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

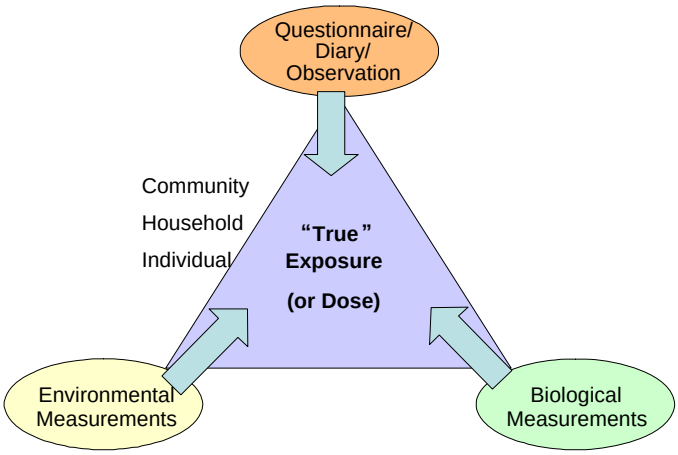

Figure 9-1. True Exposure as a Combination of Multimethod Assessments

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).