (Jeffrey Chang & Coffler, 2007).

According to the CDC (2003), most girls reach puberty between 8 and 13, and most boys reach puberty between 9 and 14. Approximately 37 percent of 7-year-old and 52 percent of 8-year-old African-American girls showed signs of precocious (early onset) puberty, while corresponding numbers for Caucasian girls were 6 percent and 16 percent, respectively (Herman-Giddens et al., 1997).

Economic and/or social burden

The total cost of evaluating and providing care to reproductive-aged women with PCOS in the United States is $4.36 billion (Azziz, Marin, Hoq, Badamgarav, & Song, 2005). There is a 15-40 fold increased risk for testicular cancer and an increased risk for infertility in men with a history of cryptorchidism, but data are too sparse to estimate economic costs (Pohl et al., 2007).

Very early or very late puberty has been associated with conditions that sometimes arise during adolescence and more often during adulthood that carry increased health care costs. Early menarche is reported to be a risk factor for breast cancer, underscoring the role of early developmental milestones as indicators for adult onset disease (Vihko & Apter, 1986). Certain girls with premature adrenarche are at risk of developing functional ovarian hyperandrogenism, PCOS, and hyperinsulinism (Vuguin, Linder, Rosenfeld, Saenger, & Dimartino-Nardi, 1999; Banerjee et al., 1998). Psychological and psychosocial disturbances are also associated with precocious puberty. Central precocious puberty often

leads to lower self-esteem, and early menarche has been associated with comorbid depression and substance abuse (Stice, Presnell, & Bearman, 2001).

Delayed puberty is associated with short stature and lack of sexual development, characteristics that may lead to emotional and social difficulties. Bone mass gain is rapid during puberty. Recent data suggest a delay in pubertal maturation may cause prolonged, possibly irreversible defects in bone mineralization that alters peak bone mass and interferes with normal bone accretion process and causes osteoporosis (Rakover et al., 2000; Moreira-Andres et al., 1998).

Preventability/malleability

Pohl et al. (2007) suggest changes in incidence of hypospadias and associated costs of medical treatment could best be affected by identification of environmental factors that may be responsible for the increase that has occurred over the past several decades. Changes in timing of puberty have been associated with increasing obesity in the U.S. population, which may also be related to environmental factors. The National Children's Study (NCS) is well positioned to evaluate the contribution of HAAs and other environmental factors to these problems, which will provide information on possible preventive measures and interventions that may be effective in reducing the incidence of adverse reproductive development and disease outcomes.

3.2 Justification for a Large Prospective Longitudinal Study

Lack of accurate information on the level and timing of past exposures to HAAs has been the principal limitation of most previous studies of the potential human impacts of known and suspected HAAs. This limitation will be addressed by the prospective design of the National Children's Study in that exposures to chemicals will be measured during pregnancy, in breast milk, and in the perinatal period before the appearance of health effects. Measurement of multiple outcomes related to single and multiple exposures and continuous or repeated exposures is possible with a large longitudinal study. The potential for cumulative effects on the reproductive system can only be discerned through the use of a large longitudinal sample that allows repeated measures of exposure and evaluation of reproductive outcomes over time. Measures of exposure or biomarkers of exposure are available for most HAAs of interest and will link exposures at specific life stages with early or late reproductive outcomes. Methods for measuring gene prevalence and expression will permit examination of genetic polymorphisms that may influence gene-environment interactions to allow assessment of genetically determined interindividual differences in susceptibility to HAAs.

Since the effects of HAAs are gender specific, it will be necessary to study exposure-outcome links separately in males and females, effectively reducing the sample size for each case to approximately 50,000. Susceptible subgroups related to genetic polymorphisms may require additional subgroup studies.

Need to study interactions

Transcriptional regulation of the Fas/FasL pathway by phthalates has been identified as critical to Sertoli cell injury, and polymorphisms in this group of genes are likely to result in differential toxicity (Yao, Lin, Sawhney, & Richburg, 2007). There is recent evidence for polymorphisms in CYP19 (aromatase) influencing age at menarche in girls (Guo et al., 2006) and several of the CYPs are affected by HAAs. Other studies have shown associations between Wnt 7a deregulation in development of the

female reproductive tract and exposure to DES (Sassoon, 1999) or PCBs (Ma & Sassoon, 2006). Changes in expression of several genes have been associated with DES exposure in a mouse model (Newbold et al., 2007). It is likely that many more potential gene-environment interactions will be revealed before children in the NCS reach the age of onset of puberty.

3.3 Scientific Merit

HAAs are chemicals that can interfere with hormonal signaling systems. HAAs may mimic, block, or modulate the synthesis, release, transport, metabolism, binding, or elimination of natural hormones. They may temporarily or permanently alter feedback loops in the brain, pituitary, gonads, thyroid, and other components of the endocrine system (Gore, 2001, 2002). Compounds with estrogenic activity were the focus of most initial concern about HAAs. Chemicals with antiestrogenic, progestogenic, androgenic, antiandrogenic, antithyroid, hypothalamic, and other effects have also come to be recognized (National Research Council [NRC], 1999). The results of endocrine disruption are often not easily detected. They may be subtle and delayed in onset and may have intergenerational effects.

History gives some notable examples of human populations exposed to high levels of HAA compounds that resulted in adverse outcomes for the exposed or their offspring. Herbst et al. (1971) observed several cases of clear cell adenocarcinoma (CCA) of the vagina in young women who had been exposed in utero one to two decades earlier to DES, a synthetic estrogen prescribed to pregnant women in the 1950s and 1960s to prevent miscarriage. In 1976 in Seveso, Italy, community-wide exposure to high concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) occurred after an industrial explosion (Mocarelli et al., 1996, 2000) causing substantially lower male-female sex ratios (0.38 at birth in offspring of the most highly exposed fathers versus 0.56 in unexposed). Sex ratio was also altered among the offspring of men exposed occupationally to TCDD in a pesticide manufacturing plant in Ufa, Bashkortostan, Russia (Ryan, Amirova, & Carrier, 2002). Accidental contamination of the Michigan food chain in 1973 by FireMaster, a fire retardant containing polybrominated biphenyls (PBBs), led to exposure of more than 4,000 persons who ingested contaminated meat and milk. Breast-fed girls exposed to high levels of PBBs in utero had an earlier age of menarche than did either breast-fed girls exposed to lower levels of PBBs in utero or girls who were not breast fed (Blanck et al., 2000).

Synthetically produced HAAs also include pesticides (e.g., DDT and endosulfan), certain plastics (e.g., bisphenol A), and other industrial chemicals (e.g., PCBs, PBDEs, and phthalates) (NRC, 1999). The United States banned some of these substances because they can interfere with endocrine function, including DDT, diethylstilbestrol, and PCBs. Evidence of the ability of HAAs to affect development and reproductive capacity came from studies of wildlife but more recently has been buttressed by in vitro studies that have begun to elucidate some of the molecular mechanisms of action of HAAs. Additionally, clinical and epidemiologic studies have begun to explore the possibility that human exposures to hormonally active compounds, particularly in utero and during early childhood, may be responsible, at least in part, for changes in semen quality, increasing incidence of congenital malformations of the reproductive organs, increasing rates of testicular cancer, and an apparent increase in incidence of precocious puberty.

Research on the human health effects of HAAs is still new. The ability to investigate the possible impacts of HAAs on human health has been limited because most previous studies have employed case-control designs with retrospective assessments of exposure. Except for studies of pharmacologic agents such as DES, most studies have been limited in their ability to accurately assess either the amount or the timing of exposure to putative HAAs. Studies to examine the possible etiologic contributions of HAAs to these trends in the case of hypospadias and cryptorchidism (Toppari et al.,

1996), testicular cancer (Carlsen, Giwercman, Keiding, & Skakkebaek, 1995), and timing of onset of puberty in young girls (Blanck et al., 2000) are needed.

Further study of the potential for genetic effects and the role of genetic polymorphisms will advance our understanding of the effects of HAAs on reproductive development and potential gene-environment interactions. The possibility of epigenetic changes in DNA following HAA exposure that may lead to transgenerational reproductive effects (Anway et al., 2005; Anway, Leathers, & Skinner, 2006; Chang et al., 2006) can be evaluated in samples collected in the National Children's Study. A number of other modes of action for HAAs have recently been reviewed by Tabb and Blumberg (2006), including several which may be investigated in adjunct or follow-up studies to the NCS.

3.4 Potential for Innovative Research

New findings

The strengths of the study include new knowledge to be generated in the following areas:

- Determine which endocrine active chemicals, at what doses, and at what times in development, contribute to the genesis of reproductive and other health problems in children and adults.
- Assess impacts of combinations of HA chemicals.
- Examine modification of HA effects by genetic polymorphisms.
- Study later impacts of early exposures and the cumulative nature of reproductive effects; for example, birth defects of the reproductive organs, alterations in onset of puberty, disease states including PCOS and endometriosis, fertility, and increased risk of breast and testicular tumors.

3.5 Feasibility

Access at critical periods of exposures/outcomes

Evaluation of children at birth, at 6-month intervals during the first 2 years of life, and at regular intervals beyond that will allow assessment of birth defects and anthropometric measures. The proposed approach can be applied successfully to study the impact of HAAs in a sample of 100,000 children with serial assessment of reproductive outcomes at birth, childhood, puberty, and adulthood; access to medical records for disease states such as PCOS; collection of maternal breast milk and maternal and child blood and urine samples at multiple time points; and serial questionnaires to assess pathways of exposure.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Bisphenol A
- Atrazine

- Organochlorines
- Lead, cadmium, mercury, arsenic
- Dioxins
- PCBs
- PBDEs

Primary/child

- Bisphenol A
- Atrazine
- Organochlorines
- Lead, cadmium, mercury, arsenic
- Dioxins
- PCBs
- PBDEs

Secondary/paternal (pilot studies/adjunct study)

- Bisphenol A
- Atrazine
- Organochlorines
- Lead, cadmium, mercury, arsenic
- Dioxins
- PCBs
- PBDEs
- Semen quality

4.2 Methods

Primary/maternal

- Blood sample
- Urine sample

- Breast milk sample

Primary/child

- Blood sample
- Urine sample

Secondary/paternal

- Blood sample
- Urine sample
- Semen sample

4.3 Life Stage

Primary/maternal

- Preconception, prenatal, and throughout nursing

Primary/child

- Birth and throughout nursing, through adolescence

Secondary/paternal

- At time of first positive pregnancy test in mother

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Physical/morphologic malformation
- Hormonal status, particularly thyroid status
- Tanner stage exam (male and female)
- Menstrual history (female)
- Spermarche (male)
- Semen quality (male) (pilot study/adjunct studies)

5.2 Methods

- Urine samples

- Blood samples
- Interview
- Direct observation by a medical professional or via medical record review
- Physical exam (or self exam)
- Semen sample

5.3 Life Stage

- Birth through age 21for birth defects and growth
- Yearly, starting at ages 6 for girls and 7 for boys until age 18 for pubertal assessments
- 18-21 years for semen sample

6. Important Confounders, Mediators, and Effect Modifiers

- **Obesity, diet, and nutrition measures:** Higher percentage of body fat increases the risk of precocious puberty; later onset in underdeveloped nations is often attributed to poor nutrition (Anderson, Dallal, & Must, 2003).
- **Smoking status of parents; urine cotinine:** Smoking, prenatal and postnatal, may reduce the age of onset of puberty (Windham, Bottomley, Birner, & Fenster, 2004). Urine cotinine is measured to examine active/passive smoking exposures.
- **Mother's menstrual and reproductive history:** Generally, the mother's menstrual history is considered the biggest predictor of age of puberty in both male and female children. Some of this effect may be seen in ethnic differences (Blanck et al., 2000).
- **Father's reproductive history:** There are genetic components for hypospadias, cryptorchidism, spermarche, and semen quality that may be related to the father's reproductive history (Pohl et al., 2007).
- **Genetic factors:** 5-alphareductase type 2 gene mutations (Silver & Russell, 1999) and androgen receptor mutations (Silver, 2000) are risk factors for hypospadias.
- **Gestational age at birth:** There is some evidence that a younger gestational age at birth is associated with greater incidence of hypospadias and cryptorchidism (Pohl et al., 2007) and is a predictor of an earlier age at menarche; however, evidence points to small for gestational age (SGA) as the predictor of precocious puberty (Adair, 2001).
- **Mother's alcohol consumption during pregnancy:** Some studies have reported an association between alcohol consumption and hypospadias (Carbone et al., 2007) and an association between later onset of puberty and maternal alcohol use, while other recent studies have reported no effect (Blanck et al., 2000).

- **Socioeconomic status and stress:** The impact of stressful sociologic factors has been related to precocious pubertal development.

- **Certain diseases or conditions:** Obesity and precocious puberty has been associated with conditions such as neurofibromatosis, hypothyroidism, polycystic ovary syndrome, etc. Delayed puberty has been associated with conditions such as sickle cell disease, thalassaemia, Celiac disease, Gaucher disease type 1, Cushing's disease, and other endocrine deficiencies.

7. Power and Sample Size

Birth defects of the reproductive system, growth, body weight, endocrine status (e.g., hypothyroidism), and puberty timing, progression, and completion will be examined in girls and boys. Subgroups include: those in rural communities exposed chronically or seasonally to pesticides; African-American girls (who usually demonstrate an earlier entrance into puberty than other races, implying unique genetic and/or environmental factors); children with certain diseases or conditions (for precocious puberty: neurofibromatosis, hypothyroidism, polycystic ovary syndrome, etc; for delayed puberty: sickle cell disease, thalassaemia, Celiac disease, Gaucher disease type 1, Cushing's disease, etc.); and populations consuming foods such as fish with high concentrations of bioaccumulative endocrine active chemicals.

In terms of gene-environment interactions, assuming 50 percent gene prevalence of polymorphisms in, for example, the Fas/FasL pathway in controls, a control-case ratio of 1, and five exposure quantiles, a sample size of 8,583 is required to detect a 50 percent increase in risk at 90 percent power. If gene prevalence is 10 percent, then 20,556 participants are required. Under these same assumptions, a sample size of 106,661 is required to detect a 20 percent increase in risk at 90 percent power (Spiegelman & Logan, 2001).

Sample size estimates based on existing methods for monitoring puberty and information about the timing of puberty available in the literature generally range between 5,000 and 20,000 children. These were based on estimates of exposure to relatively high levels of chemicals of concern set at 10 percent or 20 percent of the study population. For the most basic assessment of differences in proportions of children reaching puberty early, the NCS has sufficient power (alpha for a two-sided test of 0.05; beta = 0.20) to detect a 1.2 percent difference in the proportion of girls or boys with early pubertal attainment, assuming the prevalence of exposure is 20 percent and the proportion of children with early puberty in the controls is approximately 14 percent. Looking at subgroup analyses with a sample size of 5,000 children where approximately 15 percent are exposed, there is sufficient power to detect a 4 percent difference in the proportion of children attaining puberty early under similar assumptions. In another example, if the rates of hypospadias are approximately 30 per 10,000 among unexposed boys and 50 per 10,000 in exposed boys, the NCS has sufficient power to observe a difference when about 10 percent of the population is exposed.

Refinements in current methods for assessing puberty (e.g., by developing more objective and sensitive indicators, including biochemical and molecular biomarkers) would be expected to improve the power.

8. Other Design Issues

Ethical/ burden considerations:

- The study will need to have a formal strategy and process for effectively communicating results of physiological and biochemical measures to the child's parents and to a responsible health care provider. Any abnormalities identified in blood or anthropometric measurements would be noted and appropriate follow-up medical care recommended.
- The study also will also need to have a have a formal strategy and process for effectively communicating results of environmental monitoring to the child's parents along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings.
- Potential embarrassment about pubertal issues.
- The influence of DNA collection for genetic evaluation and RNA for gene expression profiling presents ethical questions.
- Some minimally invasive procedures are possible.
- Repeated tests are potentially burdensome.

Cost/complexity of data collection:

- It is important to have good retention rates to examine pubertal progression and completion.

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National Children's Study Locations
Vanguard locations: Study Centers awarded (bold)
Vanguard locations: Study Centers pending award (italic)
Lincoln, Pipestone, and Yellow Medicine Counties, Minnesota
and Brookings County, South Dakota
New York City (Queens)
New York
Montgomery County
Pennsylvania
Waukesha County
Wisconsin
Salt Lake County
Utah
Orange County
California
Duplin County
North Carolina
Orange County
Florida
THE NATIONAL CHILDREN'S STUDY

National Children's Study Locations

Vanguard Locations (7 total)

Orange County, CA
Lincoln, Pipestone, and Yellow Medicine Counties, MN and Brookings County, SD
Duplin County, NC
New York City (Queens), NY
Montgomery County, PA
Salt Lake County, UT
Waukesha County, WI

THE NATIONAL CHILDREN'S STUDY
HEALTH GROWTH ENVIRONMENT

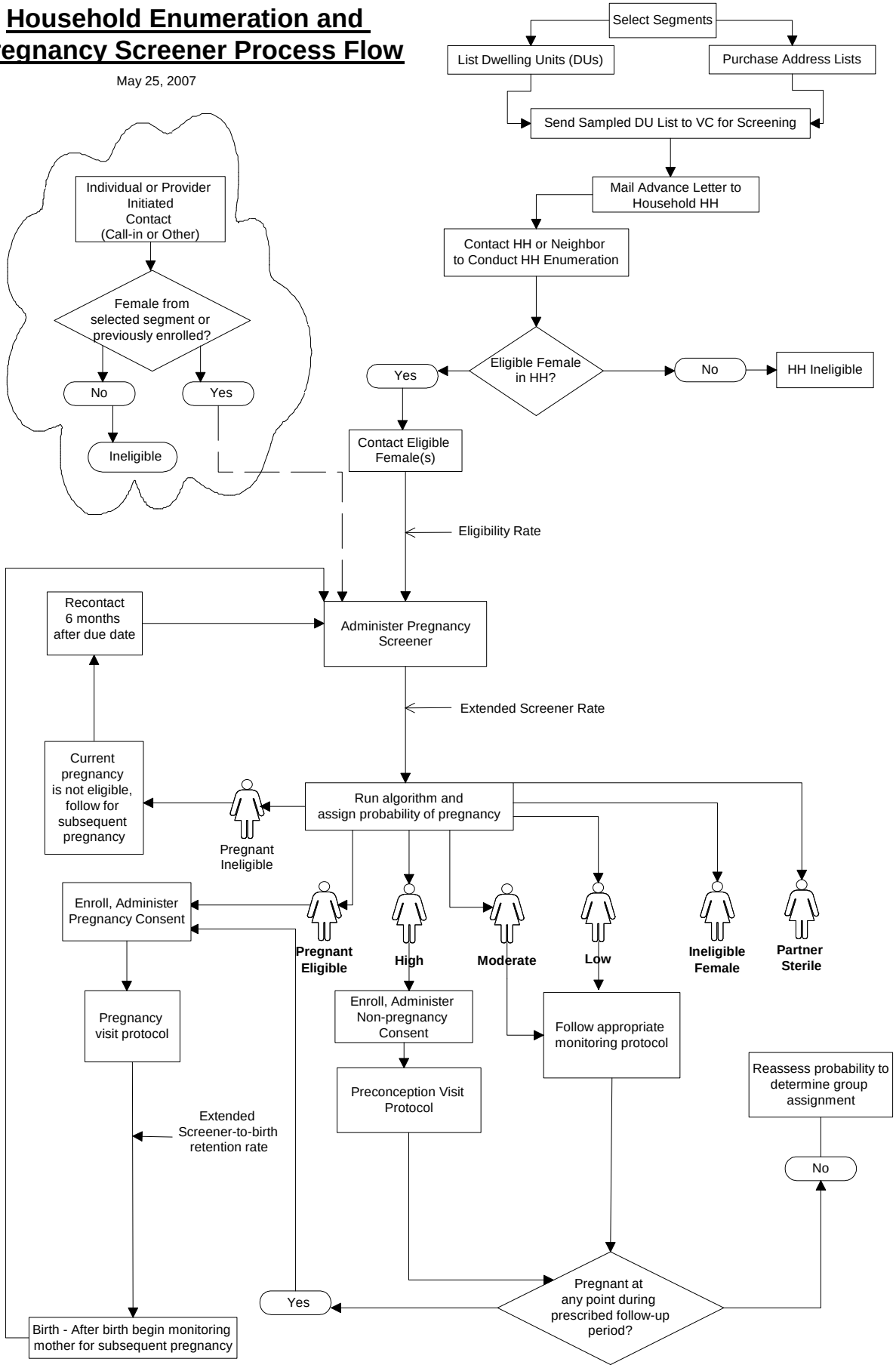
Study Locations (98 total)

Colbert County, AL
Benton County, AR
Apache County, AZ
Maricopa County, AZ
Pinal County, AZ
Humboldt County, CA
Kern County, CA
Los Angeles County, CA
Sacramento County, CA
San Bernardino County, CA
San Diego County, CA
San Mateo County, CA
Ventura County, CA
Denver, CO
Douglas County, CO
Litchfield County, CT
New Haven County, CT
New Castle County, DE
Baker County, FL
Hillsborough County, FL
Miami-Dade County, FL
Orange County, FL
Baldwin County, GA
DeKalb County, GA
Fayette County, GA
Honolulu County, HI
Polk County, IA
Bear Lake County, ID and Lincoln and Uinta Counties, WY
Cook County, IL
DuPage County, IL
Johnson, Union, and Williamson Counties, IL
Macoupin County, IL
Will County, IL
Marion County, IN
Saline County, KS
Jefferson County, KY
Jessamine County, KY
Beauregard and Vernon Parishes, LA
New Orleans, LA
Bristol County, MA
Worcester County, MA
Baltimore County, MD
Montgomery County, MD
Cumberland County, ME
Genesee County, MI
Grand Traverse County, MI
Lenawee County, MI
Macomb County, MI
Wayne County, MI
Becker, Clearwater, and Mahnomen Counties, MN
Ramsey County, MN
Stearns County, MN
Jefferson County, MO
St. Louis, MO
Coahoma County, MS
Hinds County, MS
Buncombe County, NC
Burke County, NC
Cumberland County, NC
Durham County, NC
Gaston County, NC
Rockingham County, NC
Stark County, ND
Burlington County, NJ
Middlesex County, NJ
Passaic County, NJ
Warren County, NJ
Valencia County, NM
Monroe County, NY
Nassau County, NY
New York City (Brooklyn), NY
New York City (Manhattan), NY
Cuyahoga County, OH
Lorain County, OH
Cleveland County, OK
Comanche County, OK
Marion County, OR
Philadelphia County, PA
Schuylkill County, PA
Westmoreland County, PA
Providence County, RI
Spartanburg County, SC
Bradley County, TN
Cumberland and Morgan Counties, TN
Davidson County, TN
Bexar County, TX
Childress, Collingsworth, Donley, and Hall Counties, TX
Dallas County, TX
Harris County, TX
Hidalgo County, TX
Lamar County, TX
Stephens and Young Counties, TX
Travis County, TX
Cache County, UT
Grant County, WA
King County, WA
Thurston County, WA
Marion County, WV

February 2006

Household Enumeration and Pregnancy Screener Process Flow

May 25, 2007



OVERVIEW OF NCS DATA COLLECTION AND CORRESPONDING MODALITIES

This section provides a brief description of the methods for collecting data from NCS participants and their environments and also touches on the measurement domains that can be addressed by each method. Descriptions of the actual measurements are contained in Appendix E and enumerated in the detailed tables included in Appendices F.1, G, H, and I.

1. Interview, Questionnaire, and Diary Data

1.1 In-Person Interviews

Each participant contact within the NCS will include information collected via personal interview or questionnaire. At the in-person contacts, home and clinic visits, the majority of the information will be collected using computer-assisted personal interviewing technology (CAPI). Information about topics with potential sensitivity (e.g., sexual activity) will be collected using computer-assisted self-interview techniques (CASI) with audio capabilities.

1.2 Self-Administered Interviews

At the end of each in-person contact, one or more self-administered questionnaires will be left with the participant to be completed. Dietary exposure (food frequency questionnaire) is an example of a topic to be covered by these self-administered questionnaires. During the initial years of the NCS, participants will be asked to complete paper machine-readable questionnaires that will either be picked up by local data collectors or returned to the local Study Centers by mail. However, in later years, electronic or Internet-based interview tools may be available.

1.3 Telephone Interviews

Brief telephone interviews will be conducted between in-person contacts. An important purpose of the telephone contacts is to verify participant status and tracking information. In addition, brief medical updates and information on potential frequently changing exposures (e.g., change in occupational exposures or child care) will be obtained during most calls. To minimize participant burden, telephone interviews will be brief, between 10 and 15 minutes in length. For selected contacts, automated calls may be considered, but the primary mode of administration will be interviewer-administered telephone interviews.

1.4 Diaries, Medical Provider Logs, and Family Health Records

Data collection in several stages of the NCS will include participant completion of diaries. During early pregnancy, women will be asked to complete a diary on a daily basis. In later pregnancy this will be done on a weekly basis. The diary is designed to collect information on symptoms such as nausea or abdominal cramping and to ascertain potentially important exposures such as medications, alcohol, tobacco and the consumption of potentially allergenic foods.

Similar to the self-completion questionnaires, diaries will be paper and pencil, designed to be completed and mailed in to the Study Centers. Later in the study, electronic options may be available.

Medical provider logs are similar to the diaries because they will be held and completed by the participant. Initially, they will be paper-based instruments; they may evolve to electronic or Internet-based tools depending on the development of appropriate instruments over the course of the NCS. A

medical provider log will be provided for the mother during the pregnancy period and for the child after birth. These logs will be used by the participant to record information concerning the date and type of a participant's contact with the health care system (e.g., routine care, emergency department visit) as well as the diagnosis and additional focused information relevant to the phase of the study. For instance, during prenatal care visits, maternal weight and blood pressure will be recorded; during childhood, information on immunizations will be recorded. Unlike the diaries, the medical provider log is designed to be kept by the participant. Data from the logs will be collected by the Study during in-person and telephone contacts by Study Center staff.

The family health record will be given to women at the first pregnancy visit. An electronic version of this instrument is planned, although a paper and pencil version will also be provided. The family health record is designed to allow the woman to talk with her parents and full siblings to ascertain diagnoses of selected medical conditions, such as diabetes, cancer and depression. At first, the family health record will be a paper form.

2. Biologic Samples

As summarized below and enumerated in detail in the tables included in Appendices E and G, a variety of biologic samples will be collected from all study participants at multiple points throughout the study. These specimens will be analyzed to explore a broad range of chemical and biologic exposures and participants' physiologic response to those exposures and to enable genomic investigations to provide insight into the interaction between an individual's genetic composition and specific environmental factors.

It is important to recognize that limitations in the volume of collected biologic samples (particularly blood), financial constraints, and, perhaps most importantly, the potential future importance of associations between critical outcomes and analytes currently unrecognized preclude immediate and complete analysis of those specimens. Thus, the bulk of biospecimens will be collected, processed, and stored in such a way as to allow the opportunity for later analysis in NCS subpopulations or in nested case-control studies. In general, blood (serum, plasma, and whole blood), urine, and breast milk samples will be refrigerated or frozen as soon as possible at each study site, shipped to the central repository and deep-frozen in small aliquots to minimize thaw-refreeze cycles. In addition to the biospecimens, information relevant to biologic exposures will also be collected through other modalities. For instance, history of recent infectious disease will be obtained through in-person interviews.

2.1 Blood

Blood will be drawn at the prepregnancy, first trimester and third trimester visits for the mother, at the first trimester visit for the biological father and at the 12 month visit for the child. At each visit, blood will be collected in multiple tubes (i.e., red top, purple top, gray top, metal free) to maximize the potential for later analyses (details of volumes, tube types for each visit, and potential analytes for each visit and participant are listed in Appendix G).

Examples of broad areas for which blood will be a primary source of information are:

- Maternal and child endocrine function (e.g., TSH and thyroid hormone);
- Maternal and child infection and inflammatory status (e.g., cytokine and Ig profiles, CRP);

- Nutritional and metabolic status (e.g., folate, antioxidants, HgbA1c); and
- Chemical bioburden (e.g., persistent organic compounds (PCBs, PBDEs, dioxins), bioaccumulative metals (lead, mercury, cadmium).

Whole blood will also be the primary source of genomic DNA for analyses of gene-specific polymorphisms, methylation patterns, and for the presence of exposure-specific DNA adducts. The multiple blood samples obtained from the child throughout the course of the study will enhance the value of the epigenetic and adduct analyses. The use of whole blood RNA for gene expression analyses is also being considered, though the expense associated with those samples may limit this to subpopulations of the NCS.

2.2 Urine

Urine samples will be collected from the mother several times before (for the prepregnancy cohort) and during pregnancy, from the biological father once during pregnancy, and from the child at multiple times (details of volumes and potential analytes for each visit and participant are listed in Appendix G).

Maternal urine will be a main source for evidence of exposure to non-persistent chemicals in the periconceptional period and during pregnancy. Examples of relevant exposures that can be assessed in urine samples include multiple insecticides, herbicides, endocrine active compounds and polycyclic aromatic hydrocarbons. Using amplification methods, maternal urine can also be used to assess genitourinary tract infection or colonization with chlamydia, gonorrhea, and other organisms.

The primary use of urine samples from the biological father (in the early pregnancy period) and the child (from birth through 12 months) is for chemical exposure analyses similar to those outlined for maternal urine.

2.3 Vaginal Swabs

Women will be asked to provide self-collected swabs of vaginal fluid before pregnancy (for those in the prepregnancy cohort) and at the first trimester home and third trimester clinic visits. These swabs will be processed primarily for the assessment of evidence of local inflammation, via analysis for a variety of cytokines, metalloproteinases, and other inflammatory markers. In addition, at each visit, one swab will be rolled onto a slide for later gram stain and assessment for bacterial vaginosis.

2.4 Saliva

Saliva will be collected primarily for assessment of cortisol levels as an indicator of physiologic stress and hypothalamic-pituitary-adrenal axis (HPA) function. Samples will be obtained from the mother twice during pregnancy to enable evaluation of maternal stress and pregnancy outcome, as well as the relationship of fetal exposure to stress and subsequent asthma, neurodevelopment, and other conditions. Maternal and paternal samples will be obtained at the 6 month postnatal visit as an indicator of stress, to be used in the evaluation of child development, family process, and related domains. In early childhood, saliva samples will be collected at the six month visit from the mother and the biological father and at the 12 month visit for the child for evaluation of HPA activity.

At each relevant visit, saliva collection kits will be given to the participant. Instructions will be provided for the collection of multiple (2 to 3) samples to be obtained at specific times over two days.

2.5 Hair and Fingernails

Hair and toenail samples will be used as summary indicators of exposure to persistent chemicals, predominantly heavy metals and mercury. They will be obtained from the women in the prepregnancy cohort, and from pregnant women at the first and third trimester visits as well as from the father at the first trimester visit, thus enabling estimates of fetal exposure to those compounds from conception through birth. Hair samples will be obtained from the child at the 6 and 12 month visits, whenever possible, to allow assessment of chronic early life exposure to metals.

2.6 Birth-Specific Biospecimens

Samples collected at birth include cord blood, cord and placental samples, and meconium. Biologic samples obtained at birth are key to understanding the extent to which the maternal environment relates to actual fetal exposure and fetal response to *in utero* exposures.

Placental samples, obtained using random grid sampling, will be prepared to enable histologic assessment for local inflammation or infection as well as for chemical analysis. A membrane roll will also be obtained for similar analyses; in particular, because of their low rate of turnover, the membranes are a useful indicator of long-term exposure to persistent contaminants.

Similarly, cord samples obtained close to the fetus will be collected and processed to allow for histologic and chemical analysis. The comparison of maternal, placental, and proximal cord samples can, for instance, establish gradients between maternal exposure and that "seen" by the developing fetus. While fetal inflammation is well-assessed using cord samples, the degree to which those samples are useful for assessment of certain chemical exposures, particularly lipophilic compounds, is less certain.

To maximize the collected volume, mixed venous-arterial samples of dripped cord blood will be obtained in the delivery room. The large volumes of cord blood will be useful for genetic analyses–"pure" maternal and child samples will be available to address concerns about the potential contamination of dripped samples – as well as the biologic and chemical analyses discussed previously. In addition, cord blood can be paired with neonatal heel capillary samples obtained at 24-48 hours of age, providing an opportunity to examine metabolic and physiologic changes early in the perinatal period.

Finally, meconium will be collected from the nursery and analyzed for the presence of chemical exposures that may be difficult to obtain from other samples – metabolites of organophosphate pesticides, for example – or for a summary measure of fetal exposure to other compounds, such as cotinine.

2.7 Breast Milk

In addition to its nutritive value, breast milk is a marker of maternal bioburden as well as a medium for transfer of potentially beneficial (e.g., immunoglobulins, essential fats) and harmful (PCBs, other lipophilic chemicals, infectious agents) from mother to child. Breast milk samples will be self collected at approximately 1 and 3 months, using kits provided at the in-hospital birth visit, and following the 6 month visits, using kits provided at those times. For the NCS, the primary purpose of breast milk collection is to assess for infant exposure to lipophilic persistent organic compounds such as PCBs, dioxins, furans, and others. However, samples will also be available for other analyses such as maternal transfer of lipids and antioxidants, immunoglobulin and cytokines, and additional chemical contaminants such as perchlorate.

3. Environmental Samples

As summarized below and presented in overview in Appendix E and in detail in the tables included in Appendix H, the NCS will collect a variety of environmental samples from the homes of women during the prepregnancy period as well as from the child's home after birth. Environmental samples are crucial to the quantification of the potential exposure of a child or developing fetus to substances which cannot be identified through biomarkers. In addition, the coupling of environmental samples with biologic samples from the parents and child will provide a detailed picture of the temporal relationship between multiple exposures and child outcomes, a combination critical to the development of research-based strategies to improve child health.

The laboratory analysis plans for environmental samples share the same philosophy as those for the biologic samples; analyses central to NCS hypotheses or that cannot be deferred because of technical reasons, primarily due to degradation of sample or specific analyte, will be performed in a timely fashion. Other samples will be stored in such a way as to maximize the utility of future analyses based on the evolution of research questions, analytic techniques, and the availability of funding.

This section will concentrate on samples obtained from the home(s) of NCS participants. Procedures for collecting samples from participants' child care and school environments are still being developed. Collection of samples from the community or neighborhood is outlined in Section 5.

3.1 Indoor Air

A diverse set of indoor residential air samples will be obtained at each home visit, starting with the pre-pregnancy visit. Air samples will be collected over a multi-day period to obtain an average integrated exposure. A pump will be employed to collect samples for particulate matter (PM_{10}) and related compounds (e.g., metals, elemental carbon). To increase the Study's efficiency, the collection and analysis of some air samples - ozone, for instance - will be triggered by specific information collected at the time of the visit, such as the presence of a photocopier or laser jet printer in the home.

Volatile organic compounds and nitrogen oxides (NOx) will be collected via badges placed in specified locations within the household. In addition to collections associated with the home visits, self-collection kits will be given to participants at the third trimester clinic visit and mailed to participants when the child is 24 months old. These will be deployed by the participant and mailed back to the Study Centers according to directions given at the clinic visit and supplied with the kits.

This Study currently relies on household and neighborhood air samples and does not include personal air sampling. Through time, as technology advances and as the NCS cohort develops, provisions for personal air sampling may be developed.

3.2 House Dust

Dust samples will be obtained by surface wipes, vacuum, and by accretion onto dust mats, depending on the location of the sample and the targeted analytes. For women in the prepregnancy cohort, organophosphate, pyrethroid, and carbamate pesticides residues will be ascertained by dust wipes. Starting in pregnancy, at each home visit a wider array of samples will be obtained. Wipes and mats will be obtained to allow for assessment of metals, pesticides, and other compounds. Vacuum samples, particularly during the visits after the child's birth, will be collected for potential examination of allergens, mold, and endotoxins. A bulk dust will be archived for future analysis of analytes as yet to be determined.

Similar to the self-collected indoor air samples, dust mats, wipes, and the vacuum dust collector will be provided at the third trimester clinical visit for self-collection of samples targeted for pesticide and metal analyses.

3.3 Drinking Water

Household tap water will be collected at each home visit, though the types of samples will vary somewhat depending on whether the household uses local well water or is served by a community water supply. Starting in pregnancy, water from all households will be collected for heavy metal assessment. Houses using well water will have samples collected for perchlorate and pesticide analysis starting with the prepregnancy visit. In contrast, houses served by a community water supply will have samples collected for analysis of disinfection byproducts and volatile organic compounds, starting with the first trimester visit.

3.4 Soil

Soil samples will be obtained from households in agricultural areas for ascertainment of pesticide exposure at the 1st trimester visit. Additionally, soil samples will be obtained from the perimeter and midyards of homes at the 6 and 12 month home visits. Analysis of these samples is primarily targeted toward identification of metals and pesticides potentially encountered by an infant or toddler. Samples obtained from properties with evidence of pressure treated wood (CCA) structures will be collected during pregnancy as well as during the 6 and 12 month visits.

3.5 Noise Survey

Assessment of household ambient noise, both indoor and outdoor, is scheduled for the 12 month visit.

4. Physical Examination Measures

As enumerated below and in Appendix I, a brief physical assessment of NCS participants will occur at each in-person contact. In general, these examinations and observations will comprise a limited set of standardized, objective measures directly related to the NCS priority outcomes. Most of the examination measures are designed to assess physiologic status without making a specific clinical diagnosis. For instance, pulmonary function tests will be attempted starting at the 3 year clinical visit, but auscultation of the chest for wheezing will not be included in the study protocol. Exceptions to this general principle include some of the neurodevelopmental tests; for some conditions, positive screening (such as the M-CHAT for autism) may be followed by more specific tests or exams (e.g., ADOS) that are sometimes used for clinical diagnosis.

The first clinical visit for NCS children occurs at 3 years of age. Since this document focuses on measures obtained through the 24 month visit, only measures that will be obtained during pregnancy from the mother and at the early home visits for the child will be described.

4.1 Anthropometric Measures and Body Composition

Assessment of a child's physical growth and adiposity is important in its own right, as well as being a key factor related to subsequent outcomes such as diabetes, metabolic syndrome, and onset of puberty. Parental habitus likely influences the child's status through genetic and behavioral mechanisms and the potential short and long term influences of maternal adiposity on the child's *in utero* environment are critical areas of study for the NCS.

Baseline measurements from the mother will be obtained at the prepregnancy visit and then at each contact during pregnancy. Measurements will include weight; standing height, sitting height and other segmental measures (obtained one-time only); mid-arm, hip, and waist circumferences; and triceps and subscapular skin folds. Similar measurements will be performed on the biological father at the first trimester home visit.

Physical assessment of the child will start in the fetal period via several ultrasound assessments (Section 4.2). At birth, in addition to weight and standardized length, multiple circumferential measures, including head, abdomen, mid-arm, and thigh, will be taken. Triceps and subscapular skin folds will be obtained, as well. These measurements will be repeated during the 6 and 12 month home visits.

Additional assessments of the child's body composition, including Dual Energy X-Ray Absorptiometry, Bioelectric Impedance Analysis, or bone ultrasound, are likely to be included at the later clinical visits.

4.2 Fetal Ultrasound

Up to three fetal ultrasounds will occur as part of the NCS. If an early ultrasound is not performed as part of the woman's prenatal care, or if these results are not available, an NCS-sponsored ultrasound will be obtained toward the end of the first trimester to assure accurate pregnancy data. Subsequently, all women will be scheduled to have standardized NCS-sponsored ultrasounds obtained toward the end of the second trimester (approximately 22-24 weeks) and early to mid third trimester (28-32 weeks). These scans will focus on obtaining accurate standardized measures of fetal growth. Standardized AIUM protocols will be used for standard linear and circumferential measures, such as bi-parietal diameter, femur length, and abdominal circumference. The assessment of relative lean and fat mass will be attempted using mid-thigh circumferences, recognizing that those measurements are not as standardized and tested as the more routinely used measurements of linear growth.

The use of NCS fetal ultrasounds for identification of birth defects remains under discussion. It is possible that a series of views used in routine anatomic surveys may be stored digitally for later analysis. Regardless, strict protocols regarding appropriate follow-up of questionable or suspicious findings seen during any NCS ultrasound acquisition will be adhered to.

4.3 Blood Pressure

Blood pressure measurements will be obtained at each contact with the mother before and during pregnancy, from the father at the first trimester visit, and from the child starting with the 12 month home visit. At each visit, a participant's blood pressure assessment will consist of multiple measurements obtained using a calibrated automated device.

4.4 Dysmorphology Examination and Photography

Assessment of the child for congenital anomalies is essential for the NCS, both as a priority outcome and to enable appropriate evaluation of other outcomes, such as cognitive or motor development. It is expected that the initial source for the identification of congenital anomalies will be the abstraction of the prenatal and neonatal medical records at the delivery hospital, followed by questions on subsequent child questionnaires during infancy to identify malformations, such as some cardiac defects, that may not have been diagnosed at birth. However, there is also benefit for the NCS to conduct a targeted dysmorphology observation examination. Complete medical records may not be available. Also, there is interest in obtaining more subtle morphologic measures not reported in medical records (e.g., inter-

canthal distances, ano-genital distance) that may be associated with neurodevelopmental or other outcomes.

Unfortunately, despite the proliferation of studies on birth defects, a commonly-used, general standardized dysmorphology exam suitable for a large population-based epidemiologic study is lacking. A protocol is being developed for acquisition of digital facial photographs that can be stored and later retrieved for analysis of facial features and, perhaps, morphologic measurements. The examination and photographs will be obtained at the initial neonatal visit prior to discharge and be repeated at least once at either the 6 or 12 month visit.

4.5 Neurodevelopmental Examinations and Observations

A primary focus of the NCS is the longitudinal assessment of neurologic and behavioral development, starting at birth. Some of this assessment will occur through the administration of parental questionnaires and, later in life, recording of school performance. In addition, standardized assessment and observation of developmental activities is an important part of the NCS protocol. A wide array of neurobehavioral instruments allows examination of numerous aspects of neurologic development. For the NCS, tools will be chosen that are well-known and with recognized interpretability, relatively easily applied in a standardized fashion, and suitable for use in a geographically diverse population-based epidemiologic study.

At the birth visit, initial assessment will be via the Neonatal Intensive Care Unit Network Neurobehavioral Scale. The Bayley scales for cognitive, motor, and language development will be applied starting with the 12 month home visit. In addition, at the 6 month and the 12 month visit, the child's social development will be assessed via application of a standardized interaction between the child and one parent (mother at 6 months, father at 12 months, if available). This interaction will be videotaped for later standardized assessment.

5. Community-Level Measures

A child's health and developmental trajectory are influenced not only by individual and family characteristics, but also by those of the community in which he or she is raised. The NCS will incorporate two broad types of community-level data. The first is that which is collected as a matter of course by other agencies or organizations. Examples of these data include census information, crime reports, EPA ambient air monitor data, health provider capacity, and school district test scores. A basic set of these secondary community-level data, such as census population characteristics (e.g., population density, area income distribution) will be appended onto the NCS data files. The ability to link other secondary data to the NCS data will be available to researchers; procedures for this linkage will depend on the geographic detail of the linkage (e.g., NCS segment, census block, county, state, GPS coordinates) and associated confidentiality concerns.

The second variety of community-level data incorporated into the NCS is that which will be collected by the NCS. Environmental measures, such as community water supply samples and local air monitoring for particulate matter and other pollutants, will be collected. Study personnel will assess the physical environment of participants' neighborhoods by observation, using a structured instrument such as the Irvine-Minnesota Inventory. Examples of areas to be assessed include amenability for walking, biking, and other physical activity; presence of retail establishments; industrial or chemical sites; and other measures of land use.

NCS PROTOCOL OVERVIEW AND SUMMARY OF CONTACTS
June 20, 2007 – Version 1.3

	Prepregnancy					Pregnancy								Birth				Postnatal							
	P1 Home	Materials left behind and picked up after P1[1]	P 1 Month (Phone)	P 2 Month (Phone)	P 4 Month (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1[1]	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3[1]	36 Weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2[1]	1 month visit, if needed[2] (Home)	3 months (Phone)	6-month visit (Home)	Materials left behind and picked up after 6-mo. visit[1]	9 months (Phone)	12-month visit (Home)	Materials left behind and picked up after 12-mo. visit[1]	18 months (Phone)	24 months (Phone)
Informed consent/Detailed visit information/Medical release, as needed	M					M F	M F				M			M	M		M		M			M F			
Interviews/Assessments/Questionnaires																									
In-person/Phone	M		M	M	M	M F	M F		M		M		M		M		M	M	M C		M	M F C		M	M
Self-administered questionnaire		M						M F				M				M				M F	F		M F	F	
Diaries/Medical visit logs																									
Pregnancy diary						M	M	→	→	→	→	→	→												
Medical provider visit log						M	M	→	→	→	→	→	→		C	→	→	→	→	→	→	→	→	→	→
Environmental																									
Indoor air	M					M	M												X			X			
House dust	M					M	M												X			X			
Drinking water	M					M	M												X			X			
Soil						M	M												X			X			
Visual assessment	M					M	M												X			X			
Noise survey																						X			
Indoor air (self-collected)												M													X
House dust (self-collected)												M													X
Physical exam																									
Anthropometric	M					M F	M F				M				C		C		C			C			
Blood pressure	M					M F	M F				M								C			C			
Ultrasound								M[3]		M	M														
Dysmorphology															C		C								
Physical exam																			C			C			
Observational photos															C		C								
Biospecimen collection																									
Pregnancy tests (self-collected)		M																							
Vaginal swabs	M					M	M				M														
Blood/Buccal cell[4]	M					M F	M F				M			M								C			
Blood spot (heel and cord)														C											
Urine (self-collected)		M[5]						M F			M			M					C			C			
Hair	M					M F	M F				M								C			C			
Nails						F	F				M														
Cord blood														C											
Umbilical cord														M											
Placenta														M											
Meconium															C										
Breast milk (self-collected)																	M	M		M					
Saliva (self-collected)								M				M								M F			C		
Other																									
Medical record/Chart abstraction								M[3]							M C										
Community-based food collection								M																C	
Child care locations																					CC				CC
Community/Neighborhood assessment								M												X		X			X[6]

KEY: M=MOTHER F=FATHER C=CHILD X=CHILD'S PLACE OF RESIDENCE CC=CHILD'S CHILD CARE LOCATION(S)

[1] Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail in self-collected urine sample, complete self-administered questionnaire and mail in). Time frame for completion varies and is specific to each activity.
[2] A home visit will be conducted at 1 month if certain child measures are not completed at the birth visit.
[3] This ultrasound will only be conducted for women who do not already have a first trimester ultrasound as part of routine care (see protocol).
[4] Buccal cells for DNA will be collected as a backup from the mother and father at the T1 1st or Prior visit and the child at the 36-month visit when blood is not drawn.
[5] These biospecimen collections are intended to measure environmental exposures closer to the time of conception and includes two separate collections.
[6] Update data collected at 6- and 12-month visits.

NCS RESEARCH PLAN OVERVIEW
SUMMARY OF QUESTIONNAIRE AND PSYCHOLOGICAL/DEVELOPMENTAL ASSESSMENTS
June 20, 2007 – Version 1.3

	Prepregnancy					Pregnancy								Birth				Postnatal							
	P1 Home	Materials left behind and picked up after P1[1]	P 1 month (Phone)	P 2 month (Phone)	P 4 month (Phone)	T1-1st[2] (Home)	T-Prior[2] (Home)	Materials left behind and picked up after T1[1]	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3[1]	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Predischarge (Hospital)	Materials left behind and picked up after B2[1]	1 month visit, if needed[3] (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit[1]	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit[1]	18 months (Phone)	24 months (Phone)
Informed consent/Detailed visit information/Medical release, as needed	M					M F	M F				M			M			M		M F			M F			
Interview/Assessments																									
Household composition and demographics																									
Household composition	M					M	M				M								M			M			
Age, race, ethnicity, relationship, marital status	M					M F	M				M								M			M			
Biological father information						M	M																		
Education	M					M F	M															M			
Employment	M					M F	M				M								M			M			
Income	M					M F													M			M			
Supported by family income	M					M F	M				M								M			M			
Bank account, financial problems, home ownership	M					M F																			
Food security	M					M F	M												M						
Health insurance	M					M F	M				M								M			M			
Religious affiliation						M F	M																		
Culture and acculturation						M F	M													F					
Perceived stress																									
Global perceived stress						M	M				M								M						
Racism/Discrimination						M	M																		
Life events									M		M														
Parenting stress																			M						
Work/Family stress																			M						
Social support						M	M				M								M						
Family process																									
Quality of relationships						M	M												M	M F			M F		
Domestic violence						M	M				M														
Division of labor																			M						
Health behaviors (maternal)																									
Physical activity	M					M	M																		
Maternal sleep	M					M	M												M						
Eating disorders	M					M																			
Implanted medical devices	M					M																			
Douching	M					M	M																		
Dental health	M					M					M														
Health status/Functional limitations/Impairment						M	M																		
Tobacco use						M	M				M				M		M								
Environmental tobacco smoke exposure						M	M								M		M		M			M			
Frequency/Quantity of alcohol use						M	M				M														
Binge drinking						M	M												M			M			
Illicit drug use and abuse of prescription drugs						M	M				M								M			M			
Diet and toxicant exposure through food (mother)				M																					
- Food frequency questionnaire (self-administered questionnaire)		M						M				M				M									
- 3-day checklist (self-administered questionnaire)		M						M				M													
Diet and toxicant exposures through food (child)																		M							
- Child feeding form (mailed self-administered questionnaire)																M				M			M		
- Child food frequency questionnaire (mailed self-administered questionnaire)																								M	
- Child 3-day checklist (mailed self-administered questionnaire)																				M			M	M	
- Food behaviors[4]																									
Media exposure in children																			M			M			

KEY: M=MOTHER F=FATHER C=CHILD

[1] Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete self-administered questionnaire and mail in). Time frame for completion varies and is specific to each activity.
[2] T1 prior measures and activities will be conducted with the respondents who were enrolled prior to conception and completed a P1 visit. The T1 first visit will be conducted with women who are enrolled during their first trimester of pregnancy.
[3] This visit is only conducted if certain child measures are not completed at the B2 (predischarge) visit.
[4] Measurement time frame to be determined.

NCS RESEARCH PLAN OVERVIEW
SUMMARY OF QUESTIONNAIRE AND PSYCHOLOGICAL/DEVELOPMENTAL ASSESSMENTS
June 20, 2007 – Version 1.3

	Prepregnancy					Pregnancy								Birth				Postnatal							
	P1 Home	Materials left behind and picked up after P1[1]	P 1 month (Phone)	P 2 month (Phone)	P 4 month (Phone)	T1-1st[2] (Home)	T-Prior[2] (Home)	Materials left behind and picked up after T1[1]	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3[1]	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Predischarge (Hospital)	Materials left behind and picked up after B2[1]	1 month visit, if needed[3] (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit[1]	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit[1]	18 months (Phone)	24 months (Phone)
Informed consent/Detailed visit information/Medical release, as needed	M					M F	M F				M			M			M		M F			M F			
Interview/Assessments																									
Household composition and demographics																									
Household composition	M					M	M				M								M			M			
Age, race, ethnicity, relationship, marital status	M					M F	M				M								M			M			
Biological father information						M	M																		
Education	M					M F	M															M			
Employment	M					M F	M				M								M			M			
Income	M					M F													M			M			
Supported by family income	M					M F	M				M								M			M			
Bank account, financial problems, home ownership	M					M F																			
Food security	M					M F	M												M						
Health insurance	M					M F	M				M								M			M			
Religious affiliation						M F	M																		
Culture and acculturation						M F	M													F					
Perceived stress																									
Global perceived stress						M	M				M								M						
Racism/Discrimination						M	M																		
Life events									M		M														
Parenting stress																			M						
Work/Family stress																			M						
Social support						M	M				M								M						
Family process																									
Quality of relationships						M	M												M	M F			M F		
Domestic violence						M	M				M														
Division of labor																			M						
Health behaviors (maternal)																									
Physical activity	M					M	M																		
Maternal sleep	M					M	M												M						
Eating disorders	M					M																			
Implanted medical devices	M					M																			
Douching	M					M	M																		
Dental health	M					M					M														
Health status/Functional limitations/Impairment						M	M																		
Tobacco use						M	M				M				M		M								
Environmental tobacco smoke exposure						M	M								M		M		M			M			
Frequency/Quantity of alcohol use						M	M				M														
Binge drinking						M	M												M			M			
Illicit drug use and abuse of prescription drugs						M	M				M								M			M			
Diet and toxicant exposure through food (mother)				M																					
- Food frequency questionnaire (self-administered questionnaire)		M						M				M				M									
- 3-day checklist (self-administered questionnaire)		M						M				M													
Diet and toxicant exposures through food (child)																		M							
- Child feeding form (mailed self-administered questionnaire)																M				M			M		
- Child food frequency questionnaire (mailed self-administered questionnaire)																								M	
- Child 3-day checklist (mailed self-administered questionnaire)																				M			M	M	
- Food behaviors[4]																									
Media exposure in children																			M			M			

KEY: M=MOTHER F=FATHER C=CHILD

[1] Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete self-administered questionnaire and mail in). Time frame for completion varies and is specific to each activity.
[2] T1 prior measures and activities will be conducted with the respondents who were enrolled prior to conception and completed a P1 visit. The T1 first visit will be conducted with women who are enrolled during their first trimester of pregnancy.
[3] This visit is only conducted if certain child measures are not completed at the B2 (predischarge) visit.
[4] Measurement time frame to be determined.

Preconception Visit Maternal
In-Person Questionnaire Outline[1]

Number of Items	Topic	Source
HOUSEHOLD COMPOSITION AND DEMOGRAPHICS		
4 items	**Household Composition** (listing of all household members)	NHIS 2000, New
7 items	**Age, Sex, Race, Ethnicity, Relationship** (all household members)	NHIS 2000, New
2 items	**Marital Status and Education** (highest level completed; maternal and resident partner)	NHIS 2000, Census
3 items	**Employment** (hours worked; maternal and resident partner)	SIPP, New
ENVIRONMENT		
26 items	**Housing Characteristics** (heating, ultrasonic vaporizer, electrostatic air cleaner, portable ozone generator, photocopier, proximity and usage of garage, gasoline exposure, noise, building demolition exposure)	Tucson, AHHS, AHS, ALSPAC, NHEXAS, New
13 items	**Pets and Pesticide Use** (type, application method, frequency, protective equipment worn, number and type of pets, exposure to flea/tick treatments)	AHHS, CHAMACOS, NHANES, ALSPAC
17 items	**Time and Activity** (time at home, work and in transit, typical work day and typical non-work day)	BRFSS, New
27 items	**Occupational/ Hobby Exposures** (number and type of jobs/schooling, job activities, exposure to chemicals/pesticides as part of hobby)	CHAMACOS, NESEH, NHANES
58 items	**Occupational Modules** (modules for selected occupations such as agricultural worker, barber/beautician, child care worker)	New – compilation of questions from 20 studies

[1] All questions collect maternal information unless otherwise specified.

Number of items	Topic	Source
ENVIRONMENT (continued)		
79 items	**Activity Modules** (modules related to behaviors associated with selected hobbies or activities such as wood dust exposure and personal protective equipment)	New – compilation of questions from 20 studies
30 items	**Product Use** (use of creams, lotions, cleaning products)	NHEXAS
HEALTH BEHAVIORS		
15 items	**Physical Activity** (frequency and duration of moderate and vigorous physical activity, walking and sitting during past week)	IPAQ
5 items	**Routine Health Care** (place where normally go when sick and when need routine care)	New
9 items	**Dental Health** (routine cleaning, bone loss, gum health, use of dental floss, use of mouthwash)	Bruce Dye (NHANES) proposed by Oral Health Epi Research Group
12-16 items	**Self-rated Health** (self-rated overall physical and mental health, ability to do normal activities, impairment and functional status)	Census
4 items	**Sleep** (amount of sleep during night and day during past week)	NHLBI's Assessing Child and Maternal Sleep in the Early Years (Hunt)
3 items	**Douching** (frequency and type)	Mark Klebanoff
5 items	**Implants** (type, age at insertion and history)	Medical Device Implant Supplement to the 1998 NHIS
4 items	**Eating Disorders** (past or current history)	Marion Balsam

Number of items	Topic	Source
MEDICAL HISTORY		
26 items	**Reproductive and Pregnancy History** (menarche, age at first pregnancy, pregnancy outcomes, multiple births, gender, birth weight, birth date, mode of delivery, infant death, birth defects, medical care received)	Modified CAPS, Original and modified NHIS, Ken Schoendorf, Ruth Brenner
6 items	**Maternal Birth History** (birth weight, multiple birth)	Unknown, requested by protocol review team
33 items	**Use of Medicines** (prescription, non-prescription, supplements and alternative medicine)	Original and modified NHANES 2005, New
INCOME AND HEALTH INSURANCE		
7 items	**Income** (sources of income, income level, members supported by family income; all household members)	SIPP
4 items	**Financial Stress** (bank account, financial problems, home ownership)	Fragile Families, ECLS-B
3 items	**Food Security** (ability to obtain food, type of food desired; all household members)	USDA Food Security Scale
3 items	**Health Insurance** (type and how obtained)	NHIS 2004
TRACING INFORMATION		
11 items	**Tracing Information** (moving plans, alternate contact information)	New

Preconception Visit Maternal
Mail Questionnaire Outline

Number of items	Topic	Source
ENVIRONMENT		
201 items	**Diet and Toxicant Exposure through Food** (frequency and quantity of target foods eaten in past month)	FFQ, 3-Day Checklist

Preconception Follow-up Maternal Telephone Questionnaire Outline[2]

Number of items	Topic	Source
TRACING INFORMATION		
5 items	**Tracing Information** (address and marital status updates)	New
MEDICAL HISTORY		
15 items	**Pregnancy Update** (whether pregnant/trying to become pregnant, birth control methods, when learned pregnant, length trying to become pregnant, use of pregnancy diary)	P1 HH composition and demographic graphics questions, New
HOUSEHOLD COMPOSITION AND DEMOGRAPHICS		
3 items	**Employment Update** (changes in employment status, job title or occupation)	P1 HH composition and demographic graphics questions
ENVIRONMENT		
6 items	**Occupational Exposures[3]** (changes in work or schooling, new job titles, new job industry)	New
4 items	**Pets and Pesticides** (use of insecticides or flea/tick treatments)	AHHS, CHAMACOS, NHANES
1 item	**Dietary Intake[4]** (food consumption in past month)	FDA FIRST (plus items from contaminant checklist)

[2] These questions are asked at 1 month, 2 months and 4 months following the preconception visit. The number of questions asked about each topic varies depending on the follow-up contact time period. All questions collect maternal information.
[3] The Occupational/Hobby Exposures questions are asked only at the 2 month follow-up contact.
[4] The Dietary Intake question is asked only at the 2 month follow-up contact.

Number of items	Topic	Source
ENVIRONMENT (continued)		
5 items	**Housing Characteristics**[5] (use of electrostatic air cleaners, portable ozone generators, photocopiers, exposure to gasoline)	BRFSS, AHHS, CHAMACOS, NHANES, NHEXAS
20 items	**Time and Activity**[6] (time at home, work and in transit, typical work day and typical non-work day)	BRFSS, New
INSTRUCTIONS FOR FURTHER PARTICIPATION		
3 items	**Instructions for Further Participation in the Study** (access to pregnancy tests, reminder to call when pregnant)	New

[5] The Housing Characteristics questions are asked only at the 4 month follow-up contact.
[6] The Time and Activity questions are asked only at the 4 month follow-up contact.

First Trimester Maternal
In-Person Questionnaire Outline[7,8]

Number of items	Topic	Source
ENVIRONMENTAL TRIGGERS		
2 items	**Environmental Triggers** (use of tap water as main water source, insecticide use)	NHIS 2000, New
HOUSEHOLD COMPOSITION AND DEMOGRAPHICS		
3-4 items	**Household Composition** (listing of all household members)	NHIS 2000, New
3 items	**Biological Father Information** (name and address)	New
6 items	**Age, Sex, Race, Ethnicity, Relationship** (all household members)	NHIS 2000, New
2 items	**Marital Status and Education** (highest level of education completed; maternal and biological father/ resident partner)	NHIS 2000, Census
ENVIRONMENT		
35-44 items	**Housing Characteristics** (heating, air conditioning, air filtration, clothes dryer, ultrasonic vaporizer, electrostatic air cleaner, portable ozone generator, photocopier, vacuum cleaner use, proximity and usage of garage, gasoline exposure, noise, building demolition exposure)	Tucson, AHHS, AHS, ALSPAC, NHEXAS, New
11 items	**Pets and Pesticide Use** (type, application method, frequency, protective equipment worn, number and type of pets, exposure to flea/tick treatments)	AHHS, CHAMACOS, NHANES, ALSPAC

[7] All questions collect maternal information unless otherwise specified.
[8] The number of items asked in each section may vary depending on whether the study participant was interviewed prior to becoming pregnant.

Number of items	Topic	Source
ENVIRONMENT (continued)		
29 items	**Time and Activity** (time at home, work, and in transit for a typical weekday and a typical weekend day, age of car, seatbelt use, ventilation in car)	BRFSS, New
23 items	**Occupational/ Hobby Exposures** (number and type of jobs/schooling, job activities, work/schooling hours, hobbies, ventilation systems while doing hobbies, exposure to dust, exhaust, chemicals while doing hobbies)	CHAMACOS, NESEH, NHANES
58 items	**Occupational Modules** (modules for selected occupations such as agricultural worker, barber/beautician, child care worker)	New – compilation of questions from 20 studies
79 items	**Activity Modules** (modules related to behaviors associated with selected hobbies or activities such as wood dust exposure and personal protective equipment)	New – compilation of questions from 20 studies
30 items	**Product Use** (use of creams, lotions, cleaning products)	NHEXAS
HEALTH BEHAVIORS		
16 items	**Physical Activity** (frequency and duration of moderate and vigorous physical activity, walking and sitting, during past week)	IPAQ
5 items	**Routine Health Care** (place where normally go when sick and when need routine care)	New
9 items	**Dental Health** (routine cleaning, bone loss, gum health, use of dental floss, use of mouthwash)	Bruce Dye (NHANES) proposed by Oral Health Epi Research Group

Number of items	Topic	Source
HEALTH BEHAVIORS (continued)		
12-16 items	**Self-rated Health** (self-rated overall physical and mental health, ability to do normal activities, impairment and functional status)	Census
4 items	**Sleep** (amount of sleep during night and day, past week)	NHLBI's Assessing Child and Maternal Sleep in the Early Years (Hunt)
3 items	**Douching** (frequency and type)	Mark Klebanoff
5 items	**Implants** (type, age at insertion and history)	Medical Device Implant Supplement to the 1998 NHIS
4 items	**Eating Disorders** (past or current history)	Marion Balsam
MEDICAL HISTORY		
3-23 items	**Pregnancy History** (menarche, age at first pregnancy, pregnancy outcomes, multiple births, gender, birth weight, birth date, mode of delivery, infant death, birth defects)	Modified CAPS, Ken Schoendorf, Ruth Brenner
12 items	**Use of Fertility Services** (timing/desire for pregnancy, use of pregnancy services and pregnancy drugs)	NSFG Cycle 6 2003, New
31 items	**Reproductive History** (medical care received, birth plans, doctor information)	Modified CAPS, original and modified NHIS, New
1-5 items	**Maternal Birth History** (birth weight, multiple births)	Unknown, requested by protocol review team

Number of items	Topic	Source
MEDICAL HISTORY (continued)		
1 item	**Maternal Medical History** (asthma, allergies, hypertension, diabetes, cancer, HIV/AIDS, etc.)	Modified NHANES 2004
5 items	**Family Medical History** (health conditions of mother's mother, father, full brother, full sister)	Modified NHANES 2004
36 items	**Use of Medicines** (prescription, non-prescription, supplements and alternative medicine)	Original and modified NHANES 2005, New
PERCEIVED STRESS		
10 items	**Global Perceived Stress** (control over life, self-confidence, nervousness, irritation, anger, in last month)	Cohen's Perceived Stress Scale
7 items	**Discrimination** (current feelings of unfair treatment, experience of discrimination and past history)	CARDIA
SOCIAL SUPPORT		
13 items	**Social Support** (size and satisfaction with social support network)	SSQ
MENTAL HEALTH AND COGNITION		
21 items	**Maternal Depression** (concentration, sadness, fear, loneliness, crying, sleep patterns)	CES-D Scale
FAMILY PROCESS		
13 items	**Quality of Relationships** (philosophy of life, goals, time spent together, communication, happiness)	Dyadic Adjustment Scale-7
7 items	**Domestic Violence** (frequency of physical abuse and aggressor in household)	Abuse Assessment Screen, New

Number of items	Topic	Source
TOBACCO/ ALCOHOL/SUBSTANCE ABUSE		
8 items	**Tobacco Use/ Environmental Tobacco Smoke Exposure** (smoking behavior/ tobacco use before and after becoming pregnant, average environmental exposure)	Modified NSFG Cycle 6, NHANES
20 items	**Alcohol Use** (consumption of beer, wine, hard liquor and other alcoholic beverages before and after becoming pregnant)	NIOSH CVD, CARDIA, NHANES, NSFG, New
3 items	**Illicit Drug Use/ Abuse of Prescription Drugs** (use of illicit drugs and prescription drugs, currently and in past year)	CIDI drugs, Modified WHO-Assist V3.0
RELIGION AND CULTURAL BACKGROUND		
3 items	**Religious Affiliation** (frequency of participation)	National study of youth and religion
8 items	**Culture and Acculturation** (language, country of birth, length of stay in the United States, connection to cultures outside American culture; maternal and biological father)	2000 census, ECLS-B, New
INCOME AND HEALTH INSURANCE		
2-6 items	**Income** (sources of income, income level, members supported by family income; all household members)	SIPP
0-4 items	**Financial Stress** (bank account, financial problems, home ownership)	Fragile families, ECLS-B
3 items	**Food Security** (ability to obtain food, type of food desired; all household members)	USDA Food Security Scale
3 items	**Health Insurance** (type and how obtained)	NHIS 2004

Number of items	Topic	Source
TRACING INFORMATION		
11 items	**Tracing Information** (moving plans, alternate contact information)	New

First Trimester Maternal
Mail Questionnaire Outline

Number of items	Topic	Source
ENVIRONMENT		
201 items	**Diet and Toxicant Exposure through Food** (frequency and quantity of target foods eaten in past month)	FFQ, 3-Day Checklist

First Trimester Paternal
In-Person/Self-Administered Questionnaire Outline

Number of items	Topic	Source
HOUSEHOLD COMPOSITION AND DEMOGRAPHICS		
4 items	**Age, Sex, Race, Ethnicity, Relationship** (all household members)	NHIS 2000, New
2 items	**Marital Status and Education** (highest level completed)	NHIS 2000, Census
3 items	**Employment** (hours worked)	SIPP, New
MEDICAL HISTORY		
1 item	**Paternal Medical History** (asthma, allergies, hypertension, diabetes, cancer, HIV/AIDS, etc.)	Modified NHANES 2004
5 items	**Family Medical History** (health conditions of mother's mother, father, full brother, full sister)	Modified NHANES 2004
RELIGION AND CULTURAL BACKGROUND		
2 items	**Religious Affiliation** (frequency of participation)	National study of youth and religion
7 items	**Culture and Acculturation** (language, country of birth, length of stay in the United States, connection to cultures outside American culture)	2000 census, ECLS-B, New
INCOME AND HEALTH INSURANCE		
5 items	**Income** (sources of income, income level, members supported by family income; all household members)	SIPP
3 items	**Financial Stress** (bank account, financial problems, home ownership)	Fragile Families, ECLS-B
2 items	**Food Security** (ability to obtain food, type of food desired; all household members)	USDA Food Security Scale
2 items	**Health Insurance** (type and how obtained)	NHIS 2004

Second Trimester Maternal
Telephone Questionnaire Outline[9]

Number of items	Topic	Source
MEDICAL HISTORY		
9-13 items	**Pregnancy Update** (due date, pregnancy loss, birth control, pregnancy diary)	New, Pregnancy screener
30-33 items	**Medical Update** (NCS medical provider log, doctor visits, hospitalizations, diagnosis, pregnancy symptoms, fever, vaccinations, date of any planned c-section)	New
ENVIRONMENT[10]		
10 items	**Changes in Job or Schooling** (employment and schooling update)	New
6 items	**Pets and Pesticides** (insecticide use in home, methods of application, pets update)	AHHS, CHAMACOS, NHANES
19 items	**Housing Characteristics** (heating, air filtration, renovations, use of air cleaning devices update, use of photocopier/laser jet printer update, use of stove)	Tucson, AHHS, AHS, ALSPAC, NHEXAS, New
7 items	**Commuting Activities** (how normally get to work, number of miles to work, use of seatbelts, exposure to pumped gas)	
MATERNAL MENTAL HEALTH AND COGNITION		
4 items	**Depression** (whether or not had and length of feelings of sadness or depression)	Sarah Knox

[9] All questions collect maternal information unless otherwise specified.
[10] The questions in the Environment Section only appear in the T16/17 week telephone call; they do not appear in the T36 week telephone call.

Number of items	Topic	Source
PERCEIVED STRESS		
4 items	**Life Events** (separation from spouse/partner, loss of job, financial pressure, legal problems, natural disasters)	PLES
TRACING INFORMATION		
2 items	**Tracing Information** (address update)	Pre-conception phone call

Third Trimester Maternal
In-Person Questionnaire Outline[11]

Number of items	Topic	Source
HOUSEHOLD COMPOSITION AND DEMOGRAPHICS		
5 items	**Household Composition Update** (listing of all household members)	NHIS 2000, New
7 items	**Age, Sex, Race, Ethnicity, Relationship** (all household members)	NHIS 2000, New
2 items	**Marital Status and Education** (highest level completed)	NHIS 2000, Census
3 items	**Employment Update** (hours worked; maternal and biological father/resident partner)	SIPP, New
ENVIRONMENT		
TBD	**Time and Activity** (time at home, work and in transit, typical weekday and typical weekend day)	New
MEDICAL HISTORY		
5 items	**Dental Health Update** (recent dental visits)	New
33 items	**Health Care Utilization and Current Pregnancy Update** (doctor visits, hospitalizations, diagnosis, pregnancy symptoms, fever, vaccinations, where planning to deliver baby, due date, hot tub use)	Pregnancy screener, Modified CAPS
68 items	**Use of Medicines** (prescription, non-prescription, supplements and alternative medicine)	Original and modified NHANES 2005, New
PERCEIVED STRESS		
11 items	**Global Perceived Stress** (control over life, self-confidence, nervousness, irritation, anger in past month)	Cohen's Perceived Stress Scale

[11] All questions collect maternal information unless otherwise specified.

Number of items	Topic	Source
PERCEIVED STRESS (continued)		
3 items	**Life Events** (difficult life events experienced by respondent and her spouse or partner since becoming pregnant)	Lobel's Revised Prenatal Life Events Scale
SOCIAL SUPPORT		
7 items	**Social Support** (perception of social support)	SSQ
MATERNAL MENTAL HEALTH AND COGNITION		
11 items	**Maternal Depression** (sadness, loneliness, sleep patterns, perception of how others like them)	CES-D Scale
FAMILY PROCESS		
7 items	**Domestic Violence** (frequency of physical abuse and aggressor in household)	Abuse assessment screen, New
TOBACCO/ ALCOHOL/SUBSTANCE USE		
4 items	**Tobacco Use Update** (current tobacco use)	Modified NHANES
12 items	**Alcohol Use Update** (consumption of beer, wine, hard liquor and other alcoholic beverages before and after becoming pregnant)	NIOSH CVD, CARDIA, NHANES
2 item	**Illicit Drug Use/ Abuse of Prescription Drugs Update** (current use of prescription drugs)	CIDI drugs, Modified WHO-Assist V3.0
INCOME AND HEALTH INSURANCE		
3 items	**Supported by Family Income Update**	SIPP
2 items	**Health Insurance Update** (type and how obtained)	NHIS 2004

Number of items	Topic	Source
TRACING INFORMATION		
11 items	**Tracing Information** (moving plans, alternate contact information)	New

3 Month Maternal
Telephone Questionnaire Outline[12]

Number of items	Topic	Source
MEDICAL HISTORY		
TBD	**Child Medical History** (medical events, injuries, vaccinations, car seat usage)	
TBD	**Infant Feeding** (breast feeding)	
TBD	**Persistent Crying/Colic**	
PARENTING AND CHILD CARE		
TBD	**Child Care** (including location, provider information, exposures)	
TRACING INFORMATION		
4 items	**Tracing Information**	New

[12] All questions collect maternal information unless otherwise specified.

6 Month Maternal
In-Person Questionnaire Outline[13]

Number of items	Topic	Source
HOUSEHOLD COMPOSITION AND DEMOGRAPHICS		
4 items	**Household Composition** (listing of all household members)	NHIS 2000, New
4 items	**Age, Sex, Race, Ethnicity, Relationship** (all household members)	NHIS 2000, New
2 items	**Marital Status and Education** (highest level completed)	NHIS 2000, Census
3 items	**Employment** (hours worked; maternal and biological father/resident partner)	SIPP, New
2 items	**Supported by Family Income** (number of individuals supported by family income)	
3 items	**Financial Stress** (bank account, financial problems, home ownership)	Fragile families, ECLS-B
2 items	**Food Security** (all household members)	USDA Food Security Scale
3 items	**Health Insurance** (type and how obtained)	NHIS 2004
PUBLIC POLICY		
TBD	**Program Participation**	
PERCEIVED STRESS		
10 items	**Global Perceived Stress** (control over life, self-confidence, nervousness, irritation, anger)	Cohen's Perceived Stress Scale
25 items	**Parenting Stress** (attachment, restrictions of role, sense of competence subscales)	Parenting Stress Index

[13] All questions collect maternal information unless otherwise specified.

Number of items	Topic	Source
PERCEIVED STRESS (continued)		
21 items	**Work and Family Conflict** (strains and benefits associated with combining work and family)	Work and family Conflict scale
SOCIAL SUPPORT		
12 items	**Social Support** (size and satisfaction with social support network)	SSQ
FAMILY PROCESS		
38 items	**Quality of Relationships** (philosophy of life, goals, time spent together, communication, happiness)	Dyadic Adjustment Scale, Love and relationships scale
18 items	**Division of Labor** (division of child care responsibilities within the family, division of household activities such as who pays the monthly bills and who does the grocery shopping)	My time spent as a parent
PARENTING AND CHILD CARE		
TBD	**Parenting Practices/Behaviors** (attachment to child, activities with the baby, parenting practices having to do with decisions, looking after the child and meals, attitudes about being a parent)	
TBD	**Media Exposure**	
TBD	**Child Care** (location, provider information, exposures)	
HEALTH BEHAVIORS		
3 items	**Sleep** (sleep during night and day)	NHLBI's Assessing Child and Maternal Sleep in the Early Years (Hunt)

Number of items	Topic	Source
ALCOHOL/SUBSTANCE USE		
TBD	**Excessive Alcohol Use**	
TBD	**Substance Use**	
MATERNAL MENTAL HEALTH AND COGNITION		
20 items	**Maternal Depression** (concentration, sadness, fear, loneliness, crying, sleep patterns)	CES-D Scale
ENVIRONMENT		
TBD	**Environmental Tobacco Smoke Exposure to Child**	
45 items	**Housing Characteristics** (moved, heating, air conditioning, renovations, windows open, stove/oven use, clothes dryer, ultrasonic vaporizer, garage/carport, water source, vacuum use, allergy proofing)	Tucson, AHHS, AHS, ALSPAC, NHEXAS, New
25 items	**Occupational/ Hobby Exposures** (number and type of jobs/schooling, job activities, work/schooling hours, drinking water source, exposure to smoking, activities indoors, activities outdoors, exposure to chemicals/pesticides as part of hobby)	CHAMACOS, NESEH, NHANES
11 items	**Pets and Pesticide Use** (type, application method, frequency, protective equipment worn, number and type of pets, exposure to flea/tick treatments)	AHHS, CHAMACOS, NHANES, ALSPAC
31 items	**Time and Activity** (transportation of child, seatbelt/car seat use, teething and pacifier use, mouthing of hands and objects, swimming)	BFRSS, New
Observation	**Family Home Environment**	IT-Home

Number of items	Topic	Source
MEDICAL HISTORY		
62 items	**Use of Medicines** (including prescription, non-prescription, supplements and alternative medicine for child)	Original and modified NHANES 2005, New
TBD	**Child Medical History** (medical events, injuries, vaccinations, car seat usage)	
CHILD TEMPERAMENT/EMOTIONAL REGULATION		
15 items	**Activity Level** (movement of arms and legs, squirming, locomotor activity)	Rothbart IBQ
16 items	**Fearfulness** (startle or distress to sudden changes in stimulation, novel physical objects or social stimuli, inhibited approach to novelty)	Rothbart IBQ
12 items	**Positive Anticipation and Approach** (rapid approach, excitement, positive anticipation of pleasurable activities)	Rothbart IBQ
PARENT-CHILD INTERACTION		
Assessment	**Parent Child Interaction**	Three boxes task
TRACING INFORMATION		
11 items	**Tracing Information** (moving plans, alternate contact information)	New

6 Month Maternal
Mail Questionnaire Outline

Number of items	Topic	Source
FAMILY PROCESS		
TBD	**Quality of Relationships**	Dyadic Adjustment Scale
ENVIRONMENT		
TBD	**Diet and Toxicant Exposures through Food** (frequency and quantity of target foods in past two weeks)	Child Feeding Form, Child 3-Day Checklist,
TBD	**Product Use** (use of creams, lotions, cleaning products)	

6 Month Paternal
In-Peron/Self-Administered Questionnaire Outline

Number of items	Topic	Source
FAMILY PROCESS		
38 items	**Quality of Relationships** (philosophy of life, goals, time spent together, communication, happiness)	Dyadic Adjustment Scale
PARENTING AND CHILD CARE		
TBD	**Parenting Practices/Behaviors** (attachment to child, activities with the baby, parenting practices having to do with decisions, looking after the child and meals, attitudes about being a parent)	ECLS-B
ENVIRONMENT		
TBD	**Environmental Tobacco Smoke and Take Home Exposures**	
PATERNAL MENTAL HEALTH AND COGNITION		
20 items	**Paternal Depression** (concentration, sadness, fear, loneliness, crying, sleep patterns)	CES-D Scale

9 Month Maternal
Telephone Questionnaire Outline[14]

Number of items	Topic	Source
MEDICAL HISTORY		
TBD	**Child Medical History** (medical events, injuries, vaccinations, car seat usage)	
TBD	**Infant Feeding** (breast feeding)	
PARENTING AND CHILD CARE		
TBD	**Child Care** (location, provider information, exposures)	
ENVIRONMENT		
TBD	**Housing Characteristics** (age of home, renovations, drinking and cooking water source, noise, dust, treatment of water for formula, allergy proofing)	Tucson, AHHS, AHS, ALSPAC, NHEXAS, New
2 items	**Pets and Pesticide Use** (application method)	AHHS, CHAMACOS, NHANES, ALSPAC
29 items	**Time and Activity** (transportation of child, seatbelt/car seat use, teething and pacifier use, mouthing of hands and objects)	
TRACING INFORMATION		
4 items	**Tracing Information** (contact information update)	New

[14] All questions collect maternal information unless otherwise specified.

12 Month Maternal
In-person Questionnaire Outline[15]

Number of items	Topic	Source
HOUSEHOLD COMPOSITION AND DEMOGRAPHICS		
4 items	**Household Composition** (listing of all household members)	NHIS 2000, New
4 items	**Age, Sex, Race, Ethnicity, Relationship** (all household members)	NHIS 2000, New
2 items	**Marital status and Education** (highest level completed)	NHIS 2000, Census
3 items	**Employment** (hours worked; maternal and biological father/resident partner)	SIPP, New
2 items	**Supported by Family Income** (number of individuals supported by family income)	
3 items	**Health Insurance** (type and how obtained)	NHIS 2004
PUBLIC POLICY		
TBD	**Program Participation**	
FAMILY PROCESS		
TBD	**Family Process**	
PARENTING AND CHILD CARE		
TBD	**Parenting Practices/Behaviors** (attachment to child, activities with the baby, parenting practices having to do with decisions, looking after the child and meals, attitudes about being a parent)	ECLS-B
TBD	**Media Exposure**	
TBD	**Child Care** (location, provider information, exposures)	

[15] All questions collect maternal information unless otherwise specified.

Number of items	Topic	Source
MENTAL HEALTH AND COGNITION		
TBD	**Maternal Mental Health and Cognition**	
ALCOHOL/SUBSTANCE USE		
TBD	**Excessive Alcohol Use**	
TBD	**Substance Use**	
ENVIRONMENT		
TBD	**Environmental Tobacco Smoke Exposure to Child**	
43 items	**Housing Characteristics** (moved, heating, air conditioning, renovations, windows open, stove/oven use, clothes dryer, ultrasonic vaporizer, garage/carport, water source, vacuum use, allergy proofing)	Tucson, AHHS, AHS, ALSPAC, NHEXAS, New
TBD	**Occupational/ Hobby Exposures** (number and type of jobs/schooling, job activities, work/schooling hours, drinking water source, exposure to smoking, activities indoors, activities outdoors, exposure to chemicals/pesticides as part of hobby)	CHAMACOS, NESEH, NHANES
11 items	**Pets and Pesticide Use** (type, application method, frequency, protective equipment worn, number and type of pets, exposure to flea/tick treatments)	AHHS, CHAMACOS, NHANES, ALSPAC
31 items	**Time and Activity** (transportation of child, seatbelt/car seat use, teething and pacifier use, mouthing of hands and objects, swimming)	
MEDICAL HISTORY		
62 items	**Use of Medicines** (prescription, non-prescription, supplements and alternative medicine for child)	Original and modified NHANES 2005, New

Number of items	Topic	Source
MEDICAL HISTORY (continued)		
TBD	**Child Medical History** (medical events, injuries, vaccinations, car seat usage)	New
CHILD DEVELOPMENT		
TBD	**Child Language Development**	MacArthur-Bates CDI
TBD	**Social Competence**	BITSEA
Assessment	**General Cognitive Ability, Motor Development, and Language Development** (maternal and child)	Bayley III
TRACING INFORMATION		
11 items	**Tracing Information** (moving plans, alternate contact information)	New

12 Month Maternal
Mail Questionnaire Outline

Number of items	Topic	Source
FAMILY PROCESS		
TBD	**Quality of Relationships**	Dyadic Adjustment Scale
ENVIRONMENT		
TBD	**Diet and Toxicant Exposures through Food** (frequency and quantity of target foods in past two weeks)	Child feeding form, Child 3-Day Checklist
TBD	**Product Use** (use of creams, lotions, cleaning products)	

12 Month Paternal
In-Person/Self-Administered Questionnaire Outline

Number of items	Topic	Source
FAMILY PROCESS		
38 items	**Quality of Relationships** (philosophy of life, goals, time spent together, communication, happiness)	Dyadic Adjustment Scale
PARENTING AND CHILD CARE		
TBD	**Parenting Practices/Behaviors** (attachment to child, activities with the baby, parenting practices having to do with decisions, looking after the child and meals, attitudes about being a parent)	
ENVIRONMENT		
TBD	**Environmental Tobacco Smoke and Take Home Exposures**	
MENTAL HEALTH AND COGNITION		
TBD	**IQ and Literacy**	Kaufman Brief Intelligence Test, Woodcock-Johnson Letter-Word Identification
PARENT-CHILD INTERACTION		
Assessment	**Parent-Child Interaction**	Three boxes task

18 Month Maternal
Telephone Questionnaire Outline[16]

Number of items	Topic	Source
MEDICAL HISTORY		
TBD	**Child Medical History** (medical events, injuries, vaccinations, car seat usage)	
TBD	**Infant Feeding** (breast feeding)	
PARENTING AND CHILD CARE		
TBD	**Child Care** (location, provider information, exposures)	
MAJOR LIFE EVENTS		
TBD	**Major Life Events**	
ENVIRONMENT		
7 items	**Housing Characteristics** (drinking and cooking water source, allergy proofing)	Tucson, AHHS, AHS, ALSPAC, NHEXAS, New
2 items	**Pets and Pesticide Use** (application method)	AHHS, CHAMACOS, NHANES, ALSPAC
TBD	**Occupational/ Hobby Exposures** (number and type of jobs/schooling, job activities, work/schooling hours, drinking water source, exposure to smoking, activities indoors, activities outdoors, exposure to chemicals/pesticides as part of hobby)	CHAMACOS, NESEH, NHANES
27 items	**Time and Activity** (transportation of child, seatbelt/car seat use, mouthing of hands and objects)	

[16] All questions collect maternal information unless otherwise specified.

Number of items	Topic	Source
TRACING INFORMATION		
4 items	**Tracing Information**	New

24 Month Maternal
Telephone Questionnaire Outline[17]

Number of items	Topic	Source
MEDICAL HISTORY		
TBD	**Child Medical History** (medical events, injuries, vaccinations, car seat usage)	
TBD	**Infant Feeding** (breast feeding)	
PARENTING AND CHILD CARE		
TBD	**Child Care** (location, provider information, exposures)	
MAJOR LIFE EVENTS		
TBD	**Major Life Events**	
ENVIRONMENT		
7 items	**Housing Characteristics** (heat source, age of home, renovations, windows open, ultrasonic vaporizer, gasoline storage, pumped gas, tap water source, vacuum use, noise and dust, water damage, mildew, humidifier, dehumidifier, allergy proofing)	Tucson, AHHS, AHS, ALSPAC, NHEXAS, New
11 items	**Pets and Pesticide Use** (use of insecticides, application method, or flea/tick treatments)	AHHS, CHAMACOS, NHANES, ALSPAC
31 items	**Time and Activity** (transportation of child, seatbelt/car seat use, teething and pacifier use, mouthing of hands and objects, swimming)	
TRACING INFORMATION		
4 items	**Tracing Information**	New

[17] All questions collect maternal information unless otherwise specified.

NCS PROTOCOL BIOSPECIMEN DETAILS
June 20, 2007 – Version 1.3

Summary of Biological Specimens by Person/Visit (Mother (M), Father (F), Child (C)

Prepregnancy					Pregnancy								Birth				Postnatal							
P1 Home	**Materials left behind and picked up after P1[1]**	**P 1 month (Phone)**	**P 2 months (Phone)**	**P 4 months (Phone)**	**T1-1st (Home)**	**T1-Prior (Home)**	**Materials left behind and picked up after T1[1]**	**16-17 weeks (Phone)**	**T2 (Clinic)**	**T3 (Clinic)**	**Materials left behind and picked up after T3[1]**	**36 weeks (Phone)**	**B1 Delivery (Hospital)**	**B2 Predischarge (Hospital)**	**Materials left behind and picked up after B2[1]**	**1 month visit if needed (Home)**	**3 months (Phone)**	**6 month visit (Home)**	**Materials left behind and picked up after 6 mo. visit[1]**	**9 months (Phone)**	**12 month visit (Home)**	**Materials left behind and picked up after 12 mo. visit[1]**	**18 months (Phone)**	**24 months (Phone)**
Blood (M) Vaginal (M) Hair (M) *Urine Kit (M)* [2]	Urine[3]	Pregnancy tests plus urine kit (M)			Blood (M,F) *Urine kit (M,F)* [2] Vaginal (M) Hair (M,F) Nail (F) *Saliva kit (M)* [4]	Blood (M,F) *Urine kit (M,F)* [2] Vaginal (M) Hair (M,F) Nail (F) *Saliva kit (M)* [4]	Urine (M,F) Saliva (M)			Blood (M) Urine[5] Vaginal (M) Hair (M) Nail (M) Saliva[5]			Blood (M) Urine (M) Placenta (M) Umbilical cord (M) Heel stick (C) Cord blood(C)	Meconium (C)		*Breast milk kit (M)* [6]	*Breast milk kit (M)* [6]	Breast milk (M)[6] Urine (C)[2] Hair (C) *Saliva kit (M,F)* [4]	Saliva (M,F)		Blood (C) Urine (C)[2] Hair (C) *Saliva kit (C)* [4]	Saliva (C)		

[1] **Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.**

[2] **Urine will be self collected as a first morning void (except at birth). At participant visits, a kit will be left and the participant will collect a first morning void urine the morning of the sample pickup.**

[3] **Two urine samples will be collected after P1. One will be collected shortly following the visit (the morning of the pickup of the air pumps and other environmental equipment). The second is intended to measure environmental exposures closer to the time of conception and will be collected the morning after a positive pregnancy test and frozen. Participants will be provided with shipping material to mail this sample to the repository.**

[4] **Saliva kits will be left at the visit and adult participants directed to collect 3 samples each day for 2 days following the visit and store them in a freezer. For child participants, parents will be directed to collect 2 samples each day for 2 days following the visit and store them in a freezer.**

[5] **Prior to the T3 clinic visit, participants will be sent a urine collection kit and a saliva collection kit. They will be directed to collect samples and freeze them and bring the samples to the clinic visit.**

[6] **A breast milk kit will be given at the birth visit and milk will be collected at 1 month and mailed in. A breast milk kit will be sent after the 3 month phone call to mothers who are still nursing and the participant will mail the milk sample in. For mothers who are still nursing, a kit will be sent prior to the 6 month visit for collecton prior to the visits. Milk will be picked up during the visit.**

NCS PROTOCOL BIOSPECIMEN DETAILS (continued)
June 20, 2007 – Version 1.3

Simplified Summary of Cord Blood (mL) at Birth

	Prepregnancy					Pregnancy								Birth				Postnatal									
	P1 Home	Materials left behind and picked up after P1[1]	P 1 month (Phone)	P 2 months (Phone)	P 4 months (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1[1]	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3[1]	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2[1]	1 month visit if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit[1]	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit[1]	18 months (Phone)	24 months (Phone)
Endocrine panel																										
Cortisol														X												
Cortisone														X												
Corticotropin releasing hormone														X												
Cortisol binding globulin														X												
CRH binding protein														X												
ACTH														X												
Reproductive																										
Estriol														X												
Estradiol														X												
Progesterone														X												
Infection/Inflammation/Biological																										
Hemoglobin/Hematocrit														X												
Cytokines/Interleukins														X												
Ig types and subtypes																										
Herpes simplex 1 & 2														X												
Hepatitis profile														X												
Toxoplasmosis (toxoplasma gondii)														X												
Varicella														X												
Rubella (IgM antibody)														X												
Syphilis														X												
IgE (cat, dog, cockroach, dust mite, fungi, mouse/rat urine)														X												
C-reactive protein (CRP)														X												
Homocysteine, fasting preferred														X												
RBC folate														X												
Thyroid (TSH and free t4)														X												
Antioxidant (vitamins A/E, carotenoids), fasting														X												
Vitamin C, fasting														X												
Alcohol, % carb deficient transferrin														X												
N3-N6 fatty acids														X												
Glucose metabolism																										
Fasting glucose														X												
HgbA1C														X												
Fasting insulin														X												
Insulin-like growth factor														X												
Chemicals																										
Metals: mercury, cadmium, lead														X												
Lipids, PCBs, organochlorine pesiticides, PBDE, perfluorinated compounds (PFOA,PFOS)														X												
Genetic material																										
Cryopreserved PBMCs														X												
Genomic DNA														X												
Mitochondrial DNA														X												
RNA														X												
Other																										
Guthrie card														X												
TOTAL volume needed for specified analytes (mL)														**60.7**												
# of blood tubes proposed to be collected																										
Pre-screened Lavender for metals														1- 3 mL												
Red top														3-10 mL												
Lavendar EDTA														2-10 mL; 1-6mL												
ACD tube for PBMC collection														1-10 mL												
PaxGene RNA														1-2.5mL												
Gray top NaF														1-4mL												

[1] **Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.**

Simplified Summary of Blood (mL) by Visit from Mother (M), Child (C), and Father (F)

	Prepregnancy					Pregnancy								Birth				Postnatal							
	P1 Home	Materials left behind and picked up after P1[1]	P 1 month (Phone)	P 2 months (Phone)	P 4 months (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1[1]	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3[1]	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2[1]	1 month visit if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit[1]	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit[1]	18 months (Phone)	24 months (Phone)
Endocrine panel																									
Cortisol						M F	M F				M														
Cortisone						M	M				M														
Corticotropin releasing hormone						M	M				M														
Cortisol binding globulin						M	M				M														
CRH binding protein						M	M				M														
ACTH						M	M				M														
Reproductive																									
Estriol						M	M				M														
Estradiol						M	M				M														
Progesterone						M	M				M														
Infection/Inflammation/Biological																									
Hemoglobin/Hematocrit	M					M	M				M											C			
Cytokines/Interleukins	M					M	M				M														
Ig types and subtypes																									
Herpes simplex 1 & 2 (Ig)	M					M	M				M														
Hepatitis profile (A & B) medical records	M					M	M				M														
Toxoplasmosis (toxoplasma gondii) (Ig)	M					M	M				M														
Varicella (Ig)	M					M	M				M														
Rubella (IgM antibody)	M					M	M				M														
Syphilis (Ig)	M					M	M				M														
IgE (cat, dog, cockroach, dust mite, fungi, mouse/rat urine)	M					M	M				M														
CRP	M					M	M				M														
Heat shock proteins	M					M	M				M														
Homocysteine						M	M				M														
RBC folate						M	M				M														
Thyroid (TSH and free t4)	M					M	M				M														
Antioxidant (vitamins A/E, carotenoids)						M	M				M														
Vitamin C						M	M				M														
Vitamin D (25OH)						M	M				M											C			
Alcohol, % carb deficient transferrin	M					M	M				M			M											
Omega FA (N3-N6)											M														
Glucose metabolism																									
Glucose (fasting)											M														
HgbA1C	M					M	M																		
Insulin (fasting)											M														
Insulin-like growth factor											M														
Chemical exposures																									
Metals: Mercury, cadmium, lead	M					M F	M F							M								C			
Lipids, PCBs, organochlorine pesiticides, PBDE, perfluorinated compounds (PFOA, PFOS)	M					F	F															C			
Combination of dioxins/furans and all other chemicals (excluding metals)						M	M				M			M											
Genetics																									
Cryopreserved PBMCs						F	F				M														
Genomic DNA^						M F	M F																		
Mitochondrial DNA						M F	M F																		
RNA																									
Other																									
Guthrie card, heel stick														C											
TOTAL volume needed for specified analytes (mL)																									
Mother	**26.2**					**42.6**	**42.6**				**56.9**			**33.5**											
Father						**24.2**	**24.2**																		
Child																						**13.6***			
# of blood tubes proposed to be collected																									
Pre-screened Lavender for metals	1-3 mL					1-3 mL M, F	1-3 mL M, F							1-3 mL								1-3 mL			
Red top	3-10 mL					3-10 mL M 1-10 mL F	3-10 mL M 1-10 mL F				3-10 mL			3-10 mL								2-5 mL			
Lavendar EDTA	1-10 mL					2-10 mL M 1-10 mL F	2-10 mL M 1-10 mL F				2-10 mL											1-6 mL			
ACD tube for PBMC collection						1-10 mL F	1-10 mL F				1-10 mL														
PaxGene RNA											1-2.5 mL														
Gray top NaF											1-4 mL														

[1] **Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.**

***A complete list of analytes for the child at 12 months have not been confirmed. A 6 mL Lavender tube is being proposed to be collected to allow for future determination of analytes.**

^Saliva collection for DNA will be a backup if participants refuse blood draw.

Summary of Breast Milk (mL) by Visit

	Prepregnancy					Pregnancy								Birth				Postnatal							
	P1 Home	Materials left behind and picked up after P1[1]	P 1 month (Phone)	P 2 months (Phone)	P 4 months (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1[1]	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3[1]	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2[1]	1 month visit, if needed* (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit[1]	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit[1]	18 months (Phone)	24 months (Phone)
Antioxidants: Vitamins C/E, beta-carotene																	M	M		M					
Phytoestrogens																	M	M		M					
Component: lipid, proteins, carbohydrates, enzymes, immunoglobulins, minerals, vitamins, cytokines, hormones																	M	M		M					
Chemical exposures																									
Dioxins/furans; organochlorine pesticides; PCBs																	M	M		M					
Pesticides																	M	M		M					
PBDE																	M	M		M					
Perchlorate, iodide, thiocynate, nitrate																	M	M		M					
Manganese																	M	M		M					
Phenols-bisphenol A and parabens																	M	M		M					
TOTAL volumes (mL)																	**80-100 ml**	**80-100 ml**		**80-100 ml**					

[1] **Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.**

*** Breast milk instructions and supplies will be given at birth, but the first self-collection will occur around 1 month postnatal.**

NCS PROTOCOL BIOSPECIMEN DETAILS (continued)
June 20, 2007 – Version 1.3

Simplified Summary of Urine (mL) From Mother (M), Father (F), and Child (C)

	Prepregnancy					Pregnancy								Birth				Postnatal							
	P1 Home	Materials left behind and picked up after P1[1]	P 1 month* (Phone)	P 2 months (Phone)	P 4 months (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1[1]	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3[1]	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2[1]	1 month visit if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit[1]	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit[1]	18 months (Phone)	24 months (Phone)
Illicit drug panel		M	M					M				M		M									C		
Cortisol								M																	
Preganancy tests		M	M																						
PCR for chlamydia		M	M					M				M													
PCR for gonnorhea		M	M					M				M													
Chemical exposures																									
Atrazine + pyrethoids + OPs + carbamates + ETU/PTU/EBC		M	M					M F				M		M						C			C		
Phthalates and bisphenol A/nonyl phenol		M	M					M F				M		M						C			C		
Halogenated phenols(PCP)		M	M					M F				M		M						C			C		
Perchlorate		M	M					M F				M		M						C			C		
PAH		M	M					M F				M		M						C			C		
Iodine		M	M					M F				M		M						C			C		
Urine metals (multi-element panel, includes Cd, As, etc.)		M	M					M F				M		M						C			C		
Mercury		M	M					M F				M		M						C			C		
Creatinine		M	M					M F				M		M						C			C		
Cotinine		M	M					M F				M		M						C			C		
Phytoestrogens		M	M					M F				M		M						C			C		
TOTAL volume needed for specified analytes (mL)																									
Mother		**33.5**	**33.5**					**33.5**				**33.5**		**31.5**											
Father								**31.5**																	
Child																				**31.0**			**31.0**		

[1] Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.

*** P 1 month urine sample: A first morning void urine will be collected the morning after a positive pregnancy test.**

Vaginal Swab Summary for Mother (M)

	Prepregnancy					Pregnancy								Birth				Postnatal							
	P1 Home	Materials left behind and picked up after P1[1]	P 1 month (Phone)	P 2 month (Phone)	P 4 month (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1[1]	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3[1]	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2[1]	1 month visit if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit[1]	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit[1]	18 months (Phone)	24 months (Phone)
Vaginal swabs																									
pH											M														
Bacterial vaginosis	M					M	M				M														
Antibodies	M					M	M				M														
Cytokines	M					M	M				M														
Metalloproteinase	M					M	M				M														

[1] **Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.**

NCS PROTOCOL BIOSPECIMEN DETAILS (continued)
June 20, 2007 – Version 1.3

Placenta, Umbilical Cord, and Meconium Summary at Birth

	Prepregnancy					Pregnancy								Birth				Postnatal							
	P1 Home	Materials left behind and picked up after P1[1]	P 1 month (Phone)	P 2 month (Phone)	P 4 month (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1[1]	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3[1]	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2[1]	1 month visit, if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit[1]	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit[1]	18 months (Phone)	24 months (Phone)
Placenta																									
Antibodies and cytokines														M											
Pathology														M											
Chemical contaminants														M											
Umbilical cord																									
Antibodies and cytokines														M											
Pathology														M											
Chemical contaminants														M											
Meconium																									
Cotinine															C										
Organophosphate metabolites															C										

[1] Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.

NCS PROTOCOL BIOSPECIMEN DETAILS (continued)
June 20, 2007 – Version 1.3

Simplified Summary of Hair, Nail, and Saliva Collection From Mother (M), Father (F), and Child (C)

	Prepregnancy					Pregnancy								Birth				Postnatal							
	P1 Home	Materials left behind and picked up after P1[1]	P 1 month (Phone)	P 2 month (Phone)	P 4 month (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1[1]	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3[1]	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2[1]	1 month visit if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit[1]	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit[1]	18 months (Phone)	24 months (Phone)
Hair																									
Cotinine	M					M F	M F				M								C			C			
Total Hg (assumed to be organic ethyl,	M					M F	M F				M								C			C			
Nail																									
Organic Hg (ethyl, methyl)						F	F				M														
Hg inorganic						F	F				M														
Saliva*																									
Cortisol								M				M								M F			C			

[1] **Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.**

*** Saliva kits will be left at the visit and adult participants directed to collect 3 samples each day for 2 days following the visits and store them in a freezer.**
For child participants, parents will be directed to collect 2 samples each day for 2 days following the visit and store them in a freezer.

NCS Overview of Environmental Questionnaire Items by Contact (For the Same Home)
June 20, 2007 – Version 1.3

	Prepregnancy					Pregnancy								Birth				Postnatal								
	P1 home	Materials left behind and picked up after P1	P 1 month* (phone)	P 2 month* (phone)	P 4 month* (phone)	T1-1st (home)	T-prior (home)	Materials left behind and picked up after T1	16-17 weeks* (phone)	T2* (clinic)	T3 (clinic)	Materials left behind and picked up after T3*	36 weeks (phone)	B1 delivery (hospital)	B2 Pre-discharge (hospital)	Materials left behind and picked up after B2	1 month visit if needed (home)	3 months (phone)	6 month visit (home)	Materials left behind and picked up after 6 mo. visit	9 months (phone)	12 month visit (home)	Materials left behind and picked up after 12 mo. visit	18 months (phone)	24 months (phone)
Interview																										
HOUSING CHARACTERISTICS																										
Main heating fuel source						X	X												U							
Heat source this week	X					X	X		X			X							X			X			X	
Heating/Cooling months						X	X																			
Age of home						X	X																			
Renovations/additions						X	X		U			U							U		U	U			U	
Outdoor additions																			X		U	U			U	
A/C						X	X		U			U							U			U			U	
House air filtration						X	X																			
Electrostatic, 0_3 generators, LaserJet's	X		U			X	X		X										U (Trigger)			X (Trigger)				
Windows open	X					X	X		X										X			X			X	
Stove						X	X		X			X						X	X	X	X	X				
Clothes dryer						X	X												U			U				
Vaporizer	X					X	X												U			U			U	
Garage location	X					X	X																			
Vehicle parked, warm up	X					X	U					X							U			U			U	
Gasoline storage	X					X	X																			
Pumped gas	X		X			X	X		X			X														
Drinking and cooking water source						X	X					X							X		X	X		X	X	
Tap water source	X (Trigger)					X (Trigger)																				
Vacuum cleaner						X													U			U			U	
Noise and dust																			U		U	U			U	
# water problems in last 6 mo																									X	
Mildew																									X	
Dehumidifier																									X	
Humidifier																									X	
Treatment of water used for to make formula or baby food																			X		X					
Allergy proofing																			X		U	U		U	U	
PETS AND PESTICIDES																										
Insecticides, such as Raid…	X		X	X	X	X	X		X			X			X		X		X		X	X		X	X	
Specific products in past 3-mo	X					X	X												U			X				
Method	X		X	X	X	X	X		X			X			X		X		X		X	X		X	X	
Where, who, how often applied	X					X	X												X			X				
PPE	X					X	X																			
Pets	X					X	X		U			U							U			U			U	
Flea control - including treatment of pets / flea collars	X		U	U	U	X	X		U			U							U			U			U	
OCCUPATIONAL/HOBBY																										
Number of jobs	X					X	U		U			U							U BF							
Describe job	X					X	U		U			U							U BF							
Describe hobbies	X					X	U		U			U														
Hobbies and work-at-home	X																		X			U		U		
How long Mother continued to work															X		X									
Occupational take-home exposures (short form, e.g., any household members wear dirty clothes, boots, etc home?)																			X			U		U		

KEY X = Core topic questions U = Update questions BF = Core questions if mother is breast feeding U BF = Update questions if mother is breast feeding Trigger = Question answers determine environmental sampling

*Address information updated. If participant moved, visit home and ask additional housing questions.

NCS Overview of Environmental Questionnaire Items by Contact (For the Same Home) (continued)
June 20, 2007 – Version 1.3

	Prepregnancy					Pregnancy								Birth				Postnatal							
	P1 home	Materials left behind and picked up after P1	P 1 month* (phone)	P 2 month* (phone)	P 4 month* (phone)	T1-1st (home)	T-prior (home)	Materials left behind and picked up after T1	16-17 weeks* (phone)	T2* (clinic)	T3 (clinic)	Materials left behind and picked up after T3*	36 weeks (phone)	B1 delivery (hospital)	B2 Pre-discharge (hospital)	Materials left behind and picked up after B2	1 month visit if needed (home)	3 months (phone)	6 month visit (home)	Materials left behind and picked up after 6 mo. visit	9 months (phone)	12 month visit (home)	Materials left behind and picked up after 12 mo. visit	18 months (phone)	24 months (phone)
TIME-ACTIVITY																									
Time & activity (Mother)	X		X			X	U			X		X													
Time & activity (Child)																			X		X	X		X	X
Commuting						X	U		X			X													
Transportation of child																			X		X	X		X	X
Seatbelt/carseat use	X					X	U		X			X							X		X	X		X	X
Teething and pacifier use																			X		X	X			X
Mouthing of hands and objects																			X		X	X		X	X
Swimming and other child recreational activities - location, frequency (since last contact)																			X			X			X
PRODUCT USE																						X			X
Mother use of lotions, creams, gels							X				X								BF						
Tanning lotions, creams and gels	X						X				X														
Mother use of shampoos and soaps							X				X								BF						
Mother use of products to wash clothes, towels, other laundry							X				X								X						
Products used to wash child's clothes																			X						
Bleach, dry clean	X						X				X								X						
Mother use of household cleaning products	X						X				X														
Mother use of other products in the home (special cleaners, varnish, paint mothballs)	X						X				X								X						
Use of air fresheners, candles, incense (household use)	X						X				X								X						
Sum of disinfectants	X						X				X								X						
Sum of aerosols	X						X				X														
Mother's pica behavior	X						X				X														
Lice/scabies treatment	X		X	X	X		X		X		X								X						
Lice/scabies Products	X						X				X								X						
Use of lotions, creams, gels, powders on child																			X						
Use of shampoos and soaps on child (incl. 'no tears')																			X						
Type of diapers																			X						
Baby wipes with fragrance																			X						
Environmental Sample Collection and Visual Assessment (see "Summary of Environmental Samples by Visit" Table for details)	X					X	X												X			X			
Environmental Equipment (pick-up)		X						X												X			X		
Environmental Samples (mail-in)								X				X													X

KEY X= Core topic questions U = Update questions BF = Core questions if mother is breast feeding U BF = Update questions if mother is breast feeding Trigger = Question answers determine environmental sampling

*Address information updated. If participant moved, visit home and ask additional housing questions.

NCS Protocol Summary of Environmental Samples by Contact (for the same home) (continued)
June 20, 2007 – Version 1.3

			Prepregnancy		Pregnancy					Birth	Postnatal		
			P1 home	Materials left behind and picked up after P1	T1-1st (Home)	T1 - prior (Home)	Materials left behind and picked up after T1	T3 (Clinic)	Materials left behind and picked up after T3	Birth visit (Hospital)	6 month visit (Home)	12 month visit (Home)	24 months (Phone)
Indoor Air	**Method**	**%Homes**											
PM_{10} - metals (Pb, Cd, Mn); elemental carbon[5]	pump	100	X	PU	X	X	PU				X, PU	X, PU	
VOCs	badge	100	X	PU	X	X	PU	X[8]	Return		X, PU	X, PU	X[8], return
Aldehydes and ketones	badge	100						X[8]	Return		X, PU	X, PU	X[8], return
NO_2, NOx	badge	100			X	X	PU	X[8]	Return		X, PU	X, PU	X[8], return
O_3	badge	25[4]									X, PU	X, PU	
CO	logger	100			X	X					X	X	
House Dust													
Allergens, endotoxin (+ temperature and RH)	vacuum	100						X[8]	Return		X	X	X[8], return
Mold	vacuum	100									X	X	
Metals - Pb, Mn, As	wipe	100			X (As, Pb)	X (As, Pb)					X		
Metals[3]	dust mat	100						X[8]	Return				
Pesticides: OPs, carbamates	wipe	100	X		X	X		X[8]	Return		X		
Pesticides: OCs	wipe	100						X[8]	Return		X		
Pesticides: Pyrethroids	wipe	100	X		X	X		X[8]	Return		X	X	X[8], return
Bulk dust sample for archive (potential for future analyes)	vacuum	100			X	X					X	X	
Drinking Water[5]													
Disinfection byproducts (DBPs) - HAA9	water	88[1]			X	X						X	COMMUNITY
VOCs (includes THMs)	water	88[1]			X	X					X	X	COMMUNITY
Metals - Pb, Cd, As (six-month holding time)	water	100			X	X					X		COMMUNITY
Coliforms (24-hour holding time)	water	12[2]									X		
Nitrate (24-hour holding time)	water	12[2]	X		X	X							
Perchlorate (28-d holding time)	water	12[2]	X		X								COMMUNITY
Pesticides: OPs, carbamates, atrazine	water	<12[2]	X		X	X					X	X	COMMUNITY
Pesticides: Pyrethroids	water	<12[2]	X		X	X					X	X	COMMUNITY
Soil													
Perimeter: Metals - Pb, Cd, Mn, As	soil	100									X		
Mid-yard: Metals - Pb, Cd, Mn, As	soil	100									X	X	
Mid-yard: Pesticides - OPs, carbamates, pyrethroids	soil	>10[7]			X	X					X	X	
Near CCA treated wood - Cr+6 (as total), As	soil	70										X	
Visual Assessment - Housing, Neighborhood - Indoor and Outdoor)[6]			X		X	X					X	X	X
Noise Survey - Indoor and Outdoor	SLM											X	

Notes:

X = Sample will be collected at contact. PU = Pick up sample (pump and badge samples will be left in place for six-seven days). SLM = sound level meter.

[1] Houses served by CWS only

[2] All private wells, rural communities, and/or if CWS report presence of contaminant (might limit to samples in spring/fall for pesticides and where other data indicate usage[e.g., GIS data])

[3] Deploy mat at T3 contact (self collection) and mail back at one month

[4] If electrostatic filter, ozonator, or laser printer present, plus subsample for seasonal infiltration

[5] PAH will be assessed by Qx; metals by XRF. Sample stored for possible future analyses (perhaps allergens)

[6] Some observational data will be gathered by neighborhood drive-arounds/database extraction

[7] Rural areas only

[8] Self-collected samples - Activity is initiated at in-person visit or is mailed to the participant and requires participant action (e.g., collect and mail in VOC badge). Time frame for completion varies and is specific to each activity.

NCS PROTOCOL CONTACT SCHEDULE OF PHYSICAL MEASURES
June 20, 2007 – Version 1.3

	Prepregnancy					Pregnancy								Birth				Postnatal							
Physical Measures	**P1 Home**	**Materials left behind and picked up after P1**	**P 1 month (Phone)**	**P 2 month (Phone)**	**P 4 month (Phone)**	**T1-1st (Home)**	**T1 prior (Home)**	**Materials left behind and picked up after T1**	**16-17 weeks (Phone)**	**T2 (Clinic)**	**T3 (Clinic)**	**Materials left behind and picked up after T3**	**36 weeks (Phone)**	**B1 delivery (Hospital)**	**B2 Pre-discharge (Hospital)**	**Materials left behind and picked up after B2**	**1 month visit if needed (Home)**	**3 months (Phone)**	**6 month visit (Home)**	**Materials left behind and picked up after 6 mo. visit**	**9 months (Phone)**	**12 month visit (Home)**	**Materials left behind and picked up after 12 mo. visit**	**18 months (Phone)**	**24 months (Phone)**
Anthropometric Measures																									
Maternal weight	√					√	√				√														
Maternal standing height	√					√	√																		
Maternal sitting height						√	√																		
Maternal knee height						√	√																		
Maternal mid arm circumference	√					√	√				√														
Maternal hip circumference	√																								
Maternal waist circumference	√																								
Maternal head circumference						√	√																		
Maternal triceps fold	√					√	√				√														
Maternal subscapular skin fold	√					√	√				√														
Paternal weight						√	√																		
Paternal standing height						√	√																		
Paternal sitting height						√	√																		
Paternal knee height						√	√																		
Paternal mid arm circumference						√	√																		
Paternal hip circumference						√	√																		
Paternal waist circumference						√	√																		
Paternal head circumference						√	√																		
Paternal triceps fold						√	√																		
Paternal subscapular skin fold						√	√																		
Infant recumbent length															√		√		√						
Child height																									
Infant/child weight															√		√		√						
Infant/child head circumference															√		√		√						
Infant/child mid upper arm circumference															√		√		√						
Infant/child abdomen circumference															√		√		√						
Infant/child thigh circumference															√		√		√						
Infant/child triceps skin fold															√		√		√						
Infant/child subscapular skin fold															√		√		√						
Blood Pressure																									
Maternal blood pressure	√					√	√				√														
Paternal blood pressure						√	√																		
Infant/child blood pressure																						√			
Maternal/Fetal Ultrasound																									
Early crown-rump length for dating (get results from obstetrician or send to clinic)						√	√																		
Fetal growth (biparietal diameter and head circumference, femur length, abdominal circumference)										√	√														
Fetal fat and body composition (mid-thigh circumference - total and lean compartment, abdominal wall thickness)										√	√														
Fetal measures (Doppler): Umbilical artery Systolic: Diastolic flow, uterine artery										√	√														
Infant Dysmorphology																									
Observational examination for major anomalies (facial clefts, neural tube defects, limb and digit abnormalities, ear position, shape, size, abdominal wall defects)															√		√								
2-D photos (face-frontal and profile to look at ear position and hands, other anomalies if present)															√		√								
Infant Physical Exam																									
Exam for atopic dermatitis/eczema																		√				√			
Photo (face) for rashes neonatal acne																		√				√			
Chart abstraction - Labor and Delivery																									
Exposure of fetus to hypoxia															√										
Hyperthermia															√										
Medications															√										
Interventions															√										
Uterine perfusion															√										

NCS PROTOCOL CONTACT SCHEDULE OF PHYSICAL MEASURES (continued)
June 20, 2007 – Version 1.3

	Prepregnancy					Pregnancy								Birth				Postnatal							
Physical Measures	**P1 Home**	**Materials left behind and picked up after P1**	**P 1 month (Phone)**	**P 2 month (Phone)**	**P 4 month (Phone)**	**T1-1st (Home)**	**T1 prior (Home)**	**Materials left behind and picked up after T1**	**16-17 weeks (Phone)**	**T2 (Clinic)**	**T3 (Clinic)**	**Materials left behind and picked up after T3**	**36 weeks (Phone)**	**B1 delivery (Hospital)**	**B2 Pre-discharge (Hospital)**	**Materials left behind and picked up after B2**	**1 month visit if needed (Home)**	**3 months (Phone)**	**6 month visit (Home)**	**Materials left behind and picked up after 6 mo. visit**	**9 months (Phone)**	**12 month visit (Home)**	**Materials left behind and picked up after 12 mo. visit**	**18 months (Phone)**	**24 months (Phone)**
Chart abstraction - Neonatal																									
Complications of pregnancy															√										
Type of delivery															√										
Type of anesthesia															√										
Intrapartum complications															√										
Place of delivery															√										
Type of delivery practitioner															√										
Birth and discharge weight, length, size for gestational age,															√										
APGAR score															√										
Post-partum resuscitation															√										
Hospital course															√										
Physical exam results															√										
Problems in nursery (jaundice, feeding difficulty, respiratory, sepsis, hypoglycemia)															√										
Medications/therapeutics during hospital stay															√										
Immunizations given															√										
Imaging studies performed															√										
Blood or other lab tests															√										
Procedures during hospital stay															√										
Screening tests															√										
Hearing screen															√										
Metabolic screen															√										
Psychosocial issues (bonding, cultural variations, etc.)															√										
Discharge diagnoses															√										
Follow-up prescribed (where, when, with whom)															√										

NATIONAL CHILDREN'S STUDY

List of Methods Development Studies, Reviews/White Papers, Workshops, and Publications from Methods Development Studies and Working Groups

September 17, 2007 – Version 1.3

Methods Development Studies

Title

Alternative exposure measurement designs to improve epidemiologic study designs

Application of measure of spontaneous motor activity for behavioral assessment in human infants and young children for use in risk evaluation

Assessment of neurobehavioral toxicity in human infants and laboratory animals

Community involvement methods development for the NCS

Comparison/feasibility of collecting mother-infant hair/nail and buccal cell DNA/RNA samples (mail vs. health care professional)

Demonstration of low cost, low burden exposure monitoring strategies—birth cohort

Development and testing of informed consent materials

Draft Timeline: Prepare to implement a cohort study of children's environmental health

Evaluation of disposable diapers for quantitative measurements of pesticide metabolites and creatinine in urine samples

Exposures and health of farm worker children in California

Feasibility of primary care sites for subject observation and data collection

Final Interim Report: Candidate sites, machines in use, data storage and transmission methods: testing

Feasibility of 3D ultrasound data acquisition and reliability of data retrieval from stored 3D images

Final Report: National Children's Study (NCS) estimating subject burden for potential NCS measurements

Focus group phase I: eliciting community involvement, recruitment, and retention of subjects

Focus group phase II: follow up on recruitment and retention of subjects

Interim Assessment: Current understanding of the feasibility of the 3D ultrasound volume data set capture and evaluation: Testing feasibility of 3D ultrasound data acquisition and reliability of data retrieval from stored 3D images

Interim Report: Develop a community involvement strategy: Prepare to implement a cohort study of children's environmental health for the North Carolina Pilot Project

Methods Advancement for Milk Analysis (MAMA)

North Carolina Herald Study

Overall procedures protocol and patient enrollment protocol: Testing feasibility of 3D ultrasound data acquisition and reliability of data retrieval from stored 3D images

Pilot study to validate the extraction and analysis of DNA from non-invasive DNA sources for application in environmental epidemiology studies

Reliability and validity of injury reporting

Status Report: Begin to develop complete operations manuals for the cohort: Prepare to implement a cohort study of children's environmental health for the
North Carolina Pilot Project

Tampa Asthmatic Children's Study (TACS)

Testing feasibility of 3-D ultrasound data acquisition and reliability of data retrieval from stored 3-D images

Time-integrated exposure measures to improve the predictive power of exposure classification for epidemiologic studies

Use of biomarkers of response for assessing potential sensitivity of children to adverse health outcomes from exposure to environmental chemicals: subproject 1

Use of biomarkers of response for assessing potential sensitivity of children to adverse health outcomes from exposure to environmental chemicals: subproject 2

Reviews/White Papers

Title

Administrative and other data on individuals and areas

Analysis of existing measures of children's health services and measures for the NCS

Assessment of existing linkages with local communities among potential research sites

Assessing social-emotional development in children from a longitudinal perspective for the National Children's Study: A compendium of measures

A systematic analysis of possible sampling strategies (Westat Report)

Background papers for sampling workshop

Battelle validation sampling design software—Beta release user's guide

Biomarkers database (phase I, II, and III)

Brief on estimated sample size of women needed to result in 100,000 live births

Characterizing anticipated mobility of the National Children's Study cohort

Discussion on utility of validation samples for the National Children's Study

Ethical (pro and con) issues related to inclusion of minor adolescents who are pregnant or at risk of pregnancy

Evaluation of exposure measurement methods and approaches for the NCS

Executive Summary: Characterizing anticipated mobility of the National Children's Study cohort

Executive Summary: Discussion on utility of validation samples for the National Children's Study cohort

Final Report: Biomarkers of environmental health and safety risks to children for use in a longitudinal cohort study—update

Final Report: Literature search on housing and neighborhood characteristics and conditions related to child health and development

Final Report: Use of 1) sensors and 2) Radio Frequency ID (RFID) for the National Children's Study

Final White Paper with Executive Summary: Measurement and analysis of exposures to environmental pollutants and biological agents

How retrospective information is collected and recalled

Identifying and selecting developmental measures

Lessons learned papers from the Children's Environmental Health Centers – Special issue of EHP

Longitudinal assessment of motor development in epidemiologic research for the National Children's Study

Measuring housing quality and characteristics

NCS IMS release strategy

Neuropsychological assessments in children from a longitudinal perspective for the National Children's Study

Potential impact of the NCS on priority health outcomes

Psychiatric assessments in children from a longitudinal epidemiologic perspective for the National Children's Study economic impact of the National Children's Study PowerPoint presentation

Strategies for minority recruitment in the National Children's Study: Issues of trust

Systematic review of core and potential hypotheses (Lewin Report)

Technology assistance in clinical information collection

Technology needs assessment and testing: Use of sensors and RFID for the NCS

Using the selected National Children's Study sampling area for the Age, Gene/Environment Susceptibility Study

White paper on evaluation of sampling design options for the National Children's Study

White papers on measurement of housing quality

Workshops

Title

Addressing rural children in the NCS

Ascertainment and diagnosis of birth defects: objectives, methods, and measurements

Assessing dietary intakes and patterns in women and young children: methodological issues with implications for the design of the NCS

Assessing the incidence and outcome of mild traumatic brain injury

Cancer and the NCS: opportunities and challenges

Collection and use of genetic information in the NCS

Community engagement

Dietary assessment in a prospective epidemiologic study for pregnant women and their offspring

Ethical issues in longitudinal pediatric studies

Exploration of day-specific probabilities of pregnancy

Expanding methodologies for capturing day-specific probabilities of conception workshop

Fetal and neonatal growth and development

Gene environment interaction and the regulation of behavior

Growth and body composition: objectives, methods, and measurements

Identification of measures for health care processes and outcomes in the NCS

Innovative technologies for remote collection of data for the National Children's Study workshop

International Cancer Consortium Workshop

International consultation on longitudinal cohort studies

Measuring parenting from an epidemiologic perspective

Measuring physical activity in the NCS

Measuring race/ethnic discrimination and racism from a developmental perspective

Measurement of Maternal and Fetal Infection and Inflammation Workshop

Media effects on child health and development

Medicines exposures: collection, coding, and classification

Methods for the assessment of asthma-related health outcomes

Neurobehavioral development and environmental exposures: measures for the NCS placental measurements

Psychosocial stress and pregnancy and infancy

Sampling design

Technology needs assessment and testing

Time-use data for the NCS

Use of herbal products in pregnancy, breastfeeding, and childhood

National Children's Study
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STATISTICAL ANALYSIS PLAN FOR BIRTH DEFECTS FROM IMPAIRED GLUCOSE METABOLISM

1. Background and Hypothesis Development

1.1 Introduction

The risk of congenital anomalies in offspring is higher among women who have type 1 or type 2 diabetes before or during the early stages of pregnancy (Becerra, Khoury, Corder, & Erickson, 1990). Major congenital malformations are the leading cause of mortality and serious morbidity in infants of mothers with established diabetes (Kitzmiller, Buchanan, Kjos, Combs, & Ratner, 1996). Animal models show the teratogenicity of impaired glucose metabolism throughout the range of elevated glucose, not just for the most extreme values that in humans would be called frank diabetes (Moley, Vaughn, & Diamond, 1994). However, the current literature offers no definitive findings regarding the association between lesser magnitudes of glucose intolerance and birth defects in humans. It is important to fill this gap in the understanding of human fetal development because impaired glucose tolerance (IGT) is more common than frank diabetes, so that, even if relative risks for IGT are smaller than those for frank diabetes, the population disease burden (e.g., as measured by population attributable risk) may be higher for IGT. Another reason this issue is important is that the prevalences of both IGT and pregestational type 2 diabetes are rising along with the obesity epidemic in the United States. Further, many studies looking at the effects of impaired glucose metabolism in early pregnancy on congenital anomalies have examined only known cases of diabetes, thus failing to include women who have unrecognized or undiagnosed forms of impaired glucose metabolism in analyses. (Farrell, Neale, & Cundy, 2002; Schaefer et al., 1997; Anderson et al., 2005; Kanwar et al., 2005).

In summary, while there is strong evidence of the impact of diagnosed pre-existing and gestational diabetes on pregnancy outcomes, there is inadequate information about the influence of impairment of glucose tolerance. To address this issue, the following broad hypothesis has been developed for investigation using data collected in the National Children's Study (NCS):

> Impaired glucose metabolism during pregnancy is associated with increased risk of major congenital malformations of the heart, central nervous system, musculoskeletal system, and all congenital birth defects combined.

1.2 Prevalence of Birth Defects and of Impaired Glucose Metabolism During Pregnancy

A complication in studying congenital birth defects is that there are many forms of congenital malformations, all of which are relatively rare. The three most common birth defects in the United States are major congenital malformations of the heart (0.6 percent of births) (Hoffman & Kaplan, 2002); of the central nervous system (0.3 percent of births) (Branum & Schoendorf, 2003); and of the musculoskeletal system (0.2 percent of births) (Feuchtbaum et al., 1999). Overall, an estimated 3-4 percent of all births in the United States exhibit one or more congenital malformations (Leppig, Werler, Cann, Cook, & Holmes, 1987; Lynberg & Edmonds, 1994).

IGT is defined as two-hour glucose levels of 140 to 199 mg/dL on the 75-g oral glucose tolerance test (OGTT), and impaired fasting glucose is defined as glucose levels of 100 to 125 mg/dL in fasting patients (Rao, Disraeli, & McGregor, 2004). Prevalence estimates of IGT differ depending on the test used for its diagnosis. Based on data from the 1999-2002 National Health and Nutrition Examination Survey (NHANES), approximately 26 percent of women between 20 and 39 years old have impaired

fasting glucose, 1.7 percent have diagnosed diabetes, and 0.7 percent have undiagnosed diabetes (Cowie et al., 2006). However, since pregnancy is known to increase the risk of IGT, the prevalence of IGT within the NCS sample of pregnant women will be higher. It has been estimated that about 4-7 percent of pregnancies in the United States are complicated by gestational diabetes, with possibly higher rates in some ethnic groups (Kjos & Buchanan, 1999).

1.3 Development of a Specific Hypothesis

In a clinical setting, IGT is commonly measured by using either fasting glucose alone, a fasting OGTT, or a combination of the two. While these measures would give the most accurate determination of a pregnant woman's impaired glucose metabolism, the NCS has decided for practical reasons not to collect fasting glucose measures in early pregnancy. Participant burden issues related to the unpleasantness of these tests for pregnant women and the need to retain the women and their offspring in the Study over many years has led to a decision to rely on a less burdensome test based on non-fasting blood samples collected during the first trimester of pregnancy. These samples will be used to assess Hemoglobin A1c (HgbA1c) levels, which do not depend on the fasting state of the woman.

The level of HgbA1c, also known as glycated hemoglobin or glycosylated hemoglobin, is an indicator of the person's blood sugar control during the previous 2-3 months. HgbA1c is formed when glucose in the blood binds irreversibly to hemoglobin to form a stable molecule. It possesses the lifespan of normal red blood cells (90-120 days), being eliminated from the bloodstream only when the red cells are replaced. Several studies have demonstrated that HgbA1c levels are directly proportional to the concentration of glucose in the blood over the full lifespan of the red blood cells and are not subject to the fluctuations that are seen with daily blood glucose monitoring (Shibata et al., 2005; Davidson, Schriger, Peters, & Lorber, 1999; Hassan, Johnson, Nader, Gannon, & Nuttall, 2006; Woerle et al., 2004). In healthy individuals, HgbA1c makes up approximately 4-6 percent of all hemoglobin in the bloodstream, with values as high as 12 percent in those individuals with poorly controlled diabetes. The standard of care guidelines for diabetics is to maintain the HgbA1c level at 7 percent or less (American Diabetes Association, 2007); however, it has been shown that risk of coronary heart disease increases with HgbA1c levels above 4.6 percent (Khaw et al., 2004; Selvin et al., 2005). In a study of women who required insulin therapy, HgbA1c levels measured in the first trimester were found to have a strong association with adverse pregnancy outcomes, with a value of 7.8 mg/dL in women whose children had major malformations vs. 6.7 mg/dL in the others (Schaefer et al., 1997). These results support the use of HgbA1c as an effective indicator of IGT in situations where fasting blood glucose samples cannot be obtained.

Since the adverse effects of IGT for organogenesis do not appear to be limited to specific organ systems, the primary hypothesis examines all congenital birth defects grouped together as one outcome. The primary hypothesis presented above is therefore operationalized for use with NCS data to the more specific hypothesis that is the main basis of the statistical analysis plan presented in this document:

> Elevated HgbA1c levels during the first trimester of gestation are associated with increased risk of congenital birth defects.

A secondary hypothesis concerns the association between IGT and congenital heart defects, which are the most prevalent organ system birth defects. The NCS sample size will be large enough to provide sufficient statistical power to detect sizable effects for this subset of birth defects. As a result, the following secondary hypothesis is proposed:

> Elevated HgbA1c levels during the first trimester of gestation are associated with increased risk of congenital heart defects.

1.4 Justification for This Analysis

Three aspects of the NCS make it an extremely good source of data for analyzing the specific hypothesis identified above:

- The analyses will be able to assess the effects of HgbA1c levels on a nationally representative sample of live births as well as provide national estimates of the distribution of HgbA1c levels measured in the first trimester.
- The very large NCS sample size will result in a large number of birth defects for the analysis.
- The NCS longitudinal design will provide the standardized data needed to quantify HgbA1c levels in the first trimester of pregnancy and birth defects that may be detected up to age 2 (see below).

2. The Variables Used in the Analysis

This section describes the variables that will be used in the analyses of the specific hypotheses relating first trimester HgbA1c levels and congenital birth defect outcomes, which have been discussed in Section 1 above. The section starts with precise descriptions of the methods that will be used to measure the outcome variable, i.e., presence or absence of congenital birth defects (Section 2.1), and follows with descriptions of the HgbA1c exposure variable (Section 2.2) and the covariates that will be used in the analyses (Section 2.3).

2.1 Outcome Definitions and Measurement

Definitions of Outcomes

All major congenital malformations diagnosed in live-born infants by 24 months of age will be included in this analysis (see Appendix I of the Research Plan for a complete list of diagnostic measures). There are two primary outcomes to be analyzed: (1) any major congenital malformation (approximate prevalence 3-4 percent); and (2) major congenital heart defects (approximate prevalence 0.6 percent) (Hoffman & Kaplan, 2002).

NCS field staff and photo reviewers will assess presence of major congenital malformations based on the NCS protocol. NCS research staff will use such measures as a 12-14 week ultrasound and direct examination occurring at birth, the 6-month visit, and the 12-month visit to diagnose various congenital malformations. Staff will also collect medical records in support of diagnoses of malformations reported by parents on questionnaires through 2 years of age. Utilizing these data, the NCS

will identify such birth defects as facial clefts, ear abnormalities, other head/facial abnormalities, skin and hair abnormalities, limb abnormalities, hand and foot abnormalities, congenital heart defects, chest wall abnormalities, abdominal wall defects, gastrointestinal defects, neural tube defects, urogenital malformations, and major chromosomal disorders. Reviews of medical records and maternal interview data will be used to detect the existence of birth defects that are not identified by photo review, as in the case of cardiac birth defects.

A few live-born infants will die before they reach the age of 2 years. A number of those infants who die soon after birth are expected to have birth defects. If the infant dies before hospital discharge, hospital discharge records will not be available. In an effort to identify such cases, the NCS will attempt to obtain death certificates from which to collect data regarding the presence of birth defects. Infants for whom the death certificates indicate the presence of a major congenital birth defect will be included as cases in the analysis. Those infants who die prior to discharge and whose death certificate and autopsy report do not indicate that a birth defect was present at the time of death will be recorded as not having a birth defect.

To reach a final determination and classification of a congenital birth defect, a panel of medical experts will adjudicate all reported birth defects through review of medical records and images, including sonograms. This adjudication process will also include a review of all death certificate and autopsy reports associated with each live birth.

2.2 Definition and Measurement of Exposure

Standard blood samples will be collected during the scheduled first trimester visit for all pregnant women who have been enrolled in the NCS by that time and have consented to provide the blood samples. Sample volumes will be kept to a minimum to reduce the burden to the pregnant women. Following collection of these biospecimens, they will be analyzed and/or stored in one or more repositories for future analysis. Since these blood samples will be used for a number of different investigations, their use for the current analyses must be carefully controlled.

Following the 2-year observation period during which congenital malformation diagnoses will be made for the NCS children, all children with birth defects (cases) and a sample of children without birth defects (controls) will be selected for the current analyses. If selected, an aliquot of the mother's stored first-trimester blood sample will be used to analyze the HgbA1c level. HgbA1c levels will be measured as a percentage, which may be categorized for some analyses.

2.3 Definition and Measurement of Covariates

A critical consideration in analyzing the association between first trimester HgbA1c levels and congenital birth defects is the inclusion of relevant covariates in the analyses. Potential confounders that are causally prior to the first trimester HgbA1c level must be incorporated in the analyses since they may explain away any association observed between HgbA1c levels and birth defects. Also potential effects modifiers (or interaction effects) that result in differential levels of association need to be included in order to provide meaningful interpretation of the associations under different conditions. The many important covariates that will be considered in the statistical analyses are listed below. For convenience they are grouped by type. The determination of which of these covariates are confounders and which are effect modifiers will be made based on theoretical considerations and the statistical methods described in sections 4.3 and 4.4.

2.3.1 Covariates to be Investigated

2.3.1.1 Covariates Related to Individual and Family History of Morbidity

Previous history of birth defects and/or family history of birth defects: Mothers whose first child had a birth defect are 2.4 times more likely than other women to have a second infant with a birth defect. Most of the risk is accounted for by the same defect recurring (Lie, Wilcox, & Skjaerven, 1994). Perhaps one of the strongest covariates in the association between impaired glucose metabolism and congenital birth defects is a family history of birth defects, spontaneous abortions, stillbirths, and subfertility (Jenkins et al., 2007). About 20 percent of birth defects are hereditary, resulting from the interaction of genes from one or both parents plus environmental influences. Defects may include cleft lip and palate, spina bifida, and heart defects (Shaw, Rozen, Finnell, Wasserman, & Lammer, 1998; Lott, 1996).

Pre-pregnancy and pregnancy body mass index (BMI): Obesity prior to and during pregnancy, which is a result of a complex interaction between many variables related to diet and physical activity, is associated with an increased risk of birth defects and with impaired glucose metabolism (Anderson et al., 2005). Prepregnancy obesity is associated with an increased risk for birth defects (Waller et al., 2007) and may modify the impact of glucose metabolism on the risk of congenital birth defects (Hotamisligil, 2006). It will be analyzed as both a categorical variable (underweight, normal, overweight, obese, morbidly obese) and as a continuous variable.

Previous history of gestational diabetes: A woman who experienced gestational diabetes in a previous pregnancy is at an increased risk of recurrent gestational diabetes in subsequent pregnancies and at an increased risk of type 2 diabetes mellitus in her lifetime. Birth defects have been associated with gestational diabetes, which probably reflects an association with undiagnosed type 2 diabetes.

2.3.1.2 Covariates Related to Demographic Factors

Race/ethnicity: Racial and/or ethnic factors are often associated with both variation in prevalence of birth defects (Correa, McCarter, Downing, Ferencz, & the Baltimore-Washington Infant Study Group, 1991; Canfield et al., 2006) and variation in the risk of IGT among women of reproductive age.

Socioeconomic status (SES): Because access to quality health services can be associated with the risk of IGT as well as with variation in the prevalence of birth defects, SES needs to be taken into account in an evaluation of IGT and risk of birth defects.

Area of residence: Women who reside in the same geographic area are exposed to the same environmental risk factors. Previous research has demonstrated an association between environmental air quality and both impaired first trimester glucose tolerance and congenital birth defects in offspring (Gilboa et al., 2005).

2.3.1.3 Covariates Related to Behavior/Lifestyle Factors

Smoking status: Nicotine and carbon monoxide play a role in causing adverse pregnancy outcomes (U.S. Department of Health and Human Services, 2004; Law et al., 2003; American College of Obstetricians and Gynecologists, 2000; Wang et al., 2002; Little, Cardy, & Mungar, 2004). Cigarette smoke has been shown to reduce the efficacy of folic acid in the prevention of congenital birth defects

(Shaw, Nelson, et al., 2002). Smoking is also associated with an increased risk for oral clefts, but the evidence for an association with other defects is limited and inconsistent. Whether smoking is associated with IGT is not clear.

Use of medication: Anticonvulsants can cause serious problems, including mental retardation and slow growth, in the developing fetus. Other drugs associated with birth defects include antipsychotic and antianxiety agents and certain antibiotics (Jones, 1996; Hernandez-Diaz, Werler, Walker, & Mitchell, 2000, 2001). There is also some evidence that some antidepressants may be associated with birth defects and with exacerbation of IGT, and that some antibiotics with a folate-antagonist effect can increase the risk of certain defects.

Physical activity: An increase in physical activity either prior to or during the first weeks of pregnancy is a recommended course of action for women who present with impaired glucose metabolism, both to improve glucose metabolic function and to reduce the risk of adverse health effects on the offspring from prenatal IGT (Jenkins et al., 2007).

Time of entry into prenatal care: Time of entry into prenatal care may serve as a proxy measure for quality of care for IGT and use of medications for glucose metabolism. Receipt of such care to manage impaired glucose metabolism either prior to or during pregnancy through behavior, diet, medication, etc., may affect a mother's glucose metabolism as well as overall nutritional status.

Use of nutritional supplements: Supplementation with folic acid attenuates the risk for neural tube defects (Berry et al., 1999; Bower & Stanley, 1989; Czeizel, 1993; Czeizel & Dudas, 1992; Daly, Kirke, Molloy, Weir, & Scott, 1995; Laurence, James, Miller, Tennant, & Campbell, 1981; Milunsky et al., 1989; MRC Vitamin Study Research Group, 1991), cardiac defects (Botto, Khoury, Mulinare, & Erickson, 1996; Botto, Mulinare, & Erickson, 2000; Czeizel, 1996: Czeizel, Toth, & Rockenbauer, 1996; Lewis, Van Dyke, Stumbo, & Berg, 1998; Shaw, Nelson, et al., 2002), oral clefts (Itikala, Watkins, Mulinare, Moore, & Liu, 2001; Lewis et al., 1998; Shaw, Lammer, Wasserman, O'Malley, & Tolarova, 1995; Tolarova & Harris, 1995; Yang, Khoury, Olney, & Mulinare, 1997), and urinary tract defects (Czeizel, 1996; Lewis et al., 1998; Werler, Hayes, Louik, Shapiro, & Mitchell, 1999; Yang et al., 1997). Supplementation with antioxidant vitamins appears to attenuate risk for cardiac defects (Correa, Botto, Liu, Mulinare, & Erickson, 2003).

Drug use: Recreational drug use in pregnant women has been associated with arm and leg abnormalities and central nervous system problems in offspring (Jones, 1996). Whether use of recreational drugs affects the risk of IGT is unclear.

2.3.1.4 Covariates Related to Intrinsic Factors

Cardiometabolic risk factors: The presence and level of cardiometabolic risk factors (e.g., obesity, hypertension, dyslipidemia, C-reactive protein, and other markers of low-grade inflammation), which in turn are related to behavioral factors such as diet and physical activity, in a pregnant woman's bloodstream have been shown to be associated with impaired glucose metabolism (Hotamisligil, 2006). Some of these factors have also been shown to be associated with risk of birth defects.

Lipid profile: Maternal fat-modified diets result in lower total and HDL cholesterol in infants and may be a suitable way to prevent cardiovascular disease among infants from the beginning of life (Fard, Mehrabian, Sarraf-Zadegan, & Sajadi, 2004). Central adiposity, which may be difficult to measure or detect, is associated with increased serum triglycerides and cholesterol levels, which are associated with an increased risk for IGT.

Serum inositol: The mechanisms by which hyperglycemia leads to birth defects are not clear but are probably complex and could be related to oxidative stress, low inositol levels, and other metabolic abnormalities associated with hyperglycemia. Studies in animals suggest low levels of inositol may be associated with an increased risk of neural tube defects (NTDs) and other defects (Cockroft, Brook, & Copp, 1992; Hashimoto et al., 1990; Baker, Piddington, Goldman, Egler, & Moehring, 1990; Green & Copp, 1997; Cogram et al., 2002). A recent case-control study also suggests that low serum levels of inositol may be associated with an increased risk for spina bifida (Groenen et al., 2003). Thus, in evaluating the role of hyperglycemia, it is important to take into account the inositol status of women early in pregnancy. Inositol, a sugar with important structural and functional properties, is part of the diet but is also synthesized by the body.

Gene-nutrient interactions: Methylenetetrahydrafolate reductase (MTHFR) polymorphisms appear to be associated with increased risk of birth defects (Botto & Yang, 2000). Reduced folate carrier and MTHFR appear to interact with folic acid supplementation to modify the risk of birth defects (Shaw, Nelson, et al., 2002; Shaw et al., 1998). However, there is no evidence to suggest that these gene-nutrient interactions have any association with IGT.

Parity and multiple births: The number of fetuses present in a pregnancy may be associated with risk of birth defects (greater risk among multiples than among singletons). However, whether multiple births are associated with IGT is not clear. While parity or number of births has been not been shown to be a confounder of the association between glucose metabolism and birth defects , parity of births does play a significant role in other birth outcomes, such as low birth weight.

Age of mother: While the prevalence of diabetes increases with age (Centers for Disease Control and Prevention [CDC], 2003), it is not clear that, in the absence of chromosomal disorders, congenital birth defects are associated with maternal age.

Hormone levels (e.g. cortisol): Fetuses exposed to glucocorticosteroids in the first trimester appear to have a lower median birth weight and are born at an earlier gestational age, which increases the risk for being underweight or having birth defects resulting from underdevelopment, but they have not been shown to exhibit an increased teratogenic risk (Gur, Diav-Citrin, Shechtman, Arnon, & Ornoy, 2004). However, they have been included within this discussion because of the known teratogenic effects of cortisol and other stress-related hormones in animal models (Goldman, Katsumata, Yaffe, & Gasser, 1997).

2.3.1.5 Covariates Related to Environmental Factors

Respiratory infections and febrile illnesses: Research has indicated that certain maternal infections and febrile illnesses, such as rubella and influenza, are related to birth defects (Jenkins et al., 2007). Mothers reporting any febrile illness during the first trimester of pregnancy have a two-fold higher risk of offspring with a heart defect (Ferencz, Correa-Villasenor, & Loffredo, 1997). However, there is little evidence to suggest that such infections are associated with IGT.

2.4 Data Sources for the Covariates

Data on the covariates to be used in this analysis will be obtained through various collection instruments. Data regarding a participating mother's family and personal history of birth defects, family and personal history of diabetes and/or obesity, race/ethnicity, SES, area of residence, age, smoking

status, specific medication use during critical time periods prior to and during pregnancy, use of nutritional supplements again during critical time periods, time of entry into prenatal care and physical activity levels will be collected through administration of several questionnaires. Data of biochemical origin, such as the mother's lipid profile and other cardiometabolic risk factors, serum inositol and other hormone levels, in addition to the mother's HgbA1c level, will be assessed from the first trimester blood sample. In addition, all genetic sequencing for targeted genes will also be analyzed. Data obtained from medical observation, such as parity of births, respiratory infections, febrile illnesses, sonogram results at the time of examination, will be recorded by a licensed medical practitioner. Medical record reviews will be performed to verify information obtained through the data collection efforts described above.

3. Analytic Approach

3.1 Introduction

The National Children's Study is based on a representative sample of approximately 100,000 infants live-born to women residing in the United States. These births will be sampled in 105 geographic locations (see the Research Plan for sampling details). The pregnancy status of all eligible women of child-bearing age in these areas will be monitored for 4 years. All women living within the Study locations who become pregnant during the 4-year period will be enrolled in the study as early in pregnancy as possible in order to measure in utero exposures. Some women who state that they are seeking to become pregnant will in fact be enrolled in the NCS prior to becoming pregnant.

Although the NCS is a nationally representative cohort study of births, a nested case-control approach will be used for the analysis of the association between HgbA1c and birth defects. The nested case-control analysis approach offers two important advantages over an approach that analyses the full cohort. First, there is a significant advantage in terms of cost. Since the determination of a person's HgbA1c level costs approximately $22 per individual in 2007 in some research laboratories, it would be very costly to analyze HgbA1c levels for the full cohort. By restricting the analysis to all cases and a sample of a small number of controls per case, the cost of analyzing the blood samples is considerably reduced with only a modest loss of power for detecting effects.

The second important advantage of the case-control approach is that the repository samples of non-sampled controls remain available for use in other research. Given the number of probable analyses on the first trimester of pregnancy blood samples, this is a major consideration.

It should be noted that the number of infants with birth defects and other numbers used in the calculations below are initial estimates used only for illustrative purposes. They will need to be revised based on the results obtained during the actual data collection process.

3.2 Inclusion/Exclusion Criteria

Since the NCS is confined to live births for practical reasons of data collection, the Study will collect data only on the birth defect outcome status for those pregnancies that result in a live birth. The main analyses for this investigation of the association between HgbA1c levels and congenital birth defects will thus exclude miscarriages and stillbirths (but see below).

Excluded from this investigation will be births to mothers who experience Phenylketonuria (PKU). PKU, which is a rare autosomal recessive disorder of amino acid metabolism that affects 0.01 to 0.02 percent of births in North America, is unrelated to glucose metabolism. It is most often due to

deficiency of the enzyme phenylalanine hydroxylase which causes the accumulation of harmful metabolites, including phenylketones. If the mother is untreated, PKU can lead to several neurological birth defects (Luke & Keith, 1990).

Also to be excluded are births to mothers who during pregnancy used known teratogenic drugs or medications, such as Acutane, that are associated with induction of birth defects. These exclusions will be confined to births to mothers who used medications that are known to be causally associated with birth defects to the point that they outweigh any influence that glucose metabolism may have on congenital birth defects.

Chromosomal malformations such as trisomies, most notably Down syndrome, are not suspected as being influenced by glucose metabolism. As a result, those births that result in a diagnosis of Down syndrome, or any other trisomies, will be excluded from the analysis.

Another issue to be considered relates to women with pharmacologically treated diabetes. The plan is to retain the births to these mothers in the investigation. To guard against possible biases created by doing so, two analyses will be performed: one will analyze data from all women in the investigation while the second will exclude women with pharmacologically treated diabetes.

The findings from this study need to be interpreted in the light of the above exclusions. In particular, the number of miscarriages and stillbirths is sizable and many of the have malformations. Fetal mortality rates (20 weeks or later in gestation) are estimated at 6.4 per 1,000 (National Center for Health Statistics, 2003). Pauli and Resier (1994) reported that malformation syndromes and single malformations were causes of death in 78 percent of stillborns in a representative cohort. Malins (1978) reported that fatal malformations accounted for 50 percent of fetal losses in a cohort of diabetic pregnancies. While the magnitude of fetal death associated with fetal malformations is not known, the available data indicate it may be non-trivial. Since the main analysis will include only live births, a secondary analysis into the effects of first trimester HgbA1c levels on fetal death during the second or third trimesters will be conducted. This will also be a case-control study, with the cases being those mothers whose pregnancies result in fetal death and the controls being those controls used for the main analysis. It is estimated that there will be about 500-600 fetal deaths observed in the NCS sample of pregnant women. Fetal death is the main outcome variable for this analysis because it will be known for all the women involved. In addition, an investigation will be conducted to determine the number of the fetal deaths for which fetal death records can be obtained and the extent to which these records provide reliable information on the presence of congenital malformations. If reliable information on congenital malformations can be obtained for a large proportion of the fetal deaths, then another analysis that uses congenital malformations as the outcome variable will be conducted.

3.3 Selection of Cases

Within the context of this analysis, cases are defined as children with a medically diagnosed congenital birth defect identified during the first 24 months of life. Ideally, the study would take as the sample of cases all those infants who are diagnosed as having birth defects among all 100,000 mother-infant pairs in the full NCS sample. However, the sample size available for analysis is reduced for two reasons. First, some mothers in the NCS will not have provided the prenatal repository samples collected in the first trimester for use in measuring the HgbA1c exposure being studied in this analysis. Second, outcome data on birth defects will not be obtained for a small number of the live births due to withdrawal of consent, loss to follow-up, emigration, or death before the infant is 2 years old.

As discussed in Section 2.2, the exposure measurement required for this hypothesis is HgbA1c level as measured in the first trimester of pregnancy. Under the study design, HgbA1c levels will be assessed from a blood sample obtained from pregnant women during a first-trimester data collection visit. However, some women recruited into the NCS (approximately 10 percent) will not enter the study until after the first trimester of pregnancy and therefore their HgbA1c levels during the required period will not be known. The exposure measure will also be missing for a small percentage of women (approximately 4 percent) who refuse to provide a blood sample during the first trimester visit. As a result, it is estimated that first trimester HgbA1c levels will be available for approximately 86,400 women (i.e., 0.9x0.96x100,000).

While the presence of a birth defect may be diagnosed up to 24 months of age, in the majority of cases birth defects will be detected immediately after the infant is born. Outcome data will not be obtained for some of the births (approximately 3 percent) due to withdrawal of consent, loss to follow-up, or emigration, and for some infants who die in the first 2 years of life. For live births in these categories, questionnaire data, medical records, and/or death certificates will be used to determine if a congenital birth defect was diagnosed, and, if it was, they will be classified as cases. The others will be classified as having missing outcome data (recognizing that there is a high likelihood that they do not have birth defects due to the low prevalence of the identification of birth defects later in the 2 year period). The total number of live births for whom birth defects are not diagnosed before they leave the study is estimated to be 2,930 (i.e., 0.03x100,000 less an estimated 70 births for whom outcome data is obtained from other sources). Of these, 400 are estimated to be to mothers who lack a first trimester HgbA1c measurement.

The proposed analysis will thus be based on a sample selected from the approximately 83,870 mother-infant pairs for whom both exposure and outcome data are available. As discussed in Section 3.5.1 below, weighting adjustments will be applied to account for the possibility that mother-infant pairs with complete data may have different characteristics from those for whom HgbA1c levels and/or birth defects are unknown.

Based on an assumed prevalence rate for birth defects of 35/1,000 infants, the expected number of cases for use in the analysis is about 2,995.

3.4 Selection of Controls

For reasons of economy and the preservation of first-trimester blood samples, it is proposed to include only a sample of the approximately 80,875 infants without birth defects to serve as controls in the analysis. There are three primary issues to consider when selecting mother-infant pairs as controls for the analysis: what, if any, matching variables should be used; how many controls should be selected; and what sampling methods should be used. These issues are discussed in turn below.

3.4.1 Matching Cases to Controls

Matching in case-control studies ensures that the matching factors are equally distributed between cases and controls and thus removes any confounding effects of these factors. While controlling for confounders can be done in the sample design or during analysis, the former approach can be more statistically efficient. A critical requirement in the choice of any variables used to match in design is that the matching factors must be clearly and unambiguously causally prior to the exposure measure. If any doubt exists, it is better to assess the effects of the variable in the analysis, where it is possible to include it in some analyses and exclude it from others.

The initial proposal is to match on the primary sampling unit (PSU), i.e., geographic location, and time of pregnancy (e.g., year, season). The reason for choosing PSU is to accommodate the replication methodology that will be used for variance estimation (see Section 3.5.3). Matching on the year and/or season corresponding to the start of pregnancy may help to control for various temporal and/or environmental factors. The general approach proposed is to account for all other confounders in the analysis, unless there would be a marked loss in power through doing so. To identify potential confounders that need to be included in the matching process, the associations of the potential confounders with the outcomes will be computed. If any of these associations are so strong that handling the confounders in the analysis would likely lead to a substantial loss of power compared to matching in the design, consideration will be given to using these confounders as matching factors.

3.4.2 Number of Controls per Case

The greater the number of controls per case, the greater is the power for hypothesis testing and the greater is the precision of the estimates produced. The marginal increase in power in moving from three to four controls per case is 6.7 percent, from four to five is 4.2 percent; and the marginal increases diminish rapidly thereafter (Taylor, 1986; Ury, 1975). Also, the loss of precision and power from reducing the control sample size from that of the full cohort of possible controls with complete data (83,870) to the proposed sample size of four times as large as the case sample size (i.e., 14,970) is not that great while leading to substantial cost savings and preservation of blood samples. The compromise allocation of four controls per case is therefore chosen as the one that best balances costs against benefits.

3.4.3 Methods for Selecting Controls

First, cases and controls will separately be grouped according to their values on the matching factors, such as PSU, time of pregnancy, and any other variables chosen based on the analyses of their associations with the outcomes. It is unlikely that it will be necessary to control for more than one or two other variables by matching in design. Hence, there will be several cases in each group and a sample of matching controls that is four times the size of the case group will be selected. The controls will be selected using systematic probability proportional to size (PPS) sampling, where the size measure is the inverse of the nonresponse adjusted sampling weight. Selection of the sample in this manner results in a roughly equal probability of selection for each of the matched controls within a group.

This type of selection is known as “frequency matching,” where a group of cases with similar characteristics is matched at a given ratio (1:4 in this instance) to a group of controls with the same characteristics. There are several operational and analytic advantages to frequency matching, and it is often used in survey settings (Korn & Graubard, 1999).

3.5 Weighting

3.5.1 Methodology and Rationale

The general approach adopted for the analysis is a “design-based approach” in which the estimates produced by the analysis are estimates for the U.S. population of inference, namely live births. The 100,000 live births in the full NCS constitute a national sample of live births. When this sample is appropriately weighted to compensate for unequal selection probabilities, nonresponse, and noncoverage,

the sample estimates are estimates for the nation.[1] However, a caveat, as discussed above, is that some of the sample mother-infant pairs lack data on the HgbA1c exposure measure and some lack information on the birth defect outcome measures. Steps need to be taken to compensate for these missing data in order to maintain the ability to produce national estimates.

There are a number of ways to handle the problem of the missing exposure and outcome data. One option would be to simply restrict the analysis to the mother-infant pairs for whom both exposure and outcome data are available, but selection biases may make the results unrepresentative of the U.S. population of inference. Another option would be to impute for missing data on HgbA1c levels and data on birth defects so that all infants in the NCS cohort could be used in the analysis. However, this approach would require a variance estimation method that accounts for the effects of imputation on the variances of the estimates produced, such as through the use of multiple imputations. Rather than employ the more complex imputation approach, weighting adjustments are proposed as the method for dealing with the missing exposure and outcome data. The full cohort weights of the approximately 83,870 mother-infant pairs with both exposure and outcome measures will be adjusted so that this sample is representative of the U.S. population of inference.

As discussed earlier, the full NCS sample will consist of 100,000 live-born infants, of whom approximately 86,400 are expected to have exposure measurements (HgbA1c) from their mothers' first trimesters of pregnancy. Among the 86,400 infants with exposure data, a certain number will be lost to the study before the 24-month period for assessing birth defects has elapsed. Some of these infants will have incomplete outcome data. It is possible to address both these missing data problems through carefully constructed weighting adjustments that take account of what is known about those with incomplete data (Brick & Kalton, 1996).

To compensate for missing outcome data from incomplete follow-up, adjustment factors will be calculated within selected weighting classes formed by exposure, demographic, and other variables related to missing data rates. The weighting classes would likely be formed based on length of follow-up, absence of evidence of birth defects during follow-up, and other factors such as mothers' HgbA1c levels being similar (if known) or unknown. The full NCS weights of infants with outcome data will be adjusted upwards within each weighting class to represent infants with missing outcome data in that class. For example, consider an infant who dies at age 6 months and for whom no information pertaining to birth defects is obtained from death certificates or other sources. Suppose also that the mother's first trimester HgbA1c levels are not known. This infant would be assigned to a weighting class where for all members of the class: mother's exposure is unknown; outcome data on birth defects is known; the infant lived for at least 6 months and had not been diagnosed with a birth defect up to that time (but may have been subsequently); and demographic characteristics such as socioeconomic status and mother's age are similar to those for the infant with missing outcome data. The sampling weight of the infant with missing exposure and outcome data would then be pro-rated across the members of the weighting class with missing exposure data but known status with respect to birth defects. These adjustments compensate for missing outcome data, leaving the issue of next compensating for missing exposure data.

Weighting adjustments will also be applied to compensate for infants with missing exposure data. This process need only include infants with known outcome data and their adjusted weights from the first step, since they now represent infants for whom birth defect status is unknown. Weighting classes will be formed using outcome values and other factors that are known to be related to maternal hemoglobin levels (e.g., socioeconomic status), and weighting adjustments will be computed in a manner similar to that described above.

[1]See Chapter 8 of the NCS Research Plan for a discussion on the statistical weighting methods to be used for the NCS as a whole.

These weighting adjustments assume that data on exposure and outcomes are missing at random (MAR), i.e., that the probability of the data being missing is constant within the weighting classes (Little & Rubin, 2002). The adjustments compensate for unequal probabilities of the data being missing across weighting classes, and they will reduce bias to the extent that these probabilities are unequal and the weighting classes are associated with missing data.

3.5.2 Weighting in Case-Control Studies

In most nested case-control studies, the controls are sampled at much lower rates than the cases and also different groups of controls are sampled at widely differential rates in order to make the control distribution across the groups correspond to that of the cases. In this situation, the use of standard survey weights based on the general population as the reference population (i.e., the population of inference) will lead to large variability in estimates that compare cases and controls. An alternative approach is to use the distribution of characteristics among the case population as the reference distribution. This approach is consistent with that used in most case-control studies and will be adopted here: the reference population is all cases in the U.S. population of infants. The weights described above for the cases were developed for this purpose. The weights for the control sample will be similarly developed to represent the characteristics of the case population. In this approach, the weights of controls in a group are scaled to the sum of weights of the cases in that group, thus making the reference population the case population, not the total population (DiGaetano & Rizzo, 2003).

While the use of this weighting method in the planned analysis will result in some loss of precision, the approach is preferable to an unweighted analysis of a self-selected sample, the results of which cannot safely be generalized. Effects of elevated maternal glucose levels obtained from the proposed weighted analysis will represent the average exposure effect on the national population of cases or on any specific subset of that population.

3.5.3 Replicate Weights

The NCS is based on a complex sample design involving stratification and clustering by PSUs and by segments within PSUs. One way to reflect the variance due to this type of sample design is to use replication variance estimation methods. This methodology requires the development of a set of replicate weights in addition to the set of final weights described in Sections 3.5.1 and 3.5.2.

Replicates are subsets of the sample formed so that each replicate is a representative sample of the entire study population. Typically, replicates are formed by dropping one or more PSUs out in turn. However, it needs to be noted that large PSUs that are selected with certainty are in fact strata, not PSUs. It is the sub-units within these strata that are the real PSUs, and it is these sub-units that are dropped out sequentially to form replicates. Replicate weights are computed separately for each subset so that the weighted replicate estimates represent the study population. Sample statistics (e.g., means, proportions, regression coefficients) are computed using the full sample weights and then separately for each replicate using the replicate weights. The variability in the replicate results provides the basis for estimating the precision of the estimates produced in the analysis, in a manner that reflects the sample design and the weighting process.

3.6 Imputation of Missing Data for Covariates

As explained in Section 3.5.1, missing data on HgbA1c levels and/or birth defects will be handled by weighting adjustments. In addition, covariate information may be missing due to item nonresponse. The percentage of missing data for each analysis variable will be evaluated and appropriate procedures will be used to impute for missing values. The procedure used for each variable will depend on the extent and type of missing data but possible methods include hot deck imputation, semiparametric imputation, or logical imputation based on related variables.

3.7 Power Calculations

We assume that approximately 14,970 infants will be included in this analysis. In the analyses, it is anticipated that the level of HgbA1c will be treated as a continuous variable (likely with curvilinear terms or a spline in the logistic model). Unfortunately, data on HgbA1c levels for women in the first trimester of pregnancy, the primary exposure measure, are not readily available. The power calculations have therefore been carried out by treating HgbA1c as a binary variable. The calculations use a value of 4 percent in the "high" group, as would apply if only the very high levels of HgbA1c were associated with birth defects. The power calculations presented here are therefore likely to be conservative. The table below provides estimates of power for detecting odds ratios in the range of 1.50 to 3.00 under these assumptions and also under an assumption of a design effect for odds ratios of approximately 1.30 based on the expected number of cases per PSU and estimated intra-class correlation for the NCS for birth defects.

Outcome	Rate per 1,000 births	Expected sample size	Odds ratio				
			1.50	1.75	2.00	2.50	3.00
Heart defects	6	510	0.31	0.54	0.74	0.94	0.99
All major birth defects	35	2,995	0.91	>0.99	>0.99	>0.99	>0.99

The results in this table demonstrated that this investigation has very high power for detecting clinically significant associations between elevated HgbA1c levels and all major birth defects as a group. Moreover, the analyses will also be able to support stratified analyses of subgroups of the sample in order to investigate potential effect modifiers, an important aspect of the analyses.

The statistical power to detect the effects of impaired glucose tolerance on heart defects is lower but still adequate for odds ratios greater than about 2. It may also be possible to examine as outcomes other groupings of birth defects that are suggested by the literature to be associated with gestational hyperglycemia.

4. Analysis Procedures

4.1 Overview

The data analysis will be conducted in several steps. First, data must be combined from various files to create a data file for analyzing the associations of potential confounders with birth defect outcomes (all birth defects and heart defects only). The results of these analyses will be used to guide the choice of confounders to be used in the matching. The sample of matched controls will then be selected and an analytic data file that includes all the variables to be used in the analyses will be created. Next, preliminary frequencies and descriptive statistics will be generated for all analytic variables. A

preliminary set of analyses will examine bivariate relationships between covariates and exposure, covariates and outcomes, and exposure and outcomes. The main analysis and final step of the process will involve fitting a conditional logistic regression models for the presence of birth defects given HgbA1c levels and other covariates.

4.2 Preliminary Frequencies and Descriptive Statistics

Once an analysis data set has been produced, unweighted frequencies and descriptive statistics for all key variables will be reviewed. The main purpose of this step will be to check for correct coding of the data, logical relationships between related variables, the need to collapse categorical variables, extreme values, skewed, and other distributional features that might affect data analysis, and to assess the extent of missing data.

As a result of this step, some corrections or recoding of the data may be required. Once these issues have been addressed, the changes will be verified and additional output will be reviewed as necessary.

4.3 Bivariate Relationships

The purpose of this step is to gain insight into the pairwise relationships between covariates and HgbA1c levels, between covariates and congenital birth defect outcomes (both overall and for heart defects alone), and between HgbA1c levels and birth defects. First, two-way cross tabulations will be generated for each pair of variables by categorizing continuous and many-leveled ordinal variables, as necessary. Where outcomes are involved, separate tables will be run for all birth defects and for heart defects. These frequencies will be run with and without sampling weights. Tests of association between variables will be conducted on the weighted tables using appropriate software to account for the complex sample design. These results will be reviewed by the analysis team and used to inform the model building process in the final step.

4.4 Conditional Logistic Regression Analyses

Conditional logistic regression is the primary tool for the analysis of data from matched case-control studies. It is used to assess the effects of covariates on the relative odds of a particular outcome and, in particular, to remove the influence of confounders from the estimate of effect due to exposure. The form of the regression model is

$$\log\left(\frac{\pi_{ij}}{1-\pi_{ij}}\right) = \alpha_i + \mathbf{x}'_{ij}\boldsymbol{\beta}$$

where, in the context of this analysis, π_{ij} is the probability that the j^{th} infant in the i^{th} matched group is diagnosed with a birth defect in the first 24 months of life, and $\mathbf{x}_{ij}$ is a vector of covariates that includes the status of the j^{th} infant's mother with respect to HgbA1c level in the first trimester of pregnancy, as well as all confounders. The covariate vector can include both individual-level characteristics and characteristics that are shared by all infants in the matched case-control group.

The conditional logistic regression model allows each matched group of infants to have a unique risk of birth defects by a group-specific intercept α_i, and then assumes that the covariates have a common effect on the odds of birth defects over all matched groups represented by the coefficient vector $\boldsymbol{\beta}'$.

To perform this analysis, the conditional logistic model can be fit using software that fits the discrete proportional hazards model for tied event times since both models have the same likelihood function. In this approach, the matched groups are treated as strata, case status is treated as the censoring variable (1 for cases, 0 for controls), and a dummy time variable is created (e.g., 1 for cases, 2 for controls)(see, for example, Lachin, 2000.)

Identification of an appropriate model will initially be facilitated by using a stepwise selection procedure to determine whether a covariate should enter and remain in the model. However, covariates identified by subject-matter experts as being important factors in birth defects, glucose functioning, or general health may be forced into the model. Likelihood or deviance measures will be used to assess overall model fit and residuals will be examined to address model diagnostics. Once a set of main effects has been identified, potential interactions will be tested for significance. A separate model will be fit for each of the two outcome variables. Potential effect modifiers will be investigated either by including in the model an interaction term between the effect modifier and the exposure measure, or by stratifying the model to see if the effect of exposure differs across strata defined by levels of the potential effect modifier.

These models will be estimated using the sampling weights described in Section 3.5.2, and standard errors and tests of significance will be computed based on replicate methods using software such as WesVar or SUDAAN (Westat, 2007; Research Triangle Institute, 2004). Team members will then review and interpret the results of the analyses, such as model fit statistics, estimated regression coefficients and odds ratios for each independent variable, and significance tests.

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