OVERVIEW OF NCS DATA COLLECTION AND CORRESPONDING MODALITIES

This section provides a brief description of the methods for collecting data from NCS participants and their environments and also touches on the measurement domains that can be addressed by each method. Descriptions of the actual measurements are contained in Appendix E and enumerated in the detailed tables included in Appendices F.1, G, H, and I.

1. Interview, Questionnaire, and Diary Data

1.1 In-Person Interviews

Each participant contact within the NCS will include information collected via personal interview or questionnaire. At the in-person contacts, home and clinic visits, the majority of the information will be collected using computer-assisted personal interviewing technology (CAPI). Information about topics with potential sensitivity (e.g., sexual activity) will be collected using computer-assisted self-interview techniques (CASI) with audio capabilities.

1.2 Self-Administered Interviews

At the end of each in-person contact, one or more self-administered questionnaires will be left with the participant to be completed. Dietary exposure (food frequency questionnaire) is an example of a topic to be covered by these self-administered questionnaires. During the initial years of the NCS, participants will be asked to complete paper machine-readable questionnaires that will either be picked up by local data collectors or returned to the local Study Centers by mail. However, in later years, electronic or Internet-based interview tools may be available.

1.3 Telephone Interviews

Brief telephone interviews will be conducted between in-person contacts. An important purpose of the telephone contacts is to verify participant status and tracking information. In addition, brief medical updates and information on potential frequently changing exposures (e.g., change in occupational exposures or child care) will be obtained during most calls. To minimize participant burden, telephone interviews will be brief, between 10 and 15 minutes in length. For selected contacts, automated calls may be considered, but the primary mode of administration will be interviewer-administered telephone interviews.

1.4 Diaries, Medical Provider Logs, and Family Health Records

Data collection in several stages of the NCS will include participant completion of diaries. During early pregnancy, women will be asked to complete a diary on a daily basis. In later pregnancy this will be done on a weekly basis. The diary is designed to collect information on symptoms such as nausea or abdominal cramping and to ascertain potentially important exposures such as medications, alcohol, tobacco and the consumption of potentially allergenic foods.

Similar to the self-completion questionnaires, diaries will be paper and pencil, designed to be completed and mailed in to the Study Centers. Later in the study, electronic options may be available.

Medical provider logs are similar to the diaries because they will be held and completed by the participant. Initially, they will be paper-based instruments; they may evolve to electronic or Internet-based tools depending on the development of appropriate instruments over the course of the NCS. A

medical provider log will be provided for the mother during the pregnancy period and for the child after birth. These logs will be used by the participant to record information concerning the date and type of a participant's contact with the health care system (e.g., routine care, emergency department visit) as well as the diagnosis and additional focused information relevant to the phase of the study. For instance, during prenatal care visits, maternal weight and blood pressure will be recorded; during childhood, information on immunizations will be recorded. Unlike the diaries, the medical provider log is designed to be kept by the participant. Data from the logs will be collected by the Study during in-person and telephone contacts by Study Center staff.

The family health record will be given to women at the first pregnancy visit. An electronic version of this instrument is planned, although a paper and pencil version will also be provided. The family health record is designed to allow the woman to talk with her parents and full siblings to ascertain diagnoses of selected medical conditions, such as diabetes, cancer and depression. At first, the family health record will be a paper form.

2. Biologic Samples

As summarized below and enumerated in detail in the tables included in Appendices E and G, a variety of biologic samples will be collected from all study participants at multiple points throughout the study. These specimens will be analyzed to explore a broad range of chemical and biologic exposures and participants' physiologic response to those exposures and to enable genomic investigations to provide insight into the interaction between an individual's genetic composition and specific environmental factors.

It is important to recognize that limitations in the volume of collected biologic samples (particularly blood), financial constraints, and, perhaps most importantly, the potential future importance of associations between critical outcomes and analytes currently unrecognized preclude immediate and complete analysis of those specimens. Thus, the bulk of biospecimens will be collected, processed, and stored in such a way as to allow the opportunity for later analysis in NCS subpopulations or in nested case-control studies. In general, blood (serum, plasma, and whole blood), urine, and breast milk samples will be refrigerated or frozen as soon as possible at each study site, shipped to the central repository and deep-frozen in small aliquots to minimize thaw-refreeze cycles. In addition to the biospecimens, information relevant to biologic exposures will also be collected through other modalities. For instance, history of recent infectious disease will be obtained through in-person interviews.

2.1 Blood

Blood will be drawn at the prepregnancy, first trimester and third trimester visits for the mother, at the first trimester visit for the biological father and at the 12 month visit for the child. At each visit, blood will be collected in multiple tubes (i.e., red top, purple top, gray top, metal free) to maximize the potential for later analyses (details of volumes, tube types for each visit, and potential analytes for each visit and participant are listed in Appendix G).

Examples of broad areas for which blood will be a primary source of information are:

- Maternal and child endocrine function (e.g., TSH and thyroid hormone);
- Maternal and child infection and inflammatory status (e.g., cytokine and Ig profiles, CRP);

- Nutritional and metabolic status (e.g., folate, antioxidants, HgbA1c); and
- Chemical bioburden (e.g., persistent organic compounds (PCBs, PBDEs, dioxins), bioaccumulative metals (lead, mercury, cadmium).

Whole blood will also be the primary source of genomic DNA for analyses of gene-specific polymorphisms, methylation patterns, and for the presence of exposure-specific DNA adducts. The multiple blood samples obtained from the child throughout the course of the study will enhance the value of the epigenetic and adduct analyses. The use of whole blood RNA for gene expression analyses is also being considered, though the expense associated with those samples may limit this to subpopulations of the NCS.

2.2 Urine

Urine samples will be collected from the mother several times before (for the prepregnancy cohort) and during pregnancy, from the biological father once during pregnancy, and from the child at multiple times (details of volumes and potential analytes for each visit and participant are listed in Appendix G).

Maternal urine will be a main source for evidence of exposure to non-persistent chemicals in the periconceptional period and during pregnancy. Examples of relevant exposures that can be assessed in urine samples include multiple insecticides, herbicides, endocrine active compounds and polycyclic aromatic hydrocarbons. Using amplification methods, maternal urine can also be used to assess genitourinary tract infection or colonization with chlamydia, gonorrhea, and other organisms.

The primary use of urine samples from the biological father (in the early pregnancy period) and the child (from birth through 12 months) is for chemical exposure analyses similar to those outlined for maternal urine.

2.3 Vaginal Swabs

Women will be asked to provide self-collected swabs of vaginal fluid before pregnancy (for those in the prepregnancy cohort) and at the first trimester home and third trimester clinic visits. These swabs will be processed primarily for the assessment of evidence of local inflammation, via analysis for a variety of cytokines, metalloproteinases, and other inflammatory markers. In addition, at each visit, one swab will be rolled onto a slide for later gram stain and assessment for bacterial vaginosis.

2.4 Saliva

Saliva will be collected primarily for assessment of cortisol levels as an indicator of physiologic stress and hypothalamic-pituitary-adrenal axis (HPA) function. Samples will be obtained from the mother twice during pregnancy to enable evaluation of maternal stress and pregnancy outcome, as well as the relationship of fetal exposure to stress and subsequent asthma, neurodevelopment, and other conditions. Maternal and paternal samples will be obtained at the 6 month postnatal visit as an indicator of stress, to be used in the evaluation of child development, family process, and related domains. In early childhood, saliva samples will be collected at the six month visit from the mother and the biological father and at the 12 month visit for the child for evaluation of HPA activity.

At each relevant visit, saliva collection kits will be given to the participant. Instructions will be provided for the collection of multiple (2 to 3) samples to be obtained at specific times over two days.

2.5 Hair and Fingernails

Hair and toenail samples will be used as summary indicators of exposure to persistent chemicals, predominantly heavy metals and mercury. They will be obtained from the women in the prepregnancy cohort, and from pregnant women at the first and third trimester visits as well as from the father at the first trimester visit, thus enabling estimates of fetal exposure to those compounds from conception through birth. Hair samples will be obtained from the child at the 6 and 12 month visits, whenever possible, to allow assessment of chronic early life exposure to metals.

2.6 Birth-Specific Biospecimens

Samples collected at birth include cord blood, cord and placental samples, and meconium. Biologic samples obtained at birth are key to understanding the extent to which the maternal environment relates to actual fetal exposure and fetal response to *in utero* exposures.

Placental samples, obtained using random grid sampling, will be prepared to enable histologic assessment for local inflammation or infection as well as for chemical analysis. A membrane roll will also be obtained for similar analyses; in particular, because of their low rate of turnover, the membranes are a useful indicator of long-term exposure to persistent contaminants.

Similarly, cord samples obtained close to the fetus will be collected and processed to allow for histologic and chemical analysis. The comparison of maternal, placental, and proximal cord samples can, for instance, establish gradients between maternal exposure and that "seen" by the developing fetus. While fetal inflammation is well-assessed using cord samples, the degree to which those samples are useful for assessment of certain chemical exposures, particularly lipophilic compounds, is less certain.

To maximize the collected volume, mixed venous-arterial samples of dripped cord blood will be obtained in the delivery room. The large volumes of cord blood will be useful for genetic analyses–"pure" maternal and child samples will be available to address concerns about the potential contamination of dripped samples – as well as the biologic and chemical analyses discussed previously. In addition, cord blood can be paired with neonatal heel capillary samples obtained at 24-48 hours of age, providing an opportunity to examine metabolic and physiologic changes early in the perinatal period.

Finally, meconium will be collected from the nursery and analyzed for the presence of chemical exposures that may be difficult to obtain from other samples – metabolites of organophosphate pesticides, for example – or for a summary measure of fetal exposure to other compounds, such as cotinine.

2.7 Breast Milk

In addition to its nutritive value, breast milk is a marker of maternal bioburden as well as a medium for transfer of potentially beneficial (e.g., immunoglobulins, essential fats) and harmful (PCBs, other lipophilic chemicals, infectious agents) from mother to child. Breast milk samples will be self collected at approximately 1 and 3 months, using kits provided at the in-hospital birth visit, and following the 6 month visits, using kits provided at those times. For the NCS, the primary purpose of breast milk collection is to assess for infant exposure to lipophilic persistent organic compounds such as PCBs, dioxins, furans, and others. However, samples will also be available for other analyses such as maternal transfer of lipids and antioxidants, immunoglobulin and cytokines, and additional chemical contaminants such as perchlorate.

3. Environmental Samples

As summarized below and presented in overview in Appendix E and in detail in the tables included in Appendix H, the NCS will collect a variety of environmental samples from the homes of women during the prepregnancy period as well as from the child's home after birth. Environmental samples are crucial to the quantification of the potential exposure of a child or developing fetus to substances which cannot be identified through biomarkers. In addition, the coupling of environmental samples with biologic samples from the parents and child will provide a detailed picture of the temporal relationship between multiple exposures and child outcomes, a combination critical to the development of research-based strategies to improve child health.

The laboratory analysis plans for environmental samples share the same philosophy as those for the biologic samples; analyses central to NCS hypotheses or that cannot be deferred because of technical reasons, primarily due to degradation of sample or specific analyte, will be performed in a timely fashion. Other samples will be stored in such a way as to maximize the utility of future analyses based on the evolution of research questions, analytic techniques, and the availability of funding.

This section will concentrate on samples obtained from the home(s) of NCS participants. Procedures for collecting samples from participants' child care and school environments are still being developed. Collection of samples from the community or neighborhood is outlined in Section 5.

3.1 Indoor Air

A diverse set of indoor residential air samples will be obtained at each home visit, starting with the pre-pregnancy visit. Air samples will be collected over a multi-day period to obtain an average integrated exposure. A pump will be employed to collect samples for particulate matter (PM_{10}) and related compounds (e.g., metals, elemental carbon). To increase the Study's efficiency, the collection and analysis of some air samples - ozone, for instance - will be triggered by specific information collected at the time of the visit, such as the presence of a photocopier or laser jet printer in the home.

Volatile organic compounds and nitrogen oxides (NOx) will be collected via badges placed in specified locations within the household. In addition to collections associated with the home visits, self-collection kits will be given to participants at the third trimester clinic visit and mailed to participants when the child is 24 months old. These will be deployed by the participant and mailed back to the Study Centers according to directions given at the clinic visit and supplied with the kits.

This Study currently relies on household and neighborhood air samples and does not include personal air sampling. Through time, as technology advances and as the NCS cohort develops, provisions for personal air sampling may be developed.

3.2 House Dust

Dust samples will be obtained by surface wipes, vacuum, and by accretion onto dust mats, depending on the location of the sample and the targeted analytes. For women in the prepregnancy cohort, organophosphate, pyrethroid, and carbamate pesticides residues will be ascertained by dust wipes. Starting in pregnancy, at each home visit a wider array of samples will be obtained. Wipes and mats will be obtained to allow for assessment of metals, pesticides, and other compounds. Vacuum samples, particularly during the visits after the child's birth, will be collected for potential examination of allergens, mold, and endotoxins. A bulk dust will be archived for future analysis of analytes as yet to be determined.

Similar to the self-collected indoor air samples, dust mats, wipes, and the vacuum dust collector will be provided at the third trimester clinical visit for self-collection of samples targeted for pesticide and metal analyses.

3.3 Drinking Water

Household tap water will be collected at each home visit, though the types of samples will vary somewhat depending on whether the household uses local well water or is served by a community water supply. Starting in pregnancy, water from all households will be collected for heavy metal assessment. Houses using well water will have samples collected for perchlorate and pesticide analysis starting with the prepregnancy visit. In contrast, houses served by a community water supply will have samples collected for analysis of disinfection byproducts and volatile organic compounds, starting with the first trimester visit.

3.4 Soil

Soil samples will be obtained from households in agricultural areas for ascertainment of pesticide exposure at the 1st trimester visit. Additionally, soil samples will be obtained from the perimeter and midyards of homes at the 6 and 12 month home visits. Analysis of these samples is primarily targeted toward identification of metals and pesticides potentially encountered by an infant or toddler. Samples obtained from properties with evidence of pressure treated wood (CCA) structures will be collected during pregnancy as well as during the 6 and 12 month visits.

3.5 Noise Survey

Assessment of household ambient noise, both indoor and outdoor, is scheduled for the 12 month visit.

4. Physical Examination Measures

As enumerated below and in Appendix I, a brief physical assessment of NCS participants will occur at each in-person contact. In general, these examinations and observations will comprise a limited set of standardized, objective measures directly related to the NCS priority outcomes. Most of the examination measures are designed to assess physiologic status without making a specific clinical diagnosis. For instance, pulmonary function tests will be attempted starting at the 3 year clinical visit, but auscultation of the chest for wheezing will not be included in the study protocol. Exceptions to this general principle include some of the neurodevelopmental tests; for some conditions, positive screening (such as the M-CHAT for autism) may be followed by more specific tests or exams (e.g., ADOS) that are sometimes used for clinical diagnosis.

The first clinical visit for NCS children occurs at 3 years of age. Since this document focuses on measures obtained through the 24 month visit, only measures that will be obtained during pregnancy from the mother and at the early home visits for the child will be described.

4.1 Anthropometric Measures and Body Composition

Assessment of a child's physical growth and adiposity is important in its own right, as well as being a key factor related to subsequent outcomes such as diabetes, metabolic syndrome, and onset of puberty. Parental habitus likely influences the child's status through genetic and behavioral mechanisms and the potential short and long term influences of maternal adiposity on the child's *in utero* environment are critical areas of study for the NCS.

Baseline measurements from the mother will be obtained at the prepregnancy visit and then at each contact during pregnancy. Measurements will include weight; standing height, sitting height and other segmental measures (obtained one-time only); mid-arm, hip, and waist circumferences; and triceps and subscapular skin folds. Similar measurements will be performed on the biological father at the first trimester home visit.

Physical assessment of the child will start in the fetal period via several ultrasound assessments (Section 4.2). At birth, in addition to weight and standardized length, multiple circumferential measures, including head, abdomen, mid-arm, and thigh, will be taken. Triceps and subscapular skin folds will be obtained, as well. These measurements will be repeated during the 6 and 12 month home visits.

Additional assessments of the child's body composition, including Dual Energy X-Ray Absorptiometry, Bioelectric Impedance Analysis, or bone ultrasound, are likely to be included at the later clinical visits.

4.2 Fetal Ultrasound

Up to three fetal ultrasounds will occur as part of the NCS. If an early ultrasound is not performed as part of the woman's prenatal care, or if these results are not available, an NCS-sponsored ultrasound will be obtained toward the end of the first trimester to assure accurate pregnancy data. Subsequently, all women will be scheduled to have standardized NCS-sponsored ultrasounds obtained toward the end of the second trimester (approximately 22-24 weeks) and early to mid third trimester (28-32 weeks). These scans will focus on obtaining accurate standardized measures of fetal growth. Standardized AIUM protocols will be used for standard linear and circumferential measures, such as bi-parietal diameter, femur length, and abdominal circumference. The assessment of relative lean and fat mass will be attempted using mid-thigh circumferences, recognizing that those measurements are not as standardized and tested as the more routinely used measurements of linear growth.

The use of NCS fetal ultrasounds for identification of birth defects remains under discussion. It is possible that a series of views used in routine anatomic surveys may be stored digitally for later analysis. Regardless, strict protocols regarding appropriate follow-up of questionable or suspicious findings seen during any NCS ultrasound acquisition will be adhered to.

4.3 Blood Pressure

Blood pressure measurements will be obtained at each contact with the mother before and during pregnancy, from the father at the first trimester visit, and from the child starting with the 12 month home visit. At each visit, a participant's blood pressure assessment will consist of multiple measurements obtained using a calibrated automated device.

4.4 Dysmorphology Examination and Photography

Assessment of the child for congenital anomalies is essential for the NCS, both as a priority outcome and to enable appropriate evaluation of other outcomes, such as cognitive or motor development. It is expected that the initial source for the identification of congenital anomalies will be the abstraction of the prenatal and neonatal medical records at the delivery hospital, followed by questions on subsequent child questionnaires during infancy to identify malformations, such as some cardiac defects, that may not have been diagnosed at birth. However, there is also benefit for the NCS to conduct a targeted dysmorphology observation examination. Complete medical records may not be available. Also, there is interest in obtaining more subtle morphologic measures not reported in medical records (e.g., inter-

canthal distances, ano-genital distance) that may be associated with neurodevelopmental or other outcomes.

Unfortunately, despite the proliferation of studies on birth defects, a commonly-used, general standardized dysmorphology exam suitable for a large population-based epidemiologic study is lacking. A protocol is being developed for acquisition of digital facial photographs that can be stored and later retrieved for analysis of facial features and, perhaps, morphologic measurements. The examination and photographs will be obtained at the initial neonatal visit prior to discharge and be repeated at least once at either the 6 or 12 month visit.

4.5 Neurodevelopmental Examinations and Observations

A primary focus of the NCS is the longitudinal assessment of neurologic and behavioral development, starting at birth. Some of this assessment will occur through the administration of parental questionnaires and, later in life, recording of school performance. In addition, standardized assessment and observation of developmental activities is an important part of the NCS protocol. A wide array of neurobehavioral instruments allows examination of numerous aspects of neurologic development. For the NCS, tools will be chosen that are well-known and with recognized interpretability, relatively easily applied in a standardized fashion, and suitable for use in a geographically diverse population-based epidemiologic study.

At the birth visit, initial assessment will be via the Neonatal Intensive Care Unit Network Neurobehavioral Scale. The Bayley scales for cognitive, motor, and language development will be applied starting with the 12 month home visit. In addition, at the 6 month and the 12 month visit, the child's social development will be assessed via application of a standardized interaction between the child and one parent (mother at 6 months, father at 12 months, if available). This interaction will be videotaped for later standardized assessment.

5. Community-Level Measures

A child's health and developmental trajectory are influenced not only by individual and family characteristics, but also by those of the community in which he or she is raised. The NCS will incorporate two broad types of community-level data. The first is that which is collected as a matter of course by other agencies or organizations. Examples of these data include census information, crime reports, EPA ambient air monitor data, health provider capacity, and school district test scores. A basic set of these secondary community-level data, such as census population characteristics (e.g., population density, area income distribution) will be appended onto the NCS data files. The ability to link other secondary data to the NCS data will be available to researchers; procedures for this linkage will depend on the geographic detail of the linkage (e.g., NCS segment, census block, county, state, GPS coordinates) and associated confidentiality concerns.

The second variety of community-level data incorporated into the NCS is that which will be collected by the NCS. Environmental measures, such as community water supply samples and local air monitoring for particulate matter and other pollutants, will be collected. Study personnel will assess the physical environment of participants' neighborhoods by observation, using a structured instrument such as the Irvine-Minnesota Inventory. Examples of areas to be assessed include amenability for walking, biking, and other physical activity; presence of retail establishments; industrial or chemical sites; and other measures of land use.