

**A White Paper on**  
**Measurement and Analysis of Exposures to Environmental**  
**Pollutants and Biological Agents during the National**  
**Children's Study**

***Prepared for***  
National Children's Study Federal Advisory Committee, Program Office and  
the Interagency Coordinating Committee

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## Disclaimer

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## Preface

In 1997 the President's Task Force on Environmental Health Risks and Safety Risks to Children was charged with developing strategies to reduce or eliminate adverse effects on children caused by environmental exposures. The task force proposed a longitudinal cohort study of the effects of environmental exposure (broadly defined) on the health and development of children. Subsequently, the Children's Health Act of 2000 authorized the National Institute of Child Health and Human Development (NICHD) to conduct a national longitudinal study of environmental influences (including physical, chemical, biological, and psychological) on children's health and development. To lead the planning and implementation of the study, staff and funds have been allocated by the National Institute of Child Health and Human Development, National Institute for Environmental Health Sciences (NIEHS), the Centers for Disease Control and Prevention (CDC), and the Office of Research and Development of the U.S. Environmental Protection Agency (EPA). Investigators from each of these four lead entities serve on the Interagency Coordinating Committee (ICC), which has further developed the conceptual framework for this National Children's Study. The various workgroups are charged with providing technical guidance to the Federal Advisory Committee of the NCS. Our workgroup is the Exposure to Chemical Agents Workgroup (ECAWG), charged with characterizing various means of assessing exposure for those hypotheses requiring exposure assessment (The National Children's Study Interagency Coordinating Committee, 2003).

This White Paper addresses the specific questions posed to the ECAWG and forms a framework for the development of more detailed documents. The White Paper provides examples of chemicals, chemical classes and biological agents that may be important for exposure assessment associated with specific hypotheses as part of the NCS. However, the White Paper does not wish to suggest that any of these examples of chemicals and/or biological agents will be included in the NCS. Such decisions are made as part of a separate process. Substantial expertise will be required to develop a study design and field protocols adequate to test the NCS study hypotheses. To this end, we strongly suggest that the outcome and study design workgroups, as well as individuals and groups from other public and private enterprises, work interactively to ensure the highest quality study design and implementation possible.

In this report, *Chapter 1* serves as an Introduction to human exposure assessment in general and to other exposure assessment issues, including the chemicals potentially relevant to the National Children's Study; *Chapter 2* describes the hypotheses requiring exposure assessment and our recommended considerations for sampling and analysis; *Chapters 3* and *4* describe which environmental and biological matrices, respectively, should be analyzed in order to classify exposure to a given chemical or other relevant substance in people at various life stages; *Chapter 5* describes the use of questionnaire data as a stand alone exposure assessment tool or as a supplement to analytical measurements of environmental and biological samples; and *Chapter 6* includes a synthesis of suggested exposure measurement approaches and epidemiological considerations regarding study design.

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## Executive Summary

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Estimating children's health risks requires knowledge and understanding of exposures and related dose-response relationships for individual environmental chemicals and real-world mixtures. In addition, assessment of children's exposures involves the influence of biological, physical, and social environments on the child's environmental health and the variations in exposure-related attributes by developmental stage (Needham and Sexton, 2000). This very complex task of examining and measuring the influence of biological, physical, and social environments by life stage and its impact on various health indices is a critical component of the National Children's Study (NCS).

Development of the environmental exposure assessment component of the NCS will require determining a number of factors, including

- chemical and biological agents of health concern,
- cost-effective approaches to measure these chemicals in environmental and biological matrices,
- well-designed and administered questionnaires, and
- cost-effective strategies for gathering the necessary exposure-related information with minimum burden to the participants

The principal objective of this White Paper is to provide exposure measurement guidance to the Federal Advisory Committee, the Interagency Coordinating Committee and the Program Office of the National Children's Study, so that the NCS will increase the knowledge and understanding of when, where, why, and how elevated exposures to chemical or biological agents of concern are likely to occur and among whom these elevated exposures may be found. This knowledge will then be used to relate exposures to health outcomes of interest. Furthermore, the members of the Exposures to Chemical Agents Work Group (ECAWG) of the NCS have been charged with providing the expertise and leadership that will allow the exposure-related information, collected as part of the NCS, to be standardized and linked throughout the study with potential health outcomes of interest. This White Paper is one of several inputs to a *separate* process that will select (1) the chemical and biological agents, (2) the study design, and (3) the measurement approaches to be used. The White Paper is the product of nearly two years of dedicated work by ECAWG. The expertise represented by ECAWG covers a wide range of disciplines, including environmental sciences, analytical chemistry, physics, exposure monitoring, biomonitoring, microbiology, survey sciences, and epidemiology.

For the purposes of this White Paper, "exposure" is defined as contact between an agent and a target, and contact takes place at an exposure surface over an exposure period. "Environmental exposure" is contact with 1) selected chemicals, both natural and synthetic, that are of interest in studying priority health outcomes in the NCS, and with 2) biological agents, such as arthropods and dander, that influence asthma. "Child" is anyone from 0 to 21 years of age. The White Paper focuses on those aspects related to understanding the exposure to chemicals and biological agents in the environment of the child, the pregnant mother, and the child's parents before the child was conceived. In the White Paper, we consider the role of important physical environments that affect exposures. The "physical environment" in this context refers to environmental media and pathways associated with environmental chemicals that children, pregnant women, and young adults who are not yet parents may come into contact with as a result of their normal activities, behaviors, and social interactions. The physical environment encompasses the air these individuals breathe, the food and beverages they consume, the surfaces they contact, and their activities, including where the activities occur and their duration. Ultimately, for a

biological effect to take place, first exposure to the chemical must occur. The chemical must enter the body after contact, be absorbed, and be distributed to the target organ.

Three primary methods are used to assess human exposures: 1) questionnaires and diaries, 2) environmental/personal measurements, and 3) human biological measurements (e.g., blood, urine). These methods utilized in whole or in part (depending on the exposure scenario) provide information to estimate exposure status. This exposure information can then be used in relating exposures to hypothesis-specified health outcomes. The following hypothetical relationships were selected from those available to the workgroup (December 2002) as examples that had a clear environmental component.

- *Pesticides and neurobehavioral outcomes* The persistence or widespread use of many pesticides, such as the organochlorine, organophosphate, and contemporary pyrethroid pesticides, indicates the need to include pesticides in any exposure assessment for neurological effects. However, the pharmacokinetics of organochlorine pesticides and organophosphate pesticides differ widely. For example, many organochlorine pesticides are lipophilic and have long biological half-lives; therefore, their concentrations can be measured in serum and other lipid-rich matrices several years after exposure. Organophosphate and other nonpersistent contemporary pesticides, however, are not lipid soluble and are excreted as metabolites in urine. Classifying exposure to these pesticides depends very much on the exposure scenario. Typically, it is not possible to use a single biological or environmental measure alone to classify nonpersistent pesticide exposure. Techniques that are available include measurements of pesticides in first morning void urine samples, tests of serial blood samples (and possibly meconium and saliva), and testing of home air and dust over critical life stages (pre-conception, pregnancy, birth, infancy, early childhood, and pre-school). These measurement methods should provide adequate data to test this hypothesis on pesticides and neurobehavioral outcomes.
- *Exposures to indoor and outdoor air pollution and asthma* Evidence suggests that air pollution exacerbates pre-existing asthma. The research now needs to focus on 1) the role of chronic and acute exposures to air pollutants in the development of asthma in children, and on 2) the investigation of gene–environment interactions in the etiology of asthma.
- *Exposures to bioaerosols and asthma* Environmental exposures appear to be important in both inception and attack/trigger phases of asthma. Sources of bioaerosols of concern for asthma include house dust mites, endotoxin, and arthropod and rodent antigens.
- *Undesirable outcomes of pregnancy – pre-conception enrollment and exposure assessment* The inclusion of a pre-conception cohort in the NCS is critical to understanding the effects of pre-conception and early pregnancy environmental exposures on adverse pregnancy outcomes. Chemicals of interest and the exposure scenarios dictate the use of the exposure assessment method. A wide range of assessment tools—including questionnaires, environmental sampling, and biomonitoring—are essential to understanding the complex associations between environmental exposures and undesirable pregnancy outcomes.
- *Exposures to environmental agents affecting altered age of puberty* A number of factors may be associated with altered age of puberty, including nutrition, obesity, genetic susceptibility, and environmental chemicals. All of these factors must be evaluated when assessing for the age of maturation because several chemicals have been reported to be associated with altering the attainment of puberty in people.
- *Chemical exposures and obesity* Many of the dietary, social, and environmental data collection efforts proposed to study obesity will also be useful for environmental exposure assessments.

Although a number of chemicals and exposure measurement methods are discussed in the White paper, we recognize that during the next 21 years new information will be available about chemicals and newer recommended methods for sampling them. Consequently, a portion of the environmental and

biomonitoring samples collected will need to be archived so that future technologies can be applied to these samples. Furthermore, for both cost and technical considerations, analyses of some of the stored samples may only be performed only after certain health outcomes have been confirmed to determine retrospectively the exposures of subjects with these confirmed conditions. We propose the following measurement and study design considerations to address exposures to those chemicals relevant to the hypotheses considered:

***Particulate matter, gaseous pollutants, air toxics, and metals in the air*** At present, active sampling is the only feasible way to directly perform exposure assessments on particulates. Gaseous air pollutants can be collected adequately with passive or active samplers. Passive samplers are usually the most suitable for personal monitoring, but they can also be used for microenvironmental (indoor) and central site monitoring. Particle-bound chemicals, including PAHs and other semi-volatile organics, can be collected on a filter media followed by a suitable sorbent material and then measured. Many of these chemicals, but not all of them, can also be measured in biological specimens.

***Indoor allergens and endotoxins*** Sampling for environmental agents linked with asthma requires sampling of indoor air at home and collecting floor and surface dust samples for analyses of dust mites, mold, endotoxin, and other arthropod or rodent antigens. Samples from furniture, mattress, and stuffed toy samples should also be collected at routine intervals and analyzed to assess potential exposures for a young child. Inhalation is the most important route for exposure to indoor bioaerosols. Therefore, floor and surface dust measurements are useful when these media are possible sources of dust mites.

***Metals*** Assessing exposures to metals (e.g., lead, cadmium, mercury, arsenic, beryllium, chromium, cobalt, manganese, nickel, and vanadium) will be important for studying the effects of metals on neurocognitive, neurodevelopmental, reproductive, and other developmental outcomes. Dietary ingestion is likely to be an important exposure route for most metals for all age groups. Other pathways—including ingestion of water, ingestion of dusts and soils, and inhalation of particles containing metals—may be important for some metals for some age groups, but not for others. These metals can be measured in environmental samples as well as in blood or urine samples.

***Dermal and non-dietary ingestion*** Because of their activities, infants and young children may receive higher exposure than adults to metals and pesticides by the dermal and indirect ingestion pathways. Infants and young children ingest soil and house dust from their unique mouthing behaviors and eating patterns and from frequent contacts with surfaces contaminated with chemicals resulting from use of consumer products and other indoor and outdoor sources.

***Persistent organic chemicals*** Such persistent organic chemicals as polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and polybrominated diphenyl ethers (PBDEs) are of interest as potential endocrine disrupting chemicals. In addition, concerns associated with persistent organic pesticide (POP) exposure include adverse neurodevelopment in children. Although diet among young children is generally the most important route of exposure, POPs are found in all the environmental media, usually at relatively low levels. Assessment of exposures to these chemicals in health studies can be accomplished by biomonitoring, such as analysis of blood or milk samples.

***Non-persistent pesticides*** Measurement of non-POPs is especially important for the NCS hypotheses related to neurodevelopment. Non-POPs are chemicals that are metabolized and excreted from the body in hours or days and do not accumulate in body tissues such as fat. Studies have reported a high level of correlation between non-POP levels in personal air and indoor air samples and between indoor air levels and levels in carpet dust, handwipes, and surfaces in the home. Dermal exposure and indirect ingestion may thus be important routes of exposure to pesticides following residential pesticide use,

particularly for children. Exposure assessment for non-persistent pesticides typically requires biomonitoring, environmental monitoring and collection of survey information.

In this White Paper we discuss the three main approaches to exposure analyses, collection of survey information, environmental measurements, and biomonitoring— that are recommended for assessing exposures to chemical and biological agents during the NCS.

**Survey instruments** Survey instruments, such as questionnaires, time-activity diaries and product usage information, are expected to be a key component of any planned exposure study design for the NCS. These instruments will be used to enroll or reject a potential participant, gain understanding about the participant's family, family structure and relationships, education, occupational and residential history, type and nature of potential exposures, activity and behavioral profiles, and medical- and health-related information. The survey instruments attempt to gather exposure-related information to be used to characterize the locations, activities, sources, and factors that go into an exposure analysis. Furthermore, this information is needed to make inferences to the larger cohort of participants, including those not selected for special-study monitoring, or for time periods when monitoring was not feasible.

**Environmental monitoring** Environmental monitoring provides information about the concentration of the chemical(s) to which humans are potentially exposed and the potential routes of exposure. The measurement of a chemical agent or its transformation product in an environmental medium provides information that can be used to track a chemical from its source into the environment (e.g., air, water, food, soil, and dust, etc.). Environmental data are more readily applicable for assessing exposure pathways with one environmental medium (e.g., air); multimedia exposures require increased measurements (and thus increased costs), and application of models for predicting estimated human exposure and internal dose. When paired with questionnaires to provide information on the timing, duration and frequency of exposure, environmental measurements offer researchers useful information on the nature of exposures and potential dose.

**Biomonitoring** Biomonitoring is the measurement of the parent chemical and its metabolite or adduct in a body fluid or tissue. Biomonitoring data are used to assess the total absorbed dose but the data may not differentiate the different sources or routes of exposure. In health studies, biomonitoring data are most accurately applied for assessing exposure to chemicals, such as persistent organic chemicals, that have a long biological half-life. For nonpersistent chemicals, additional information on the exposure scenario is needed to properly interpret the data to relate exposures to health effects. Some chemicals, such as some of the criteria pollutants, cannot be measured by current biomonitoring techniques. The two primary matrices used to assess human exposure to chemicals are urine and blood, although breast milk is often monitored because it is a primary source for the infants' exposure to certain chemicals.

In developing an exposure assessment plan for NCS, researchers have to evaluate carefully a number of study design considerations along with the quality assurance/quality control requirements of the study.

**Study design** Obtaining high quality exposure information is vital to the investigation of complex linkages between exposures to different environmental agents and various health indices that will be measured in the NCS. However, the measurement of exposures to a diverse set of environmental chemicals and biological agents by critical life stage is a complex task. Technical and feasibility considerations dictate that environmental exposures be quantified by three different methods: direct environmental or personal measurements; analyses of biological samples (e.g., blood, urine, hair, saliva); and indirect methods such as survey instruments, time-activity diaries, or GIS techniques. In addition to technical and practical concerns, participant burden, sample collection, and analysis costs also influence the type of exposure assessment technique that is appropriate for a study of the size of the planned NCS. A key objective of the planned NCS is to assure that the final study design has sufficient statistical to

power for testing various hypotheses on different types of associations between exposures to selected chemical and biological agents and measured health outcomes in children. Given the large sample size, the long duration of the planned NCS, and the potentially high costs and burden associated with environmental sampling, collecting detailed longitudinal exposure information across the entire cohort and at all time periods to support the multiple hypotheses relating environmental exposure to potential adverse health outcomes will be difficult. Well-designed sub-studies, however, can be carried out within the NCS cohort by using only a small fraction of the sample size to estimate and adjust for exposure measurement errors. These well-designed sub-studies will have sufficient power to characterize the relationship between exposure and health outcome for most hypotheses. This methodology allows the exposure-response relationship to be tested on the whole cohort, while the detailed validation sub-samples provide the relationship between different exposure measures. Potential methods for selecting a sub sample include stratified sampling (e.g., specific sampling of high-exposure or high-risk individuals), multistage sampling, two-stage case-control studies, and other outcome dependent designs, case cohort designs, and nested case-control studies.

***Quality assurance/quality control (QA/QC) considerations*** The ultimate success of an environmental exposure program depends on the quality of the data collected, analyzed, and used in decision making. Quality depends significantly on the adequacy and the effective implementation of the Quality Assurance system, which ensures that the data satisfy stated expectations or specifications. The QA system encompasses each of the planning, implementation, and assessment steps of the study. Included in the QA system for the exposure component of the NCS are the use of standard operating procedures (SOPs), documentation of the measurement methods and protocols used, processes for data verification and validation procedures, methods to establish and achieve the data quality objectives, development of and instructions for following analysis protocols, and methods to use to provide data quality assessment reports. The QA/QC plan must consider all aspects of the exposure assessment including the questionnaire, the selection and collection of samples, the effects of shipping and storage of both the short and long-term samples, the analyses of the samples within and among laboratories, and the analyses and reports of the data. Some quality assurance measures to consider about the long term storage of biological specimens include periodic testing of the specimens to evaluate for any deterioration, separation of individual specimens into multiple vials to prevent artifacts from repeated freeze-thaw cycles, maintenance of multiple vials from the same specimen at different freezers to prevent the total loss of the specimen because of mechanical failure, and selective analysis of specimens that were processed and stored in a similar manner.

This White Paper is divided into six chapters. The first chapter is an introduction to human exposure assessment in general and to other exposure assessment issues. The four subsequent chapters (1) identify the hypotheses requiring exposure assessment and the recommended considerations for sampling and analysis, (2) present the environmental and biological matrices to be collected and analyzed to classify exposure to a given chemical or other relevant substance in children at various life stages, and (3) discuss the survey methodologies that will be needed to serve as a stand-alone exposure assessment tool or as a supplement to analytical measurements of environmental and biological samples. The final chapter provides a synthesis of suggested exposure measurement approaches and epidemiological considerations regarding a study design.

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# 1. Introduction

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## 1.1 Background

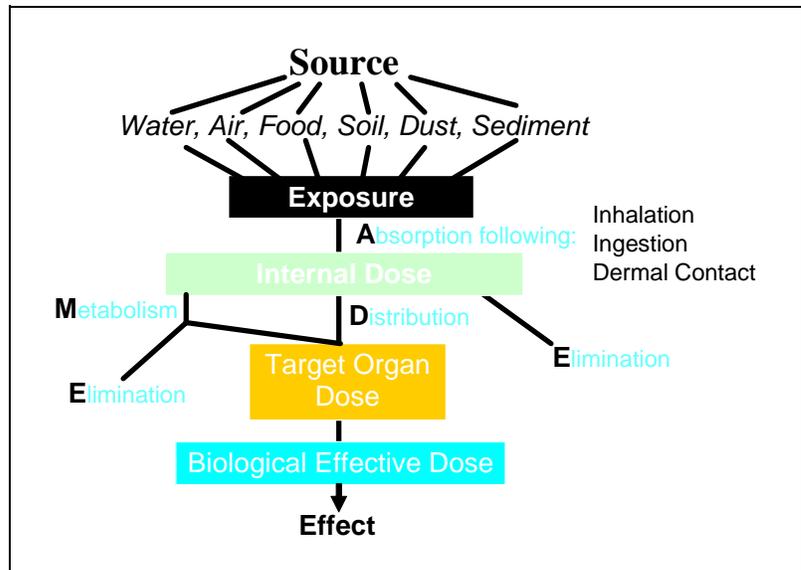
When examining a population for adverse health impacts with—at least in part—likely environmental origins, linking those impacts with exposure(s) to environmental agents (i.e., chemical, biological, and physical agents) is essential. The frequency, duration, and even timing of these exposures can be fundamentally important to understanding the root cause of adverse health impacts. Childhood exposures may be especially meaningful. Many researchers, citing *in utero* through 2 years of age as the critical or most susceptible time period, believe that exposures this early in life can lead to health impacts that manifest themselves at various stages of life, even well into adulthood. Other researchers point to pre-pubertal exposures as also being significant. For example, Mocarelli (2002) showed that pre-pubertal males highly exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin later fathered predominantly female children. Various means are available to assess children’s exposures to environmental agents (Needham and Sexton, 2000). However, before discussing these methods, we must examine the pathways of these agents that lead to exposure.

Exposure is defined as contact between an agent and a target; contact takes place at an exposure surface over an exposure period (IPCS, 2002; WHO, 2004; Zartarian et al., 1997). In the NCS, the agents of concern are selected environmental chemicals and biological agents; the targets are children; the exposure surfaces are the external surfaces of the children (i.e., skin, mouth, and nasal passage); and the exposure period is the child’s lifetime or a defined portion of that lifetime. Dose is the amount of agent that enters the target after crossing

an exposure surface. If the exposure surface is an absorption barrier, the dose is an absorbed dose; otherwise it is an intake dose (IPCS, 2002; WHO, 2004; Zartarian et al., 1997). The continuum often used to describe the human exposure pathway (*Figure 1-1*) starts with the agent at its origin or its source, which, for example, can be a chemical manufacturing plant, automobile exhaust, or a chemical waste site. The agent can undergo various fate (e.g., transformation to another chemical) and transport (e.g., long-range air transport or leaching from soil into groundwater) steps in the environment, which can lead to multiple intermediate sources in the pathway for a given agent; eventually

humans may have contact with the environmental media containing the agent or its environmental transformation products. The exposure mass may pass through membranes (based on membrane absorption coefficients and other pharmacokinetic factors) and enter into the body’s circulatory system by three routes — ingestion, inhalation, and dermal absorption. This absorbed dose of the agent or metabolite [or its reaction product (adduct)] is also known as the internal dose. This internal dose can be either directly

**Figure 1-1. Source to Exposure to Health Effects Pathway**



eliminated (usually a minor route); distributed within the body to other organs including the target organ(s); metabolized and eliminated (usually in urine); metabolized and distributed within the body to other organs including the target organ; or some combination of these (Needham et al, 2002). A portion of the dose at the target organ may be biologically effective (biologically effective dose) (Needham et al., 1992). 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, is called an exposure assessment (IPCS, 2002; WHO, 2004; Zartarian et al., 1997); certainly for health studies the term “exposure assessment” includes assessing the dose within the body.

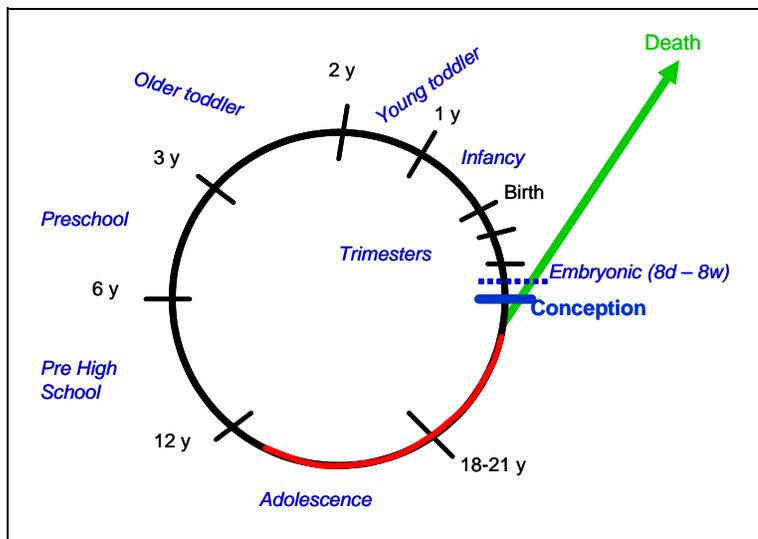
No single method is universally effective in assessing exposures to environmental agents. Because the NCS will assess exposures to children at various ages and life stages, impact or burden to the participating individual becomes a factor, especially when attempting to assess exposures during the three trimesters of gestation (*in utero*) and early childhood life stages (**Figure 1-2**).

Children are more susceptible to different agents at different periods of development; therefore, determining the timing of exposure is extremely important. Also, “best” methods for assessing exposure to a given chemical at one life stage may not be as effective in assessing exposure to

that same chemical at a different life stage. The reasons for the change in “best” methods with age include for example, relevant exposure pathways and media might change by life-stage, or within the realm of biomonitoring, the measurement of certain chemicals in blood may not be possible for a one year old because of the burden but that measurement would be available for a 12 year old (see **Chapters 3 and 4** for more information). All of the exposure assessment methods seek to gain information on the concentrations of the agent(s) to which the person(s) may have been exposed and the duration and frequency (time component) of that exposure. Using this information, researchers can construct exposure indices, which are used to estimate an individual’s exposure and ultimately dose within a population. Finally, there is little doubt that exposure assessment techniques will improve during the course of this study. Determining “gaps” in existing exposure methods and developing more cost-efficient methods to meet study objectives will be essential. The following paragraphs discuss the three main methods of assessing exposures: questionnaires and other indirect means, environmental monitoring, and biomonitoring.

Effective **questionnaires** help researchers acquire necessary information—such as demographic, lifestyle activities, medical history, potential exposure history, environmental and social stressors, and other relevant data—in a clear, unbiased manner without unduly burdening the interviewee (e.g., lengthy questionnaires tend to bore interviewees and provide inaccurate information). In exposure assessment, researchers generally use questionnaires to acquire two types of information for developing exposure indices: likelihood of the occurrence of exposure to the chemical and the frequency/duration of contact with the chemical. Questionnaires can also provide much-needed information on factors that affect the chemical’s pharmacokinetics—which can influence the biologically effective dose—and pharmacodynamics—which can influence a chemical’s effects. These factors include demographics (e.g., age, sex,

**Figure 1-2. Life Stages of Interest in NCS**



and race/ethnicity); environmental and behavioral stressors; nutritional status; and other exposures, including medications and food supplements. Although questionnaires can be used as a standalone tool, they are most often paired with other exposure assessment methods. Because they provide information unavailable through other methods, questionnaires are essential in any study of human exposure to chemical agents. However, they often provide no actual concentration data for the chemical/biological agent in the environment and in humans.

Information from other indirect methods, such as geographic information systems (GIS) and videotaping, should be considered, but such information is also limited by not providing actual concentration data for the agent in environmental and human specimens. However, videotaping has the advantages of tracing a given individual throughout his/her activities in daily life and observing potential contacts with the agent of concern and the frequency/duration of these contacts. Videotaping is particularly useful for recording the potential for transferring an agent from the outer surfaces of the body into, for example, the mouth; *i.e.*, for recording such actions as hand-to-mouth activity. GIS uses computerized maps to integrate potential exposure data (e.g., from estimated pollution data) into a spatial form so that the data can be analyzed geographically. However, analyses and interpretation of such data can be complex and in some cases may not be generalizable to all types of exposure scenarios. GIS data are often used when more direct monitoring data are not available. In the future, no doubt GIS information on both potential exposures and the occurrence of disease will be mapped globally, nationally, regionally and locally. We caution, however, that while this type of mapping information may be of benefit to the NCS, measurements in environmental or biological samples should be performed to validate exposure assessments derived from GIS or other questionnaire-based data.

**Environmental measurements**—the measurement of a chemical agent or its transformation product in an environmental medium—provide information that can be used to track a chemical from its source throughout the environment (e.g., air, water, food, soil, dust, etc.). In exposure assessment, environmental monitoring provides information about the concentration of the chemical(s) to which humans are potentially exposed and potential routes of exposure. Environmental data are most useful for exposure pathways with one environmental matrix (e.g., air); multimedia exposures require increased measurements (and thus increased costs), and the resulting data are more difficult to model for the purpose of predicting human exposures and internal doses. When paired with questionnaires to provide information on the duration and frequency of exposure and the timing of the exposures, environmental measurements offer researchers useful information on the potential dose. However, researchers must then develop models to estimate the amount of the chemical that gets into the body and becomes the internal dose (Ott, 1985; Ryan, 1991; Özkaynak, 1999; MacIntosh et al., 1995; Zartarian et al., 2000; and Burke et al., 2001). Researchers must also consider the burden to the study population. In addition to the questionnaire's burden, study subjects must also bear the burden of any necessary environmental monitoring equipment, such as personal air monitors and home air or water monitoring devices. However, many developments in monitoring personal exposures to air-borne chemicals and particulates are ongoing. These developments include portable chemical sensors and military-designed clothing ranging from bracelets to smart-shirts, which will allow not only for the assessment of chemicals in the air but also chemicals coming into contact with clothing; furthermore, the clothing devices can denote physiological changes, such as heart rate and EKG.

Assessing personal exposures in health studies such as the NCS often relies upon partial information on measured concentrations of chemicals in various microenvironments of concern. As a result, the use of limited outdoor or indoor monitoring information can lead to exposure misclassification biases, which in turn may result in loss of statistical power or the potential for obtaining a null result (Özkaynak, 1986; Özkaynak and Spengler, 1996). To minimize errors in estimating personal exposures, researchers must identify key sources, media, routes, and pathways of concern for each environmental pollutant and then determine an optimum sampling and analysis plan. These plans should take into account the stability of

the chemical in the environment, as well as if and how it is bound, suspended, or in solution. In practice, both budgetary and technical constraints limit the extent of an environmental monitoring program. Such a program's actual cost depends on the chemical, the number and type of matrices to be monitored, and the frequency of monitoring.

Environmental monitoring is useful when researchers assess exposure to chemicals whose toxicity can vary depending on the route by which they enter the body. For example, manganese and polycyclic aromatic hydrocarbons that are bound to particulate matter are potentially more toxic when inhaled than when ingested. Monitoring methods that do not account for this fact might incorrectly assess the toxicity of such an exposure.

The primary goal of an environmental epidemiological study such as the NCS is to link the biologically effective dose with the adverse health outcome of interest. Although measuring the biologically effective dose would be the most accurate assessment of exposure in such a health study, these measurements are often impossible to obtain. For example, samples cannot be taken from a target organ such as the liver. As a result, researchers often can not measure the biologically effective dose on the exposure continuum (*Figure 1-1*) but instead attempt to measure the absorbed dose or internal dose. Such measurements are called biological monitoring or biomonitoring, and they provide information on the absorbed dose independent of the environmental pathway or route of exposure. The internal dose measurements may be used to estimate the biologically effective dose using pharmacologically-based pharmacokinetic models (Mason and Wilson, 1999).

Procedures for collecting biological samples range from the invasive, such as drawing blood, to the minimally invasive or non-invasive, such as collecting urine samples. In situations that allow for an invasive procedure, drawing blood is one of the most effective methods of biomonitoring. Regardless of the route of exposure, the chemical must be absorbed into the bloodstream and circulate to the tissues prior to an effect (except in cases of direct inhalation effects on the lung and blistering agents on skin). Blood is also a "regulated" matrix in that a human maintains a constant amount of blood per kilogram of body weight; therefore, measurements can be "normalized" to this amount. Urine, which serves as a "sink" for many chemicals, is also an effective matrix for biomonitoring. Chemicals are generally found in the urine not only as their original "parent" structure but, more frequently, as metabolites. Measuring these metabolites to assess exposure can, however, be problematic because 1) multiple chemicals may form the same metabolite and 2) the environmental transformation product (for example, for organophosphorous pesticides) may be the same chemical as the metabolite, thereby increasing the chance of misinterpretation. Also, urine, unlike blood, is not a regulated matrix, and therefore concentrations of environmental chemicals or their metabolites in urine are often adjusted for the concentration of creatinine in that urine sample.

For persistent chemicals (those that have "long" half-lives on the order of months or years in the environment and in humans), biomonitoring data provide information about the types and amounts of chemicals that are internalized by people. These persistent chemicals are generally measured in blood or its components (e.g., serum and plasma), adipose tissue, or human milk. Biomonitoring is generally considered the best method for assessing human exposure to persistent chemicals, assuming the sample collection medium is feasible. Biomonitoring is less effective for assessing human exposure to nonpersistent chemicals, especially when the exposures are not constant or very infrequent (episodic). Exposure to some chemicals and physical agents—including particulate matter, asbestos, some of the criteria pollutants (e.g., oxides of nitrogen), and allergens—cannot be assessed by biomonitoring. Also, the non-specificity of the metabolite biomarker (depending on the chemical and the biological matrix used) for certain chemicals makes determining the actual chemical to which the population was exposed difficult.

Regardless of whether data from questionnaires, environmental monitoring, biomonitoring, or a combination of these techniques are used for exposure assessment, these data need to be modeled and linked to the biologically effective dose (Figure 1-1) and beyond to adverse effect (disease) data. Another approach that can potentially be used is to move through the exposure continuum (Figure 1-1) and into the effect portion of the continuum. This approach involves monitoring for endogenous changes (an effect) in the body [e.g., by using molecular profiling to note changes in messenger RNA, proteins (proteomics), and endogenous metabolites (metabolomics)] (Wilson and Suk, 2003). Once these changes are noted, we again work back on the exposure continuum to focus on the agent(s) that can be linked to these changes. This approach has advantages, but certainly the specificity of linking certain stressors (e.g., psychosocial and nutritional) in addition to exposures to environmental chemicals with such changes is unclear at this time.

## 1.2 Analytical Methods Used in Environmental and Biological Monitoring

Monitoring data have been gathered on environmental samples, animal samples, and human samples on geographic scales ranging from global to local (community). These survey data should be utilized by study designers of the NCS. However, we think that for the NCS, personal monitoring data should be basis for the exposure assessment. This means that the monitoring is either done with environmental samples, biological samples, or personal samplers. Regardless, the analytical method for actually measuring the amount of the chemical or degraded product/metabolite consists of three major steps: *sample preparation*, which generally involves the separation of the chemicals of interest from other chemicals in the matrix; *analysis*, which may involve further separation (e.g., by chromatography), but always involves detection and quantification; and *data analysis*. Generally, sample preparation is the most matrix-dependent of the three steps. During method development, study designers should choose methods that allow for the monitoring of multiple chemicals (which may have many different chemical/physical properties), but are accurate, precise, specific, calibrated, and rugged/robust. NCS designers should also consider cost and throughput. For example, when measuring chemicals in a matrix at extremely low concentrations (e.g., dioxin in blood at parts per quadrillion levels), designers should normally choose a method that uses specific cleanup procedures and high-resolution gas chromatography/high-resolution mass spectrometry with isotope dilution techniques for quantification. However, these methods have extremely high costs (about \$750-\$1000 per sample) and relatively low throughput. Other methods that require less sample preparation and use equipment available in more laboratories have lower costs and increased throughput, but even these methods are not inexpensive (costing somewhere in the low hundreds of dollars per sample). For the NCS, designers should consider using lower-cost screening procedures, such as immunoassays. In general, these procedures have higher throughputs (depending on the degree of sample preparation) and require less expensive equipment, but suffer from problems associated with cross reactivity and thus lack of specificity. The main point of this discussion is that the analytical method that is chosen must have the necessary characteristics to successfully address the question.

Once the analytical data have been generated, researchers must determine how to report and statistically treat concentration levels that are below the limit of detection (LOD). The LOD is the lowest concentration of a chemical that the analytical method can measure and is determined by the measured value that differs in a statistically significant manner from having “zero” chemical in the sample, based on one of such procedures used to treat such problem (Taylor, 1990). The efficiency of the analytical method in preparing extracts free of potential interferants (but still recovering a high percentage of the analytes of interest) and the sensitivity of the instrumental system affect the LOD for each method. Researchers should determine the LOD in each laboratory for each instrument (instrumental LOD) and for each method (method LOD). Frequently, the method LOD is calculated for each and every sample analyzed.

When multiple laboratories are involved in a study, they should all use the same, or at least comparable, methods to maintain similar LODs. Similarly, sample weights should be the same or similar.

When calculating measurements lower than the LOD, researchers generally report the concentrations as “nondetectable” with the LOD given. However, for parametric statistics, researchers must assign a number for each sample. To circumvent this problem, researchers have used values ranging from the most conservative value of zero to one-half of the detection limit concentration, to the detection limit divided by  $\sqrt{2}$ , to the most “liberal” value—the detection limit itself. Researchers have also used more sophisticated modeling methods to estimate the concentration levels for nondetectable results. These results can occur because of the lack of analyte in a sample or because of a high method LOD, which can result from such factors as insufficient sample size or from characteristics of the analytical method. For example, researchers often measure multiple analytes by mass spectrometry using selected ion monitoring and the more masses (such as analytes) measured in a run, the higher the LOD for all of the analytes (Needham and Wang, 2002).

Regardless of the analytical method chosen, researchers must be able to demonstrate the method’s accuracy, precision, specificity, linearity and range, limit of detection, and ruggedness/robustness. They must then establish a quality assurance/quality control (QA/QC) program to easily allow the detection of systematic failures in the methodology and to ensure that these defined requirements are being maintained over time and among laboratories (Needham et al., 2002). The testing procedures can include proficiency testing to ensure accuracy as measured against a known reference material, repeat measurements of known materials to confirm the validity of an analytical run and to measure analytical precision, “round robin” or interlaboratory studies to confirm reproducible measurement values among laboratories, regular verification of instrument calibration, daily assurance of minimal laboratory contamination by analyzing blank samples, and cross-validations to ensure that multiple analysts and instruments obtain similar analytical values. Additionally, some U.S. public health laboratories have been certified by the Health Care Finance Administration (HFCA) to comply with all QA/QC parameters outlined in the Clinical Laboratories Improvement Amendment (1988). The QA/QC measures are applicable to not just the analytical method but to all aspects of the measurement process, including sampling design, sample collection, transport and storage of samples, analytical method determination, and data reporting. Often overlooked in longitudinal studies, which require the collection and long-term storage of environmental and biological samples, is the effect of long-term storage on the sample and the agent. Matrix-based quality control samples containing the agent at known or “analytically assigned” concentrations should be stored under the exact conditions as the study samples and periodically monitored. Therefore, all aspects of the measurement process must be subject to a stringent QA/QC protocol. Also, any new analytical method or any change in the measurement process must be documented and validated against the method being used. Many parameters for implementing or improving a quality assurance program have been published (Taylor, 1990; Schaller, 1991).

### 1.3 Study Design Considerations

The exact strategy for exposure monitoring directly depends on study design. For example, a long-term longitudinal study of 100,000 children may yield fewer direct exposure measures for each child, whereas a series of smaller studies allows for more direct exposure measures. Regardless of study design, individual exposure assessment will play a vital role in the NCS because many of its hypotheses associate individual exposures with adverse health outcomes. Assessing exposure and the resulting dose concentrations as accurately and as totally as possible is thus crucial to proving or disproving the hypotheses. NCS study designers should be wary of using population based reference concentration data—such as that gathered from the National Health and Nutrition and Examination Survey (NHANES) and its *Second National Report on Human Exposure to Environmental Chemicals*, the Continuing Survey of Food Intake by Individuals (CFSII), and the National Human Activity Pattern Survey (NHAPS)—for

exposure and dose assessment, but should instead use personal exposure data gathered specifically for the NCS. The results of these studies should, however, be used in designing the NCS protocols. Questionnaires will be essential; videotaping and GIS may provide additional specificity and accuracy. Exposure pathways through one primary environmental medium should generally be monitored; however, researchers should consider the importance of route to potency of the exposure. Biomonitoring should be used when possible to assess total exposure to an individual or to validate and calibrate any exposure index derived by other means. Regardless of the type of analytical monitoring, designers should emplace long-term and interlaboratory quality control/quality assurance methods and continuously monitor the performance of the method and the integrity of the sample under the storage conditions. In addition, designers should recognize that exposure assessment techniques are still evolving and for certain chemicals, especially air-borne chemicals, real-time, continuous sampling and monitoring will be more widely available and less burdensome.

## 1.4 Structure and Content of the White Paper

This paper presents and prioritizes the various means of assessing human exposure to a multitude of environmental chemical/biological agents. The paper is divided into six chapters: **Chapter 1** introduces human exposure assessment in general and other exposure assessment issues. **Chapter 2** identifies hypotheses requiring exposure assessment and the recommended considerations for sampling and analysis. **Chapters 3** and **4** identify the environmental and biological matrices, respectively, to be collected and analyzed to classify exposure to a given chemical or other relevant substance in children at various life stages. **Chapter 5** discusses survey methodologies that will be needed to serve as a stand-alone exposure assessment tool or as a supplement to analytical measurements of environmental and biological samples. Finally, **Chapter 6** synthesizes suggested exposure measurement approaches and epidemiological considerations regarding a study design. **Table 1-1** lists substances of potential interest to the NCS (organized by chemical classes). We recognize that this is a dynamic list and thus will change over time since, no doubt, there will be new information in the future on this list of chemicals and recommended methods for sampling them. Consequently, it is important to plan upon storing a portion of the environmental and biomonitoring samples to be collected for archiving purposes, so that future technologies can be applied to these samples. Furthermore, for both technical and cost-considerations, it is possible that analyses of some of the stored samples can be performed only after confirmation of certain health outcomes, in order to determine retrospectively the exposures of study subjects with these identified conditions. The remainder of this paper uses this current list as a reference to assist NCS designers in developing exposure indices for accurately estimating exposures during the life stages of interest.

**Table 1-1. Chemicals and Chemical Classes of Potential Interest to the NCS**

General Class/Chemical Agent
<b>Persistent Organic Chemicals</b>
PCBs
Hydroxy PCBs
PCDDs/Fs/coplanar PCBs
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin
1,2,3,7,8-Pentachlorodibenzo-p-dioxin
2,3,7,8-Tetrachlorodibenzo-p-dioxin
1,2,3,4,6,7,8,9-Octachlorodibenzofuran
1,2,3,4,6,7,8-Heptachlorodibenzofuran
1,2,3,4,7,8-Hexachlorodibenzofuran
1,2,3,6,7,8-Hexachlorodibenzofuran
1,2,3,7,8,9-Hexachlorodibenzofuran
1,2,3,7,8-Pentachlorodibenzofuran
2,3,4,6,7,8-Hexachlorodibenzofuran
2,3,4,7,8-Pentachlorodibenzofuran
2,3,7,8-Tetrachlorodibenzofuran
3,3',4,4',5,5'-Hexachlorobiphenyl
3,3',4,4',5-Pentachlorobiphenyl
3,4,4',5-Tetrachlorobiphenyl
3,3',4,4'-Tetrachlorobiphenyl
Organochlorine pesticides
Chlordane and metabolites
DDT and metabolites (DDE, DDD)
Dieldrin
Aldrin
Endrin
Kepone
Heptachlor and metabolites
Hexachlorobenzene
Hexachlorocyclohexanes (including alpha, beta, and gamma isomers)
Mirex
Octachlorostyrene
Pentachlorobenzene
Pentachloronitrobenzene
<i>trans</i> -Nonachlor
Toxaphene
Perfluorinated Chemicals
Perfluorooctanoic sulfonic acid
Perfluorooctanoic acid
Brominated Flame Retardants
Polybrominated diphenyl ethers (PBDEs)
2,4,4'-Tribromodiphenyl ether
2,2',4,4'-Tetrabromodiphenyl ether
2,2',4,4',6-Pentabromodiphenyl ether
2,2',4,4',5-Pentabromodiphenyl ether
2,2',3,4,4'-Pentabromodiphenyl ether
2,2',4,4',5,6'-Hexabromodiphenyl ether
2,2',4,4',5,5'-Hexabromodiphenyl ether
2,2',3,4,4',5',6-Heptabromodiphenyl ether
2,2',3,4,4',5,5',6-Octabromodiphenyl ether
2,2',3,3',4,4',5,5',6,6'-Decabromodiphenyl ether

**Table 1-1. Chemicals and Chemical Classes of Potential Interest to the NCS (continued)**

<b>General Class/Chemical Agent</b>
Hexabromocyclododecane beta, delta and gamma isomers Polybrominated biphenyls (PBBs)
<b>Nonpersistent Nonvolatile Chemicals</b>
Pyrethroid insecticides
Permethrin
Cypermethrin
Deltamethrin
Resmethrin
Allethrin
Bioallethrin
Cyfluthrin
Fenvalerate
Esfenvalerate
Sumithrin
Miscellaneous pesticides
Hydramethanone
Phenoxy carb
Sulfuramide
Imidacloprid
Abimectin
Amitrol
Fipronil
Paraquat
Diquat
Pendamethalin
Phytoestrogens
Isoflavones
Daidzein
Genistein
Formononetin
Glycitein
Biochanin-A
Lignans
Secoisolariciresinol
Matairesinol
Pinoresinol
Lariciresinol
Syringaresinol
<b>Nonpersistent Semi-volatile Organic Chemicals</b>
Organophosphorus insecticides
Azinphos methyl
Chlorethoxyphos
Chlorpyrifos
Chlorpyrifos methyl
Coumaphos
Dichlorvos
Diazinon
Dicrotophos
Dimethoate
Disulfoton
Ethion

**Table 1-1. Chemicals and Chemical Classes of Potential Interest to the NCS (continued)**

General Class/Chemical Agent
Fenitrothion
Fenthion
Isazaphos-methyl
Malathion
Methidathion
Methyl parathion
Naled
Nitrofen
Oxydemeton-methyl
Parathion
Phorate
Phosmet
Pirimiphos-methyl
Sulfotepp
Temephos
Terbufos
Tetrachlorviphos
Carbamate insecticides
Carbaryl
Propoxur
Carbofuran
Benfuracarb
Carbosulfan
Furathiocarb
Pirimicarb
Bendiocarb
Aldicarb
Methomyl
Herbicides
Salts and esters of 2,4,5-trichlorophenoxyacetic acid
Salts and esters of 2,4-dichlorophenoxyacetic acid
Atrazine and other chlorotriazines
Alachlor
Acetachlor
Butachlor
Metolachlor
Other pesticides
Endosulfan I and II
Methoxychlor
Bis-dithiocarbamates and metabolites
Sulfonyl ureas
Ureas
DEET
Dicofol
Iprodione
Vinclozolin
Trifluralin
Naphthalene
Halogenated phenols
Dichlorophenols
Trichlorophenols
Pentachlorophenol
Triclosan
Tetrabromobisphenol-A

**Table 1-1. Chemicals and Chemical Classes of Potential Interest to the NCS (continued)**

General Class/Chemical Agent
Polycyclic aromatic hydrocarbons
Benzo[a]pyrene
Benzo[a]anthracene
Benzo[c]phenanthrene
Chrysene
Fluoranthene
Fluorene
Phenanthrene
Pyrene
Naphthalene
Phthalates
Dimethyl phthalate
Diethyl phthalate
Dibutyl phthalate
Dibenzylbutyl phthalate
Di-2-ethylhexyl phthalate
Di-n-octyl phthalate
Di-isononyl phthalate
Alkyl phenols
Bisphenol-A
Nonylphenol
Octylphenol
<i>tert</i> -Butylphenol
Tobacco Smoke
Cotinine
Naphthalene
<b>Nonpersistent Volatile Organic Chemicals (boiling point &lt;250 at 1 atm)</b>
Acrylamide
Acrylonitrile
1,1, 1-Trichloroethane
1,4-Dichlorobenzene
1,3-Butadiene
1,3-Dichloropropene
1,1,2,2-Tetrachloroethane
2-Butanone
Acetone
Acetaldehyde
Acrolein
Benzaldehyde
Benzene
Chloroform
Crotonaldehyde
Ethylbenzene
Ethylene dibromide
Ethylene dichloride
Ethylene oxide
Formaldehyde
Hexanal
Hexane
Isobutyraldehyde
Methylene chloride
Methyl ethyl ketone
Methyl- <i>tert</i> -butylether

**Table 1-1. Chemicals and Chemical Classes of Potential Interest to the NCS (continued)**

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<b>General Class/Chemical Agent</b>
Pentanal
Propanol
Propylene dichloride
m,p-Xylene
o-Xylene
Styrene
Tetrachloroethene
Toluene
o-Tolualdehyde
m-Tolualdehyde
p-Tolualdehyde
Trichloroethylene
Vinyl chloride
<b>Bioaccumulative Inorganic Chemicals</b>
Lead
Mercury
Cadmium
<b>Nonbioaccumulative Inorganic Chemicals</b>
Antimony
Arsenic
Barium
Beryllium
Cesium
Chromium
Cobalt
Manganese
Molybdenum
Platinum
Thallium
Tungsten
Iron
Nickel
Vanadium
Perchlorate
<b>Criteria Pollutants</b>
NOx
SOx
CO
lead
ozone
particulate matter
<b>Bioallergens</b>
Dust mites
Arthropods/rodents
Endotoxins
Pollen
Mold/mildew
Pet dander

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## 2. Relevant Chemical and Biological Agents for Different Study Hypotheses

This section discusses selected chemicals or chemical classes that are anticipated to play a role in the investigation of main NCS hypotheses, based on those provided by the NCS Interagency Coordinating Committee (ICC) to the December 2002 Study Assembly meeting. The ICC selected these hypotheses (see *Table 2-1*) based on findings from 20 NCS working groups, as reviewed and reported by the NCS Advisory Committee (NCSAC), and on independent reviews of the children's environmental health literature and comments from the broad-based Study Assembly. During the 2002 meeting, ECAWG members participated in inter-working group meetings for those hypotheses likely to involve exposure measurements or collection of exposure-related information (e.g., activity levels or dietary intake).

**Table 2-1. List of Priority Outcomes and Core Hypotheses for the NCS, November 2003**

<p><b>I. Pregnancy Outcome</b>  <b>Hypothesis:</b> Among women without diabetes before pregnancy, impaired glucose metabolism during pregnancy is proportional to risk of major congenital malformations of the heart, central nervous system, musculoskeletal system, and all birth defects combined.  <b>Hypothesis:</b> Intrauterine exposure to mediators of inflammation due to infection of either vaginal, cervical, uterine, or of more distal sites (e.g., periodontal disease), is associated with an increased risk of preterm birth.</p> <p><b>II. Neurodevelopment and Behavior</b>  <b>Hypothesis:</b> Repeated low-level exposure to nonpersistent pesticides in utero or postnatally increases risk of poor performance on neurobehavioral and cognitive examinations during infancy and later in childhood, especially, for certain agents, among those with genetically decreased paraoxonase activity.  <b>Hypothesis:</b> Prenatal infection and mediators of inflammation are risk factors for neurodevelopmental disabilities, such as cerebral palsy and autism.  <b>Hypothesis:</b> Infection and mediators of inflammation during pregnancy and the perinatal period are associated with increased risk of schizophrenia.</p> <p><b>III. Injury</b>  <b>Hypothesis:</b> Repeated head trauma has a cumulative adverse effect on neurocognitive development.</p> <p><b>IV. Asthma</b>  <b>Hypothesis:</b> Exposure to indoor and outdoor air pollution and bioaerosols (including allergens, endotoxin, and mold) is associated with increased risk of asthma.  <b>Hypothesis:</b> Respiratory viral infection early in life is associated with increased risk of asthma.  <b>Hypothesis:</b> Maternal stress during pregnancy is associated with increased risk of asthma.  <b>Hypothesis:</b> Antioxidant constituents of diet decrease risk of asthma.  <b>Hypothesis:</b> Early exposure to bacterial and microbial products decreases risk of asthma (hygiene hypothesis).  <b>Hypothesis:</b> Access to health care and management of asthma are strongly related to asthma hospitalization.</p>	<p><b>V. Obesity and Physical Development</b>  <b>Hypothesis:</b> Impaired maternal glucose metabolism during pregnancy is directly related to risk of obesity and insulin resistance in offspring.  <b>Hypothesis:</b> Intrauterine growth restriction as determined by serial ultrasound examination is associated with subsequent risk of central obesity and insulin resistance in offspring, independent of subsequent body mass index.  <b>Hypothesis:</b> Breast milk feeding, compared with infant formula feeding, and breastfeeding duration are associated with lower rates of obesity and lower risk of insulin resistance.  <b>Hypothesis:</b> Dietary predictors of obesity and insulin resistance include reduced intake of fiber and whole grains, and high glycemic index.  <b>Hypothesis:</b> Environmental factors such as distance to parks, availability of walking routes in the neighborhood, and neighborhood safety are associated with risk of obesity and insulin resistance.  <b>Hypothesis:</b> Social, behavioral, and family factors that affect development of dietary preferences and physical activity patterns early in childhood determine risk of childhood obesity and insulin resistance.  <b>Hypothesis:</b> In utero and subsequent exposure to environmental agents that affect the endocrine system (bisphenol A, atrazine, and lead) results in altered age at puberty.</p>
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Although these hypotheses will likely change as the study evolves, the NCSAC has recommended that the NCS should be hypothesis-based, and the ICC provided this set of hypotheses to define a

framework for study design. Thus, the working group used this set as the starting point to identify general types of exposure measures needed in the NCS, as described below. The purpose of this section is to discuss exposure measurement considerations for a number of chemicals or chemical classes that the work group has identified, based in part on discussions with other working groups at the December 2002 Study Assembly meeting, to be important to consider during the epidemiologic investigation of environmental factors which may be contributing to adverse health outcomes. The selective list of chemicals included in this section are expected to serve as either main effects, effects modifiers, or confounders in the epidemiologic analyses of pollution and health data in NCS. This list is not intended to be an exhaustive list of all environmental chemicals which may be considered during the NCS investigation. A full range of chemicals that may be included during the NCS study is discussed in **Chapter 3** on general exposure measurement methods. **Table 1-1** summarizes the extensive list of chemicals that may be included under the six main NCS hypothesis categories: pesticides and neurobehavioral outcomes, exposures to air pollutants and asthma, exposures to bioaerosols and asthma, exposures to pollutants and pregnancy outcomes, chemical exposures and obesity, and exposures affecting age of puberty.

None of the proposed study hypotheses postulates an association between environmental chemical exposures and elevated risks for obesity or insulin resistance. However, other chemical-related hypotheses require collection of similar samples or information basic to testing obesity hypotheses. For example, information on dietary intake, activity patterns, and exercise can be used to form estimates of exposure and intake for some chemical contaminants. Biological samples proposed for assessing obesity and insulin resistance include blood, urine, and breast milk. These media can also be used for measurement of exogenous chemical exposures. Thus, the study design should be developed to accommodate both the obesity and exposure assessment needs. This white paper document, however, does not address this chemical exposure – obesity relationship to any significant extent.

## 2.1 Pesticides and Neurobehavioral Outcomes

***Hypothesis:*** *Repeated low-level exposure to nonpersistent pesticides in utero or post-natally increases risk of poor performance on neurobehavioral and cognitive examinations during infancy and later in childhood, especially, for certain agents, among those children with genetically decreased paraoxonase activity (reviewed in Eskenazi et al., 1999; Berkowitz et al., 2004; Costa et al., 2003).*

### 2.1.1 Background

#### *Exposure*

Approximately 1 billion pounds of agricultural pesticides are used annually in the United States and approximately 85% of households store at least one pesticide in their homes (Adgate et al., 2000; U.S. EPA, 2003; U.S. EPA 2003). Recent biological monitoring studies indicate that pesticide exposures are widespread in the U.S. population and in children (Adgate et al., 2001; CDC, 2001; Curl et al., 2003; Curl et al., 2002; Fenske et al., 2000; Koch et al., 2002; Loewenherz et al., 1997; Lu et al., 2000; O'Rourke et al., 2000; Shalat et al., 2003). Information about pesticide exposures to pregnant women, however, is limited. Berkowitz et al. (2003) found urinary metabolites of pyrethroids, chlorpyrifos, and pentachlorophenol in a cross sectional study of urban women (Berkowitz et al., 2003). Data from the fourth National Health and Examination Survey (NHANES IV) include 96 pregnant women, 94% of whom had detectable levels of organophosphate (OP) urinary metabolites (CDC, 2003). Whyatt et al. (2003) and Perera et al. (2003) found diazinon and chlorpyrifos in blood samples from pregnant women living in New York City, NY and separately documented contamination of air samples in their home environments (Perera et al., 2003; Whyatt et al., 2003). Whyatt and Barr (2001) reported positive detections for OP pesticides in meconium, and a recent pilot study found pesticides in amniotic fluid (Bradman et al., 2003; Whyatt and

Barr, 2001). Overall, these studies indicate that detectable pesticide exposures are occurring to women of childbearing age, pregnant women, fetuses, and young children.

### *Paraoxonase (PON1)*

Prior data suggest that the effects of organophosphates may be modulated by paraoxonase activity. Human paraoxonase (PON1) is a polymorphic, high-density lipoprotein (HDL)-associated esterase that hydrolyzes the toxic metabolites of several organophosphate pesticides and nerve agents (Brophy et al., 2001; Li et al., 2000). PON1 is polymorphic in humans with single nucleotide polymorphisms (SNP's) found in the coding and non-coding regulatory regions. PON1 status is defined by 1) genotype or gene frequency at PON1<sub>Q192R</sub>, and 2) phenotype, as measured by PON1 activity via enzyme or PON1 expression assays (Brophy et al., 2002; Davies et al., 1996). The efficiency of detoxification depends on polymorphisms at position 192 (Q/Q; Q/R; R/R), with RR genotypes with significantly lower activity and presume increased sensitivity to OP pesticides (Li et al., 2000). Gene frequencies vary by ethnicity. For example, recent studies report gene frequencies of Q=0.6, R=0.4; Q=0.7, R=0.3; and Q=0.3, R=0.7 for different Latino, Caucasian, and African-American populations, respectively (Davies et al., 1996; Brophy et al., 2002). Finally, PON1 levels change ontogenetically, with newborns having much lower activity that increases in the first years of life (Costa et al., 2002). Berkowitz et al. (2004) recently found an association between chlorpyrifos metabolites in maternal urine samples during pregnancy and infant head circumference at birth but only among infants of mothers with low paraoxonase activity (Berkowitz et al., 2004). By emphasizing PON1, Hypothesis 2.1 focuses the study principally on the organophosphate pesticides. However, planners for the NCS should consider expanding the focus to include exposures to other potentially neurotoxic pesticides, such as pyrethroids and carbamates. Exposures to these pesticides are widespread, whereas residential use of chlorpyrifos and diazinon, the two organophosphates that have been most widely used for residential pest control, has recently been banned by the U. S. Environmental Protection Agency. In addition, polymorphisms in other metabolically important genes (e.g., various P450 enzymes, GSTM1, GSTP1, etc.) may be relevant to this hypothesis but are addressed by the Gene-Environment Workgroup.

### *Toxicity and Epidemiology*

The primary effects of OP and carbamate pesticide exposure are on the nervous system. These pesticides interfere with the metabolism of acetylcholine (ACh) by inhibiting acetylcholinesterase (AChE). ACh accumulates at the neuronal junctions resulting in the continued stimulation and then suppression of neurotransmission. Unlike OPs, carbamates do not irreversibly inhibit AChE, and their effect is quickly reversed after excretion (Keifer and Mahurin, 1997). Pregnancy is a time of increased risk because plasma AChE activity is already reduced during the first two trimesters (Evans et al., 1988; Howard et al., 1978). OPs can also damage the CNS through non-cholinergic mechanisms that involve alterations in the expression and function of nuclear transcription factors that control cell replication, differentiation, and apoptosis (Dam et al., 2003). Developing organisms may be more sensitive than adults to the effects of OPs (Moser and Padilla, 1998). Although immature animals recover more rapidly from AChE inhibition (Garcia et al., 2002), tests on young rodents show greater susceptibility to OPs that decreases with age (Benke and Murphy, 1975; Brodeur and DuBois, 1963; Moser, 2000; National Research Council, 1993; reviewed in Eskenazi et al., 1999). The lethal dose in immature animals is only 1% of the adult dose (Pope, 1991; Pope and Chakraborti, 1992; Whitney et al., 1995). Young animals may be susceptible due to lower activity of detoxifying enzymes (paraoxonase or chlorpyrifos-oxonase) that deactivate OP metabolites (Atterberry et al., 1997; Benke and Murphy, 1975; Davies et al., 1996; Lassiter et al., 1998; Mortensen et al., 1996; National Research Council, 1993; reviewed in Eskenazi et al., 1999). In the developing organism, AChE inhibition may affect essential functions such as cell replication and differentiation, axonogenesis and synaptogenesis (Bigbee et al., 2000; Dam et al., 1999; Dam et al., 1999; Lauder and Schambra, 1999).

Nervous system ontogeny can be divided into: 1) early brain development, i.e., first trimester in humans; and 2) the brain growth spurt, i.e., third trimester through the first 2 years of life in humans, when there is extensive axonal and dendritic growth, synaptogenesis, proliferation of glial cells, and myelination (Campbell et al., 1997; Dam et al., 1999; Dam et al., 1998; Dam et al., 2003; Garcia et al., 2002; Slotkin, 1999; Song et al., 1998). Toxins introduced during the early period can cause malformations, whereas those introduced in the later period can result in behavioral and cognitive changes, such as in learning and memory (Drachman, 1977; Eriksson and Talts, 2000; Karczmar, 1975). In rodents, the cholinergic transmitter system undergoes rapid development during the later phase, i.e., the first three to four weeks after birth (Coyle and Yamamura, 1976). Given the diversity of mechanisms and target tissues, the developing brain is likely to be vulnerable into childhood (Dam et al., 2003; Garcia et al., 2003; Moser and Padilla, 1998; Qiao et al., 2002). Many organophosphate insecticides are lipophilic and readily cross the placental boundary (Richardson, 1995; Whyatt et al., 2003). Considerable evidence in animals links exposure to OPs *in utero* or the early post-natal period with adverse neurodevelopment (reviewed in Eskenazi et al., 1999; Landrigan et al., 1999). Specifically, neurobehavior effects have been seen following perinatal exposures to chlorpyrifos, diazinon, dichlorvos, sumithrin, and trichlorfon. These effects may be due to direct impact on the fetal cholinergic system, cellular intermediates (e.g., adenylyl cyclase through noncholinergic mechanisms), and direct targeting of neural cell replication, differentiation, axonogenesis, and synaptogenesis (Campbell et al., 1997; Dam et al., 1998; Qiao et al., 2002; Slotkin, 1999; Song et al., 1997). In the only published study in humans, Guillette et al. found that 4 and 5 year-olds in a Mexican agricultural community with high OP and organochlorine pesticide use showed decreased stamina, hand-eye coordination, drawing ability and 30-minute recall as compared to children from a nearby ranching community with low usage (Guillette et al., 1998). There was no direct measure of exposure, no control for confounding, interviewers were not blinded to group status, and the assessment tools had not been validated.

Other major pesticide classes that are replacing home and some agricultural uses of OP and carbamate pesticides are pyrethroid and neo-nicotinoids. Pyrethroid exposures to pregnant women have been documented in one study (Berkowitz, 2003), but population-based biomonitoring data have not been published at this time. Pyrethrins and their synthetic derivatives, pyrethroids, have become the dominant home pesticide class. Both of these pesticide classes are neurotoxic but are generally less acutely toxic than the organophosphate and carbamate pesticides. Pyrethroids are synthetic insecticides that are chemically similar to naturally occurring pyrethrins, but are modified to be more stable in the environment (Soderlund et al., 2002). Use of pyrethroids is increasing rapidly (Soderlund et al., 2002). Like most other classes of insecticides, the pyrethroids are acute neurotoxicants (Aldridge, 1990; Bradbury and Coats, 1989; Vijverberg and van den Bercken, 1990). Specifically, they alter the permeability of excited nerve cells to sodium ions and cause repetitive nerve impulses which can vary between a few dozen for the less toxic non-cyano-pyrethroids to up to a thousand for the more toxic cyano-pyrethroids. They also have other neurobiological actions, including affects on central GABA, noradrenergic and dopaminergic or cholinergic neurotransmission (Mandhane and Chopde, 1997). In general, pyrethroids are considered among the lower toxicity insecticides in part because mammals have much higher levels of the enzymes that detoxify pyrethroids than do insects and they are rapidly metabolized and excreted in mammalian systems. However, detoxification enzymes involved in metabolism of pyrethroids are much lower during fetal and early post-natal development than they are later in life (Cantalamessa, 1993; Sheets, 2000), suggesting that young children may be more susceptible to adverse effects than adults.

In humans, pyrethroids are metabolized by esterases, mainly in the liver, and the metabolites are renally eliminated with a half-life of about 6 hours (Heudorf and Angerer, 2001). Acute high doses of pyrethroids can cause nervous system effects such as incoordination, tremors, vomiting, diarrhea and irritability. Pyrethroids are also skin and respiratory irritants. Experimental data in laboratory rodents has shown that the pyrethroid esfenvalerate can cause short-term behavioral effects in adult rodents (Mandhane and Chopde, 1997).

Nicotinyls, such as imidacloprid, cause a blockage in the nicotinic neuronal pathway that is more abundant in insects than in warm-blooded animals (making it selectively more toxic to insects than warm-blooded animals). This blockage leads to the accumulation of acetylcholine, resulting in the insect's paralysis, and eventually death.

In summary, exposures to neurotoxic non-persistent pesticides are widespread. Prenatal and early life exposures, especially through the end of the brain growth spurt at 3 years of age, may be important in relation to later adverse neurodevelopmental outcomes.

### 2.1.2 Key Pesticides of Interest

Based on these findings, the NCS Exposure and Neurobehavioral Outcome Inter Working Group 2-1 (December, 2002, Baltimore, MD), recommended that exposure assessment for Hypothesis 2.1 should focus on class-wide and specific OP, carbamate, pyrethroid, and neo-nicotinoid pesticides and other current use agricultural pesticides, but should exclude consideration of fungicides and herbicides. However, to the extent that exposures to current-use herbicides and fungicides are widespread and some may have endocrine disrupting potential, exposure assessment to these compounds should be considered (see *Section 3.6*).

### 2.1.3 Exposure Measures

Fetal exposure routes occur via the placenta or directly across the amniotic sac (para-placental exposure). Child exposures occur via ingestion, inhalation, and dermal absorption. Possible exposure assessment methods include measuring pesticides in environmental media such as house dust, air, surface wipes, food (including formula and breast milk), and water. Biomarkers include measurements of pesticides in urine, blood (maternal and child), cord blood, breast milk, meconium, saliva, and possibly other biological samples.

By definition, non-persistent pesticides do not accumulate in the body and are generally excreted within hours and days, often via water-soluble metabolites in urine. Biological exposure markers tend to reflect low-level, transient exposures that are highly variable. Because young children spend the majority of their time indoors at home (Tsang and Klepeis, 1996, Silvers et al., 1994, Wiley et al., 1991), most studies evaluating environmental indices of exposure have focused on the home environment. These measurements may characterize levels of indoor contamination, but the link between environmental measures and actual exposure are not yet adequately defined. For both biological and environmental exposure markers, relatively few data are available characterizing within subject (or home) and between subject (or home) variability and thus the ability of a given sample or series of samples to classify exposure for an epidemiologic study. To date, no single environmental or biological marker has been identified which can be used to classify exposure for longitudinal epidemiologic studies. Notwithstanding these current limitations, substantial progress has and is being made to identify key biological and environmental exposure markers (see *Sections 3.3* and *3.6* and *Chapter 4* for more details). Hypothesis 2.1 requires pesticide exposure assessments during the pre-natal period and early childhood. Because assessment of peri-natal exposures is essential for testing the study hypothesis, frequent sampling should take place during pregnancy and the early post-natal period. Annual sampling during childhood may be adequate for exposure assessment but this needs to be validated. *Tables 2-2* and *2-3* summarize proposed sampling periods and media.

### *Discussion of Exposure Measures*

As noted above, the ability of any individual measure to classify exposure on a longitudinal basis is unknown. Measurement of pesticide metabolites in urine offers many advantages over other potential exposure biomarkers. Urine is easy and non-invasive to collect, and laboratory methods are available to

**Table 2-2. Potential Pre-conception, Pregnancy, and Peri-Natal Sample Collection for Non-Persistent Pesticide Analysis**

	Precon-ception	Trimester			Perinatal Period
		First	Second	Third	
<sup>a</sup> Maternal urine*	•	•	•	•	•
<sup>a</sup> Maternal blood*			• <sup>1,2</sup>		• <sup>2</sup>
<sup>a</sup> Cord blood*					• <sup>2</sup>
Meconium					•
<sup>a</sup> Colostrum/breastmilk					•
<sup>b</sup> Maternal saliva	•	•	•	•	•
<sup>b</sup> Dietary assessment <sup>++</sup>			•		
<sup>a</sup> Home air <sup>3</sup> sample*		•			
<sup>a</sup> Home composite dust sample <sup>3*</sup>		•			
Other home environmental samples <sup>3,4,*</sup>	Special studies	Special studies	Special studies	Special studies	Special studies
Outdoor environmental samples <sup>5,*</sup>	Special studies	Special studies	Special studies	Special studies	Special studies
<sup>a</sup> Questionnaire	•	•	•	•	•
<sup>a</sup> Ecologic analysis (e.g., GIS) <sup>3</sup>	•	•	•	•	•

Notes:

- a Measures that have been used in prior epidemiologic studies.
- b Measure that are more experimental or costly.
- 1. Blood collection piggy-back on glucose tolerance test. Blood samples crucial for PON1 status, ACHE.
- 2. Blood collection that is normal part of medical care. Blood samples crucial for PON1 status, ACHE.
- 3. We recommend that a composite dust sample be collected for each home lived in during pregnancy. Other environmental samples should be considered for special studies of selected participants.
- 4. For example, surface wipe.
- 5. For example, ambient air samples in agricultural area (see text).
- \* Media with existing laboratory methods for likely target pesticides (e.g., urine, dust, air, food).
- ++ Duplicate diet sampling, food frequency questionnaire, or other method (see text).

measure many different pesticide- and class-specific metabolites. Collection from adults is straight-forward, and pediatric urine bags can be used with very young children (in one study 88% of six month olds provided samples during assessments (Bradman, 2004). Urine, however, is an unregulated body fluid, and varies in concentration within individuals, resulting in variable metabolite concentrations (Wessels et al., 2003). (However, the urine of very young children (e.g., <12 months) may be less variable because they feed and urinate frequently). Creatinine-adjustment of urinary metabolites is a standard method for accounting for urine dilution; however recent studies suggest that creatinine adjustment in pregnant women and children may not be appropriate (Boeniger et al., 1993). Due to high intra-individual variability, spot urine samples, which are easy to collect, may not accurately reflect total daily pesticide excretion and may therefore misclassify exposure. To date, no studies have evaluated the ability of single or serial spot urine samples to classify daily or chronic pesticide exposure levels. Further, no studies have evaluated the ability of 24 hour urine samples to classify chronic exposures. However, several urinary validation studies are ongoing and should be published within the next two years. One recently published study suggests that first morning void samples are likely to more accurately represent total daily exposure (Kissel et al., 2004). Existing literature evaluating spot versus 24 hour urine samples for nutrients, renal function measures, and some toxicants are mixed (Boeniger et al., 1993; Brandle et al., 1996; Chitalia et al., 2001; Dong et al., 1996; Evans et al., 2000; Finco et al., 1997; Hinwood et al., 2002; Kawasaki et al., 1982; Kieler et al., 2003; Lee et al., 1996; Luft et al., 1983; Neithardt et al., 2002; Tsai et al., 1991; Woods et al., 1998). Additionally, metabolites in urine may reflect exposure to metabolites in the environment rather than the parent compound (Duggan et al., 2003; Wilson et al., 2003). For example, TCPy, the specific metabolite for chlorpyrifos, and several dialkyl phosphates, the class-specific metabolites for many organophosphates, have been found in food samples. (Wilson et al., 2003; Lu et al., in press).

**Table 2-3. Young Child Sample Collection for Non-Persistent Pesticide Analysis**

	Months					Years			
	3	6	9	12	18	2	3	4	5
<sup>a</sup> Urine <sup>1*</sup>	•	•	•	•	•	•	•	•	•
<sup>a</sup> Blood <sup>2*</sup>				•	•	•			
<sup>a</sup> Breast milk	•	•	•	•					
<sup>b</sup> Saliva <sup>3</sup>							•	•	•
<sup>b</sup> Dietary assessment <sup>++</sup>	•	•	•	•	•	•	•	•	•
<sup>a</sup> Home air sample <sup>4*</sup>	Each home/yr								
<sup>a</sup> Home dust sample <sup>4*</sup>	Each home/yr								
Other home environmental samples <sup>4,5,*</sup>	Special studies								
Outdoor environmental samples <sup>5,*</sup>	Special studies								
<sup>a</sup> Questionnaire		•		•	•	•	•	•	•
<sup>a</sup> Ecologic analysis (e.g., GIS) <sup>4</sup>	•	•	•	•	•	•	•	•	•

Notes:

- a Measures that have been used in prior epidemiologic studies.
  - b Measure that are more experimental or costly.
  - 1. Pediatric urine bag or diaper sample for non-toilet trained children. If not diaper, spot samples or multiple spots. Methods to measure pesticides in diapers under development.
  - 2. Blood collection at young age piggy-back on CDC recommended lead screen at 12 and 24 months. Blood samples crucial for PON1 status, ACHE.
  - 3. Choking hazard for saliva collection for children less than 3 years with current protocol.
  - 4. For each home lived in.
  - 5. For example, surface wipe, clothing dosimeter, hand wipe.
  - 6. For example, ambient air samples in agricultural area (see text).
- \*Media with existing laboratory methods for likely target pesticides (e.g., urine, dust, air, food).  
 ++ Duplicate diet sampling, food frequency questionnaire, or other method (see text).

CDC has developed laboratory methods for testing many parent pesticide compounds in blood (see **Chapter 4**). Because blood is a regulated fluid, pesticides in blood are likely to accurately reflect recent exposure, especially on a cross-sectional basis (Wessels et al., 2003). However, laboratory methods are not available for many pesticides in blood, including many with either specific- or class-specific metabolites in urine. Blood is invasive to collect. Collection, however, can be timed to coincide with medically scheduled blood collections, such as during the pregnancy glucose tolerance test (26 weeks gestational age), delivery, and during 12 and 24 month lead screens. Laboratory methods are also under development for pesticides in saliva and meconium, although validated methods are available for only a few compounds. Pesticides in saliva (depending on the protein-binding capacity) should reflect blood plasma and therefore recent exposure (Barr, 2003; Fenske and Lu, 2001; Lu et al., 1997; Lu et al., 1998). Current saliva collection methods, which use a cotton sponge, could pose a choking hazard to very young children. Meconium, the first bowel void of the newborn, is a concentrated mixture of swallowed amniotic fluid, cells, bile, and other materials, and likely accumulates in the third trimester. Measurement of pesticides in meconium could provide an integrated measure of fetal exposure in the third trimester (Ostrea et al., 2002; Whyatt and Barr, 2001).

Although biomonitoring provides direct measures of exposure, results may reflect transient, variable exposures. Additionally, the non-persistent compounds targeted by Hypothesis 2.1 have short half-lives in the body (hours to a few days). Thus, we expect high within-individual variability relative to between-individual variability in biological measurements, which may limit the ability of intermittent biological measurements to provide an effective cumulative exposure measure over a period of weeks, months, or years. Additionally, a single day biological measurement cannot be used to back-calculate the pattern and

duration of individual exposures. Measuring pesticides in proximate exposure pathways can confirm the presence or absence of an immediate exposure source, particularly for media where contaminants are stable over time. As noted in *Section 3.6*, pesticide levels in air and dust are often correlated, and, particularly in indoor air, stable over time except for short-term elevations immediately following an indoor application. Thus, measurements of pesticides in dust and air can be used to augment biomonitoring data. More detailed environmental assessments (e.g., wipes, clothing dosimeters, etc.) may be warranted for special studies. Finally, measurements of pesticides in ambient media, such as outdoor air, may be warranted in some circumstances. For example, measurements of outdoor air in agricultural areas with heavy pesticide use may provide additional information on exposure pathways (young children spend most of their time indoors at home; thus, exposure assessment should focus on this environment).

As discussed in *Section 3.6*, several studies suggest that diet is a major source of pesticide exposure to children. Approaches to dietary pesticide exposure assessment include both sample collection and testing and questionnaire-based evaluations. The key questions that these methods address are “how much and what types of food are being eaten” and “what are the pesticide levels in these foods when eaten? (after preparation and handling). Duplicate diet studies, usually over 24 hours, are the primary approach used to directly measure dietary pesticide exposure. These studies provide short-term cross-sectional exposure assessment, and may be especially useful for younger children less than six months of age with stable diets (breast milk, formula, some cereals). Duplicate diet studies may underestimate dietary exposure if study designs do not account for contamination of foods from indoor sources, such as handling of food by children who also contact contaminated surfaces or dust. Studies that involve food sample collection can place substantial burdens on participants, especially low-income families. Questionnaire methods, including the use of 24 hour food recall and food-frequency questionnaires and diaries can be used to estimate the types and amount of food individuals are eating. A number of federal programs also collect statistics on food consumption rates nation wide (including the Continuing Survey of Food Intake for Individuals (CFSII) and the National Health and Nutrition Examination Surveys (NHANES) (See Section 3.3.2.3). This information can then be linked to national food pesticide residue data (such as programs operated by USDA [<http://www.ams.usda.gov/science/pdp/>] and California [<http://www.cdpr.ca.gov/docs/pstrsmon/rsmonnu.htm#resimon>]) to estimate individual exposures. Finally, questionnaires can also be used to classify broad food consumption patterns that potentially relate to exposure and nutrition (e.g., the transition in young children from liquid to solid foods and consequent increasing consumption of potentially contaminated produce).

In summary, there is no single biological or environmental measure that can be used to classify pesticide exposure. Measurements of pesticides in first morning void urine samples (or possibly several spot or 24 hour samples) combined with tests of serial blood samples, possibly meconium and saliva, and environmental testing of home air and dust over critical life stages (pre-conception, pregnancy, birth, infancy, early childhood, and pre-school) should provide adequate data to test Hypothesis 2.1.

#### 2.1.4 Other Environments

As noted above, young children spend the majority of their time indoors at home. Pregnant women, however, may be in occupational environments outside the home into the third trimester and many children enter day care at young ages. As children get older, day care is more likely, and time in pre-school, kindergarten, and school is likely as children reach ages 3-6 (Tsang and Klepeis, 1996, Silvers et al., 1994, Wiley et al., 1991). Total exposure assessment methodologies might involve the collection of environmental samples in these locations. However, the effort and cost for these activities are likely beyond the scope of the NCS except for specialized studies. Questionnaire-based exposure assessment, potentially combined with ecologic methods such as GIS-based analyses, should be used to assess potential exposure-risk factors associated with these environments.

### 2.1.5 Potential Confounding Chemical Exposures/Effect Modifiers

Potential confounding chemical exposures/effect modifiers identified by the Exposure and Neurobehavioral Outcome Inter-Working Group 2-1 (December, 2002, Baltimore, MD)

- Chemical
  - Heavy metals (*e.g.*, Hg, Pb, Cd, As)
  - POPs (persistent organic pollutants including organochlorines) (see **Section 3.5**)
  - Possible endocrine disrupters include a wide variety of compounds, including some POPs. (see **Section 3.5**).
  - PAHs- polyaromatic hydrocarbons
  - Methanol/Solvents/VOCs-volatile organic compounds
  - Drugs of abuse (*e.g.*, ethanol, marijuana, cocaine)
  - Cigarettes and second hand smoke
  - DBP-disinfection byproducts of water purification
  - Carbon monoxide
  - Pharmaceuticals/ medications/ dietary supplements (positive and negative influences)
  - Drugs used during delivery

## 2.2 Astma and Exposures to Indoor and Outdoor Air Pollution

**Hypothesis:** *After adjusting for potential confounders, the incidence of asthma in children is positively associated with chronic exposures to air pollutants including PM, ozone, and other criteria air pollutants. In addition, exposures to air pollution exacerbates existing disease. Therefore, the number and severity of asthma attacks in children increases with chronic air pollutant exposures.*

### 2.2.1 Background

Asthma appears to develop early in life, with asthma incidence increasing dramatically by 75% between 1980 and 1994. The reasons for this increase are unknown, but may be related to chronic exposures to air pollutants or aeroallergens, which are thought to cause asthma as compared to acute exposures which are thought primarily to exacerbate existing disease (Sunyer, 2001).

To date, much of the epidemiological asthma research has focused on the acute effects of air pollution and aeroallergen exposures. Since the 1950's, researchers have consistently shown that acute air pollution (PM<sub>2.5</sub> and sulfur dioxide) exacerbates asthma and also may increase its incidence (Schwartz and Dockery, 1992; Pope et al., 2000). Furthermore, children who live near a busy road have been shown to be at increased risk of wheezing, a symptom of asthma (Venn et al., 2001). More recently, researchers have demonstrated associations between wheeze or asthma incidence and both dust mite (Platts-Mills et al., 2001) and cockroach allergens (Finn et al., 2000). Consistent with these findings, the asthma symptoms of adults exposed nasally to both diesel exhaust particles (DEP) and dust mite allergen were significantly worse than when exposures were limited to dust mite allergens alone (Diaz-Sanchez et al., 2000). In addition, much smaller-than-usual amounts of allergen caused symptoms when combined with DEP, which by themselves had no effect (Diaz-Sanchez et al., 2000). These results suggest that the combined effects of allergen and air pollutant exposures may be greater than either alone.

The long-term effect of these air pollutant exposures on asthma incidence and severity are not well understood. For air pollutants, the chronic asthma studies have shown increased prevalence of respiratory symptoms for areas with higher air pollutant levels (Sunyer, 2001). For example, a national (including 53

urban areas) cross-sectional study evaluated the association of citywide mean concentrations of total suspended particles (TSP), obtained from the U.S. EPA network of population based monitors, and chronic bronchitis in the National Health and Nutrition Examination Survey (NHANES). The difference in TSP between the adjusted means of the upper and lower quartiles was  $65 \mu\text{g}/\text{m}^3$ , and the odds ratio (OR) for bronchitis across this range of exposure was 1.6 after adjusting for age, race, sex and smoking (Schwartz, 1993). A longitudinal study of 3,914 non-smokers residing in California (The Seventh Day Adventist Study) estimated exposure to  $\text{PM}_{10}$  using monitoring stations linked to residential addresses. Statistically significant but small positive associations were observed between  $\text{PM}_{10}$  and severity of asthma, as well as with development of overall airway obstructive disease, chronic productive cough and increased severity of airway obstructive disease (Abbey et al., 1995).

These studies suggest that chronic exposures to air pollution increase the incidence and severity of asthma. However, further data are needed to examine this issue directly. In particular, the roles of chronic allergen and air pollution exposures on asthma need to be examined simultaneously, which will require the collection of new data and the prospective follow-up of children.

### 2.2.2 Exposures

There is now ample evidence that outdoor air pollutants exacerbate pre-existing asthma but the evidence for outdoor pollutants increasing the incidence of asthma or allergic diseases in children is limited (Brauer et al., 2001; von Mutius, 2001). An earlier study conducted in Kanawha Valley, WV showed the role of outdoor traffic-related VOCs in increased reporting of respiratory illness, and respiratory and asthmatic symptoms in pre-school children (Ware et al., 1993). A more recent study by Brauer et al. (2002) examined the role of air pollution from traffic and the development of respiratory infections and asthmatic and allergic symptoms in children living in the Netherlands. A birth cohort study of 4,000 children under 2 years of age was conducted in multiple regions of the Netherlands. This study showed positive associations between various respiratory symptoms and physician diagnosed asthma, and particulate matter and traffic pollutants (soot,  $\text{NO}_2$ ). A sensitivity analysis of the pollution and health data revealed a stronger association with traffic and asthma diagnosed before one year of age. (Brauer et al., 2002). A similar type of a study also conducted with a birth cohort of children under two years of age in Germany showed association between traffic-related air pollution and symptoms of cough (Gehring et al., 2002). These recent findings in young children support the importance of further assessing the role of outdoor particulates,  $\text{NO}_2$ , VOCs and motor vehicle pollutants, in either causing or exacerbating pre-existing asthma. Because the indoor risk factors for asthma such as house dust mite and cockroach and maternal smoking are already established (Nelson, 2000; Gold, 2000; Arshad et al., 1993), monitoring of exposures for the asthma hypothesis has to include a comprehensive indoor and outdoor measurement component. Moreover, a differentiation between different sources of particulate matter and air toxics will be critical in identifying the causal agents that are linked with either the development or exacerbation of asthma. In addition to characterizing the role of gene–environment interactions, it is essential to identify the role of indoor allergens, such as dust mites, mold and endotoxin, as part of the planned monitoring study design. A number of these indoor allergens and pollutants, such as ETS and gas combustion appliances, may either play a direct role or serve as an effect modifier in the study of environmental factors, which may initiate or trigger asthma. As a result, researchers should combine indoor and outdoor monitoring—relying upon either active or passive sampling for PM, elemental carbon,  $\text{NO}_2$ , VOCs,  $\text{O}_3$ —with time-activity diary information and with household source questionnaires for indoor pollution sources (e.g., cooking, cigarette smoke, etc.) to develop reliable estimates of personal exposure for the study participants.

## 2.3 Asthma and Exposures to Bioaerosols

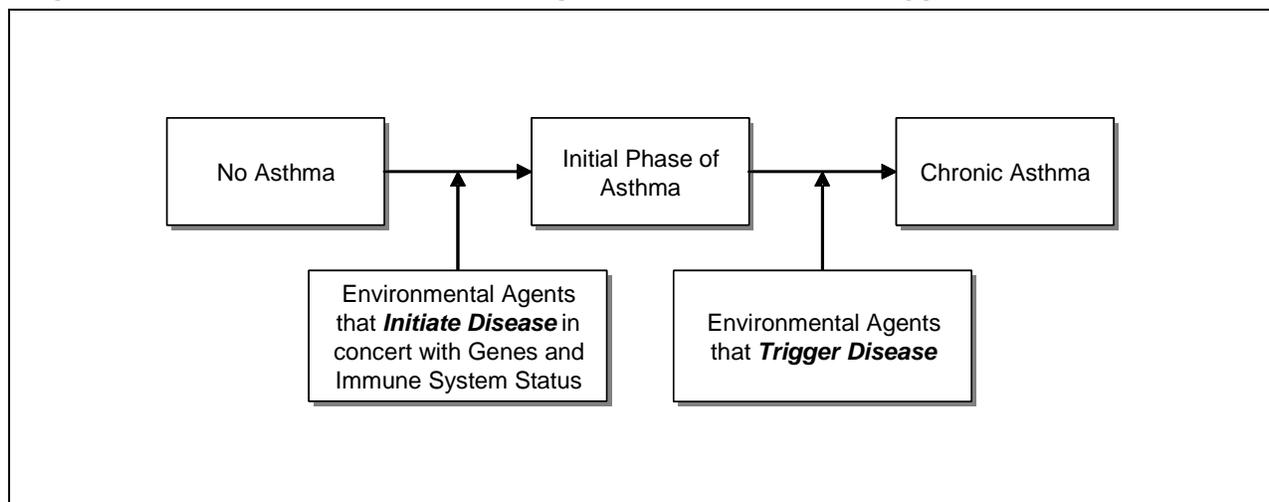
**Hypothesis:** Exposure to indoor and outdoor air pollution and bioaerosols (including allergens, endotoxins and mold) is associated with an increase in asthma.

### 2.3.1 Background

#### *Etiology*

Asthma afflicts the genetically pre-disposed; specific manifestations are shaped by environmental exposures and individual response. Some investigators believe environmental exposures early in life determine phenotypes (Th1 or Th2) of the CD4 lymphocytes affecting cell-mediated immunity. Exposures following birth are common in undeveloped nations and result in the Th1 phenotype. Th1 immunity may account for reduced asthma and atopy in developing regions of the world. By contrast, limited exposures may be experienced by infants born in developed nations. Then the enhanced Th2 phenotype experienced in utero and following birth persists. Humoral immunity is established with characteristic IgE production (IL 4 and IL 10) and proliferation and activation of eosinophyls. Persistence of the Th2 phenotype is associated with the development of atopy and asthma. Complex gene-environment interactions further complicate this scenario. Among people possessing the Th2 phenotype, genes recognizing a given environmental exposure vary. As a result, the same outcome (asthma/atopy) may be associated with different environmental agents across a population. Environmental exposures appear important in both the inception and attack/trigger phases of asthma. It is unknown whether agents identified as attack triggers for chronic asthma are the same environmental agents that initiate the disease. This leaves us with a dilemma –What environmental factors should be measured to evaluate the “cause” of asthma? *Figure 2-1* shows the initiation, progression of asthma and triggers of this disease.

**Figure 2-1. Role of Environmental Agents that Initiate and Trigger Asthma**



### 2.3.2 Exposures

There does not appear to be a single, universal exposure factor that initiates asthma; many exposures have been implicated. It is possible that any agent recognized by a genetically susceptible individual during critical development of the immune system may be implicated. Therefore almost every environmental agent known will have to be assessed and they vary widely by season, type and amount. Sources of bioaerosols of concern for asthma typically include house dust mites, endotoxin, arthropod and rodent excreta. There are two common house dust mites in the United States; *Dermatophagoides*

*pterronyssinus* (Dp) and *Dermataphagoides farinae* (Df). In tropical and subtropical environments, *Blomeia tropicalis* (Bt) may also be an issue particularly with recent immigrants from hot and humid areas. Sampling for these allergic agents is complex and assumes that the individual (child and mother/father) is genetically susceptible. Collection methods vary depending on the media and type of analysis as described further in **Section 3.2**. A number of measurement limitations also complicate characterization of exposures of concern for asthma. A number of the limitations and caveats are also provided with the additional information in **Section 3.2** tables.

### 2.3.3 Exposure Measures

As mentioned before, parental asthma and pre-natal exposures may play a role in the initiation of asthma. Exposures in early childhood to indoor allergens of concern (e.g., dust mites, mold, endotoxin, etc.) could alter immune system responses to either the initiators or triggers of asthma. Because indoor bioaerosol levels and their viability can vary seasonally, it is desirable to collect indoor air, dust and furniture/mattress/stuffed toy samples frequently over the course of a year. Ideally, quarterly samples, starting with pre-conception and through age 3, are recommended. Fewer annual samples after age 3 may be considered. **Tables 2-4** and **2-5** summarize the types of environmental and biological samples that should be collected for the study of environmental causes of asthma.

**Table 2-4. Pre-conception, Pregnancy, and Peri-natal Sample Collection for Allergen Analysis<sup>1</sup>**

	Precon-ception	Trimester			Peri-natal Period
		First	Second	Third	
Home air sample	•	•	•	•	•
Home surface dust sample	•	•	•	•	•
Home floor dust sample	•	•	•	•	•
Mattress, furniture, pillow, stuffed toys	•	•	•	•	•
Food-liquid					
Food-solid					
Biological and other samples (e.g., blood, serum for specific IgE, placenta, cord blood, skin test)	•	•			
Questionnaire <sup>2</sup>	•	•	•	•	•

Notes:

1. Primary person of interest is mother. A one time blood /serum analysis on the father for genetic analysis for polymorphisms and specific IgE should also be considered.
2. Questionnaires on indoor mold/mildew, pets, allergic sensitizations and seasonal allergies

**Table 2-5. Young Child Sample Collection for Allergen Analysis<sup>1</sup>**

	Months					Years			
	3	6	9	12	18	2	3	4	5
Home air sample	•	•	•	•	•	•	•	•	•
Home surface dust sample	•	•	•	•	•	•	•	•	•
Home floor dust sample	•	•	•	•	•	•	•	•	
Mattress, furniture, pillow, stuffed toys	•	•	•	•	•	•	•	•	•
Food-liquid	•*	•	•	•	•	•	•	•	•
Food-solid		•	•	•	•	•	•	•	•
Biological and other samples (e.g., blood, serum for specific IgE, placenta, cord blood, skin test)	•**	•**	•**	•**	•**	•**	•**	•***	•***
Questionnaire <sup>2</sup>		X		X	X	X	X	X	

Note:

1. Primary person of interest is the child
  2. Questionnaires on indoor mold/mildew, pets, allergic sensitizations and seasonal allergies
- \* Unknown. Possible breast milk specific IgE?  
 \*\* Blood/serum specific IgE  
 \*\*\* Skin test for specific IgE

## 2.4 Undesirable Outcomes of Pregnancy—Pre-conception Enrollment and Exposure Assessment

The text contributions in *Chapter 2* of this white paper are directly related to ICC core hypotheses. This section on undesirable outcomes of pregnancy—pre-conception enrollment and exposure assessment—is an exception. Although the ICC does not have a core hypothesis that directly relates to this topic, both pre and early conception exposure measures may turn out to be relevant to a number of the core hypotheses’ current outcomes. This section provides considerations for measures that could be implemented for women who are enrolled prior to conception. This pre-conception enrollment approach is suggested and supported in the January Environmental Health Perspectives papers referenced in this section from the Fertility and Early Pregnancy and Birth Defects Work Groups. This approach is also recommended by the NCS Sampling Workshop panel (report available on the NCS web site at: <http://www.nationalchildrensstudy.gov/events/workshops/samplingdesign032004.cfm>) and supported by the NCS Federal Advisory Committee.

The inclusion of a pre-conception cohort in the NCS is critical to understanding the effects of pre-conception and early pregnancy environmental exposures on adverse pregnancy outcomes. By definition, pre-conception exposures are exposures that occur prior to conception (i.e., the exposures to eggs and sperms before they form zygotes). At least two characteristics make pre-conception exposure assessment unique and challenging. First, both maternal and paternal exposure should be considered. Second, knowledge of both the timing of conception and the timing of exposure is crucial. In this section, we review available research on early pregnancy (i.e., < 5 weeks gestation) and pre-conception exposure assessment relevant to the study of health effects of environmental chemicals.

As identified by Chapin et al. (2004a), the chemicals of interest for the hypotheses related to undesirable outcomes of pregnancy are provided below. As mentioned before, this list is not necessarily complete, and decisions on what chemicals to include have not been made. The list does not imply that the NCS federal agencies have determined that these chemicals cause adverse human health risks (except as noted in separate regulatory actions, e.g., National Ambient Air Quality Standards, lead regulations, specific pesticide bans):

- Chemicals with  $t_{1/2} = <3$ days = phthalates (DEHP, DBP, butylbenzyl, dihexyl), organophosphates, phytoestrogens, triazines, vinclozolin and fungicides, bisphenol A, trichloropropane, formamide, acrylamide, and nonylphenol
- Chemicals with  $t_{1/2} = >3$ days are TCDD, PCB's, dieldrin and other OC's, and metals (Pb, Hg, As, Cd, etc.).

Assessing pre-conception and pre-natal exposures to these environmental chemicals will require the use of a range of exposure assessment tools, including questionnaires, environmental sampling, and biomonitoring. The ability to use a specific exposure tool, or mix of tools, to assess exposure to specific environmental chemicals depends on the nature of the chemical of interest. This section focuses on pre-conception exposures. Other sections in this white paper address methods to assess post-pregnancy pre-natal exposures (e.g., utility of questionnaires, environmental and ecological measures, and biomarkers).

The utility of questionnaires as an exposure assessment tool is covered fully in this white paper (refer to **Chapter 5**). There are strengths (e.g., ease of use, low cost, low burden on participant) and limitations (e.g., incomplete exposure picture, recall bias) to this approach to assessing exposure, but use of a well-crafted and validated questionnaire will be a part of a comprehensive exposure assessment strategy for a pre-conception cohort.

Environmental sampling will also play a role in an exposure assessment strategy for a pre-conception cohort. Specifically for chemicals that have physical properties or metabolic properties in human systems that make them poor candidates for biological sampling, environmental sampling is critical. The potential use of environmental sampling for the chemical exposures identified for these hypotheses is reviewed in **Chapter 3** of this white paper. Generally speaking, this sampling may include personal sampling (provides most subject-specific exposure data, but is very resource intensive and has highest burden on participant), area sampling in the home or work environment (provides less subject-specific exposure data, is resource intensive, but has a lower participant burden), or ambient sampling in the larger area or region (provides the least subject-specific exposure data, but requires the least resources and has the lowest participant burden).

Recent studies in different countries such as China, the Czech Republic, Brazil, Mexico, and the United States have shown a relationship between ambient air pollution and adverse birth outcomes, specifically low birth weight, intrauterine growth retardation, pre-term birth, and fetal mortality (Bobak and Leon, 1999; Bobak, 2000; Dejmek et al., 1999; Liu et al., 2003; Perera et al., 1998; Ritz et al., 2002; Perera et al., 2003; Pereira et al., 1998; Ritz et al., 2000; Ritz and Yu, 1999; Rogers et al., 2000; Wang et al., 1997; Woodruff et al., 1997; Xu et al., 1995). Similar findings have been found when the relationship was explored for residential exposures in rural Guatemala (Boy et al., 2002), in occupational settings (Chen et al., 2000), and when the exposure variable was proximity to traffic (Wilhelm and Ritz, 2003). Although many of these studies are from countries other than the U.S., including several from the developing world where environmental exposures are generally higher than what is commonly seen in equivalent study populations and environments in the U.S., the exposure and health effects associations, as well as the exposure assessment lessons learned from these studies, are important to the NCS in the current discussion. A variety of exposure assessment tools were used in these studies, including

questionnaires, ecologic city-wide regional environmental sampling, and residential environmental sampling. However, information on the magnitude and impact of personal pre-conception and pre-natal exposure to air pollutants on fetal growth and development were not assessed. Most of the etiologic studies investigating pre-conception exposures have been ecologic, lacking personal exposure measures. Etiologic epidemiology studies that focus more effectively on personal pre-conception and pre-natal exposure to chemicals (in the case of the examples above, air pollution) are necessary to advance the science of pre-conception and pre-natal chemical exposures and health effects.

Biological samples will also play a role in an exposure assessment strategy for a pre-conception cohort. The potential use of biological sampling for the chemical exposures identified for these hypotheses are reviewed in **Chapter 4**. Chapin and Buck (2004) argue for the need and feasibility of a pre-conception cohort for the NCS, with a particular focus on biological sampling as the primary exposure assessment tool. The authors make the point that adequate forethought and planning will result in a study that can shed new light on the earliest determinants of children's health and thereby fill data gaps. Specific to exposure assessment for a pre-conception cohort, the mini-monograph does a sufficient job of critically reviewing several of the critical issues, including: i) why pre-conception enrollment is important; ii) that pre-conception enrollment is entirely feasible; iii) that the collection of relevant data coupled with the sound analysis of specific end points will significantly increase our confidence in the answers (Chapin and Buck, 2004).

In the second article of the mini-monograph, Chapin et al. (2004b) review the evidence that shows the importance of the *in utero* environment (including chemical and hormonal levels) to the ultimate health of the child and even of the aging adult. The authors summarize the evidence that shows this impact begins with conception. The article goes on to discuss how “only a full life-cycle evaluation will help us understand these impacts, and only such an understanding will produce logically prioritized mitigation strategies to address the greatest threats first” (Chapin et al., 2004). The article concludes that “explicit assessment of such exposures and factors before and around the time of conception is indispensable for a real understanding of the determinants of health in our children, and, by extension, the next generation of adults” (Chapin et al., 2004). The limited literature available on the topic of pre-conception measurements of exposure and body burden is reviewed in this article. In discussing the needs and challenges of developing an adequate exposure assessment strategy for a pre-conception cohort in the NCS, the authors make the case that making explicit measurements of exposure and body burden in a couple before conception is necessary. In contribution to making this case, the authors present a review of articles that discuss the limited dependability of recall and the limitations of questionnaires. The authors also specifically focus on the need for making concurrent measurements of body burden because of the changing nature of xenobiotics. Citing MacIntosh et al. (1999), the authors discuss the phasing out or persistent organic pollutants and the subsequent introduction of xenobiotics with shorter half-lives, noting the fact that a biological sample taken in late pregnancy or after birth cannot be considered representative of *in vivo* levels at the time of conception or during early pregnancy. Specific to the exposure assessment-relevant issues, Chapin et al. (2004) conclude that “Only by collecting biological samples and actually measuring what the internal exposures are in women and men prior to and at the time of conception will we be able to definitively say which, if any, of these exposures are meaningful.”

In the third article of the mini-monograph, Buck et al. (2004), in an attempt to study the utility and feasibility of prospective pregnancy study designs, conducted a systematic review of the literature to summarize relevant information regarding the planning, implementation, and success of previously published prospective pregnancy studies. Specific to the exposure assessment-relevant issues, the authors report that in the studies they reviewed, a high percentage of women provided urine (57-98%) and blood (86-91%) specimens and most male partners (94-100%) provided semen samples. In summary, Buck et al. (2004) conclude that the data support the feasibility of this design.

In the fifth article of the mini-monograph, Rockett et al. (2004) present a study of the value of home-based collection of biospecimens in reproductive epidemiology. In this review, the authors examine biospecimens (urine and blood) that have been successfully collected from the home environment and also review related issues such as storage and transportation of biospecimens, and promising new approaches for collecting less frequently studied biospecimens (including hair follicles, breast milk, semen, and others). Specific to the exposure assessment-relevant issues, the authors conclude that, with caveats, the use of home-based collection of biospecimens in epidemiologic studies should assist investigators in obtaining high participation rates and thereby minimize exposure misclassification bias.

In summary, an exposure assessment scheme, in the context of pre-conception cohort, will include the use of questionnaires, environmental sampling, and biological sampling. Although a questionnaire may be used as an overall exposure assessment tool for all exposures of interest, the choice of when to use biological and/or environmental sampling to assess exposure will depend on the chemical of interest. In the case where the chemical is such that biological sampling is a feasible tool (e.g., nicotine, polychlorinated biphenyls, select pesticides), the collection of biological samples will allow the measurement of internal exposures in women and men prior to and at the time of conception, which will enable researchers to definitively say which, if any, of these exposures are meaningful (Chapin et al., 2004). But in cases where the chemical is such that biological sampling is not an option (e.g., particle and gaseous air pollutants), environmental sampling will be used to assess exposure.

## 2.5 Exposures to Environmental Agents Affecting Altered Age of Puberty

**Hypothesis:** *Pre-conception, in utero and subsequent exposure to environmental agents that affect the endocrine system results in altered age at puberty.*

### 2.5.1 Background

#### *Epidemiology*

There is recent evidence suggesting that puberty in U.S. children is starting at an earlier age compared to previous years (Herman-Giddens et al., 2001; 1997; Freedman et al., 2003; Anderson et al., 2003). This is of general interest because the extent to which this is occurring in this population has not been well characterized, and such findings have potential influences within our society. In cross-sectional studies of populations in the U.S. from 1988 to 1994, it is reported that the mean age of onset for breast development for girls was 9.5 to 9.7 years, which is about 1 to 2 years earlier than the observations of previous investigators (Lee et al., 2001). This approximates to 14% of the population achieving Tanner stage 2 or greater for breast development at the age of 8 years (Lee et al., 2001). In U.S. boys sampled from 1988 to 1994, the median age of onset for genital development ranged from 9.5 to 10.4 years, depending on race/ethnicity, which is about 1 year lower than that previously described (Herman-Giddens et al., 2001). This corresponded to about 32% to 58% of the boys attaining Tanner stage 2 at age 9 years. These observed changes in pubertal development are not isolated to the U.S., but have been noted in other countries as well (Parent et al., 2003; Proos et al., 1993; Huen et al., 1997; Viner et al., 2002; Karlberg et al., 2002). To properly define the significance of these findings to the population, researchers need more refined investigations for characterizing the time trend in pubertal development because of the limitations in study design of these prior reports (Lee et al., 2001; Viner et al., 2002; Reiter and Lee, 2001). Nutrition, genetic pre-disposition, and environmental chemical exposure are factors associated with pubertal change, and they can be evaluated during this process.

The early onset of puberty has clinical and social importance to the population. The early onset of thelarche is associated with an early diagnosis of breast cancer in susceptible populations (Hamilton et al., 2003) and adult obesity (Biro et al., 2003). Breast cancer is associated with an early age of onset of menarche as well. Girls attaining menarche after the age of 13 years were observed to have a one third decreased risk for breast cancer compared to those attaining menarche at a younger age (Garland et al., 1998). This effect was also characterized by defining a two year delay in the age of menarche to a 10% decrease in risk for breast cancer (Hsieh et al., 1992). These observations imply the need for surveillance and intervention when these people are identified. Additionally, such information can affect diagnostic criteria (Kaplowitz et al., 1999; Chalumeau et al., 2002). The social impact is that children will need to learn how to adapt to maturing bodies, and our public school system will need to re-evaluate the timing and structure of health education classes.

The factors associated with altered pubertal development are numerous, and they include nutrition, genetic susceptibility, and environmental chemical exposure (Parent et al., 2003). When the early onset of puberty was originally noted in immigrant children, it was attributed to improved nutrition and well-being. Although these remain as the primary factors determining the onset and progression of puberty, there are other considerations coming forward. These include genetic or host susceptibility and environmental chemical exposure.

The suggestion that susceptible populations exist according to pubertal development was noted among racial/ethnic groups (Wu et al., 2002; Anderson et al., 2002; Sun et al., 2002; Freedman et al., 2002). African-American girls were reported to develop either thelarche or pubarche at the age of 9.5 years, which was 1 to 2 years earlier than Non-Hispanic white girls (Herman-Giddens et al., 1997). This trend in race/ethnic groups for the age of onset for puberty was noted in boys as well (Herman-Giddens et al., 2001; Sun, 2002). Although obesity was associated with the early onset of puberty in these racial/ethnic groups, it was less of a determining variable in African-American girls--suggesting the involvement of other factors (Kaplowitz et al., 2001). In a study looking at serum leptin levels in girls aged 8 to 17 years, African-Americans were noted to have higher leptin levels than Caucasians--even after controlling for obesity, age and serum insulin levels (Wong et al., 1998). Leptin regulates fat distribution in the body and was shown to increase in serum level before gonadotropin levels in girls and boys (Garcia-Mayor et al., 1997). The signaling pathway for leptin in the development of puberty is not known, and further work is necessary to define this mechanism and the difference in leptin levels among racial/ethnic groups.

### *Environmental Chemicals*

Several environmental chemicals are known to have hormonal activity and their effects are well demonstrated in experimental laboratory studies (Goldman et al., 2000; Stoker et al., 2000). The hormonal effects of these chemicals can be categorized by estrogenic, androgenic, and thyroidal activity. Depending on the chemical's hormonal activity, they may either delay or accelerate the onset of puberty. The effects of these chemicals on the reproductive health of people are of a general concern to the community (Longnecker et al., 2003; Landrigan et al., 2003); however, they are largely unknown because this area of focus remains a relatively new area of investigation. The chemical class that is best described in this area is the polychlorinated biphenyls (PCBs). The PCBs and their hydroxylated metabolites are noted for their effects on the thyroid regulatory pathway which can affect neurodevelopment (Meerts et al., 2002; Jacobson et al., 1996). Aside from the PCBs, several other chemicals with hormonal activity are associated with health effects following exposure, and some of them are discussed below. (As noted elsewhere in this document, the discussion of these chemicals does not imply that the National Children's Study has determined that these chemicals either cause these health effects or that they will be included in the study.)

### *Lead*

Lead was recently observed to be associated with delayed age of onset of puberty in girls in the United States (Wu et al., 2003; Selevan et al., 2003). In a cross-sectional survey of 1,706 girls between the ages of 8 and 16 years from 1988 to 1994, increased blood lead levels were associated with a decreased likelihood for the attainment of either pubarche or menarche, but not for thelarche (Wu et al., 2003). Girls with lead levels in the ranges of 2.1 to 4.9 ug/dL and 5 to 21.7 ug/dL were about 50% and 80%, respectively, less likely to reach these measures of puberty than those with lead levels between 0.7 and 2.0 ug/dL. It was also observed in younger aged girls (8 to 12 years) that the percentage of them attaining pubarche by age decreased with increasing blood lead levels, and those who did not attain either pubarche or menarche had higher blood levels than those who achieved these pubertal endpoints. By age 13 years, nearly 100% of the girls attained puberty regardless of their blood lead level.

When the same population was analyzed by racial/ethnic groups, a higher blood lead level (3 ug/dL vs. 1 ug/dL) was associated with delayed onset of pubarche, thelarche and menarche in African-American girls (Selevan et al., 2003). The onset of thelarche was delayed by about 4 months and the attainment of the completion of thelarche by 2 months in African-American girls. Menarche was delayed by about 4 months. The increase in blood lead level was associated with a decreased likelihood (about 60%) for the attainment of a successive Tanner stage for the development of breast and pubic hair in African-Americans as well. Similar delays in thelarche and pubarche were observed in Mexican-American girls, except they were smaller in magnitude. There were no differences in pubertal outcomes in Non-Hispanic white girls in relationship to blood lead levels. Also, it was observed in this population that blood lead levels were different by racial/ethnic groups. The levels were highest in African-Americans (geometric mean, 2.1 ug/dL, [95CI: 1.9-2.3]) and lowest in Non-Hispanic whites (1.4 ug/dL, [1.2-1.5]), with the Mexican-Americans (1.7 ug/dL, [1.6-1.9]) being in the middle. Underlying differences in growth or hormonal regulation among the racial/ethnic groups may explain the observed pubertal effects in association with lead (Wong et al., 1998).

Chronic exposure to lead has been demonstrated to affect the hypothalamo-pituitary-gonadal axis by altering serum levels of gonadotropic and androgenic hormones. In occupational males, prolonged exposure at the workplace was associated with decreased serum testosterone (Rodamilans et al., 1988). Serum leuteinizing hormone level was found to be lower in both occupational males (McGregor et al., 1990) and children (aged 11 to 13 years) from the general population (Vivoli et al., 1993) with high lead levels. The effects of exposure to environmental lead on the levels of estrogens, androgens, and gonadotropins in younger aged children and their pubertal development remains to be determined. Particular attention needs to be paid to the timing of exposure during development and the contribution of race/ethnicity to such findings.

### *PBB*

Polybrominated biphenyls (PBB) were evaluated for their effect on puberty in girls born to mothers exposed to cattle contaminated with Firemaster™ in 1973 (Blanck et al., 2000). Firemaster™ was a fire retardant that was inadvertently mixed with cattle feed, and PBB 153 was the major congener. In a retrospective analysis, it was determined that there was a significant interaction between *in utero* and breastfeeding exposure and pubertal outcome. Girls born to mothers with high serum PBB levels ( $\geq 7$  ug/L) and who were breastfed, developed menarche about 1 year earlier than girls who were not breastfed. This finding was unaffected by the maternal serum PBB level of the girls in the latter group, which suggest the contribution of post-natal exposure to altered pubertal development. The estimated increased risk for the earlier onset of menarche was estimated to be about 3 to 4 in girls born to mothers with high PBB serum levels and who were breastfed compared to girls born to mothers with low PBB serum levels ( $\leq$  LOD) and who were not breastfed. The likelihood for the attainment of pubarche

increased for girls who were exposed to increasing *in utero* PBB levels and breastfed. There was a slight increase in likelihood for the early development of thelarche with increasing PBB levels. This effect was less pronounced than that of the other measures for pubertal development, which may be due to the small sample size of the study. The mechanism by which PBB affects pubertal development is unclear, but animal studies demonstrate PBB to be able to alter pubertal development (Harris et al., 1978); however, in a delayed fashion. PBB has been demonstrated to promote estrogen hepatic metabolism during the pre-inatal period (Bonhaus et al., 1981) and to decrease serum thyroid hormones (NTP, 1983; Meserve et al., 1992). The latter effect may contribute to the early development of puberty through altered hormonal regulation (Doufas et al., 2000; Weber et al., 2003).

### *PCB and Dioxin*

The PCBs and polychlorinated-p-dioxins (PCDDs) were demonstrated to be associated with altered pubertal development in boys and girls (Den Hond et al., 2002). In boys, genital and pubic hair maturation were about 3 times more likely to be at lower stages of development in association with increased serum PCB 138 and PCB 153 levels, respectively. In girls, breast development was about 2 times more likely to be at a lower stage of development in association with higher serum levels of dioxin-like compounds (i.e., coplanar and mono-*ortho*-PCBs, PCDDs, and PCDFs) with aryl hydrocarbon receptor activity. The mechanisms involved in these observations are undefined, but may be related to the estrogenic activity of PCB 153 (Wojtowicz et al., 2001) or the antiestrogenic effect of the dioxin-like chemicals (Safe et al., 1998). In 594 children who were recruited for the North Carolina Infant Feeding Study from 1978 to 1982, increasing PCB levels in human milk at birth (for the cohort, range: 0.5 to 5.5 ug/g lipids, median 1.7 ug/g lipids) were not associated with varying time to attainment of the Tanner stages in boys or girls (Gladen et al., 2000). However, increasing PCB milk levels were associated with increased body weight adjusted for height at the time of puberty in Caucasian girls.

### *Organochlorine Pesticides*

These chemicals were evaluated in two investigations. In a retrospective analysis of Belgian children diagnosed with idiopathic pre-cocious puberty, serum p,p'-DDE (a metabolite of DDT) was found to be higher in immigrant children compared to non-immigrant children (Krstevska-Konstantinova et al., 2001). About 75% of the immigrant children were from developing countries (e.g., Latin America, Asia, and Africa) and arrived by adoption, which was not significant as an independent variable when evaluating for differences in serum p,p'-DDE levels. Seven other organochlorine pesticides (DDT, lindane, heptachlor, aldrin, endrin, and hexachlorobenzene) were measured and found to be below the method's limit of detection in the study population. The authors suggested the contributory role of DDE to sexual pre-cocity in the foreign-born children because of the lack of an identifiable cause in this population. Further investigations are necessary to clarify the relationship between nutritional status and these chemicals. In the North Carolina Infant Feeding Study (see above), increasing DDE levels in human milk at birth (for the cohort, range: 0.3 ug/g lipids to 23.8 ug/g lipids, median 2.4 ug/g lipids) were not associated with varying time to attainment of the Tanner stages in boys or girls (Gladen et al., 2000). There was an association between increasing DDE milk levels at birth and increasing height and body weight at the time of puberty in boys. Endosulfan exposure was recently found to be associated with delayed pubarche and genital maturity in male children in a case-control study (Saiyed et al., 2003). The exposed children (n=117) lived near a cashew plantation that applied endosulfan by air, and they had higher total endosulfan serum levels (7.5 ug/L vs. 1.4 ug/L), lower serum testosterone levels adjusted for age and serum leuteinizing hormone level, and higher serum leuteinizing hormone levels adjusted for age when compared to children in the control group (n=90). These hormonal findings suggest a gonadal disorder. However, congenital genitourinary disorders were more frequent in the exposed children (5% vs. 1%), which may independently contribute to altered hormonal levels. The findings in this study need to be further investigated with populations of larger size and by controlling for confounders.

### *Phthalates*

The phthalate class of chemicals is known for their use as plasticizers in a variety of products, such as consumer products and medical devices. They can have estrogenic or androgenic activity, depending on the phthalate. Of the phthalates with estrogenic activity, their estrogenicity can be variable (Harris et al., 1997). Di-(2-ethylhexyl) phthalate (DEHP) is commonly used in soft plastic parts and has been demonstrated in experimental models to decrease ovarian estrogen production by a receptor mediated mechanism through its metabolite, mono(2-ethylhexyl) phthalate (MEHP) (Lovekamp-Swan et al., 2001). In a case-control study, girls diagnosed with pre-mature thelarche were found to have higher serum phthalate levels than their controls (Colon et al., 2000). Although this study suggests these chemicals affected pubertal development in the case population, further investigations are necessary to better clarify this relationship.

### *Phytoestrogens*

The phytoestrogens are naturally occurring environmental chemicals with notable estrogen activity, and they include classes of chemicals such as coumestan, isoflavones, prenyl flavonoids, and lignans. These chemicals are commonly found in fruits, vegetables and legumes (e.g., soybeans). In a case-control study of 120 pairs of children, the intake of soy-based formula increased the likelihood for the development of premature thelarche before the age of 2 years, but not in children diagnosed beyond this age (Freni-Titulaer et al., 1986). Thus, suggesting that various factors were most likely contributing to early breast development in this study population, depending on the age of onset. Other factors predictive of the diagnosis of premature thelarche in the younger age group included maternal history of ovarian cysts and the consumption of fresh chicken. Additional studies evaluating this source of exposure are necessary to define the significance of these observations.

Despite the findings of the various studies presented in the above section, the extent to which environmental chemicals contribute to changes in pubertal development in people remains largely unknown. This is because of the limitations in the designs of the investigations from which these observations were made, and the few number of chemicals studied to date that are related to populations. A focused investigation could attempt to answer some of the questions regarding environmental chemical exposure and maturation. For example, what is the significance of the timing of exposure to a chemical during a child's growth on pubertal development? Current studies in this area do not allow for such conclusions because of either their cross-sectional sampling or retrospective analysis.

The observations from either cross-sectional or retrospective study designs presume the study participants in the population were exposed to the chemical at the same time during their development, and that the only difference in the magnitude of their exposure is reflected in the level of the chemical in the biological matrix. An example of where the latter situation becomes challenging is with human milk. When infant exposure is assessed from human milk consumption, both the changing level of the chemical in the milk during the breastfeeding period and the duration of breastfeeding must be considered. The timing of exposure to a chemical is very difficult to ascertain from these study designs. This is because the random sampling of blood or urine from an individual does not allow for the determination of when the exposure occurred, which could have been at any time prior to the procurement of the specimen. However, exposures to apparent events (e.g., Seveso, Italy industrial explosion in 1976) in combination with elevated levels are exceptions to this. For a persistent chemical, this "timing" is more of a problem than for a non-persistent chemical because of its long half-life. This can make it difficult to assess for either the occurrence of subacute exposures or the source of the exposure when location has changed. The lack of consideration for these concerns can lead to either misclassification of a study participant for a critical window of exposure, or increased variance in the population for a given effect.

These study design issues are best resolved with a prospective longitudinal design, which can be accomplished for environmental chemicals during the evaluation of other factors more closely associated with pubertal development. Nutrition and genetic pre-disposition are important contributors to growth and maturity requiring further investigations. Although it is generally accepted that nutrition affects pubertal development, there remain unresolved issues because this association is variable among studies and recent evidence suggests gestational size contributes to puberty as well (Luo et al., 2003). The extent to which birth height and body mass index relates to nutritional status in determining the timing of puberty is not known. As more work is done to resolve such questions, it would be important to control for environmental chemical exposure.

### 2.5.2 Strategies for Testing Hypothesis

#### *Sampling Periods*

The timing for the collection of exposure data (environmental, questionnaire, and biological) depends on two general factors, including the adverse health effect being monitored and the environmental chemical that is under evaluation. In the evaluation of sexual maturation, it is important to monitor for the periods during the formation of the sexual organs (*in utero*), when a significant amount of chemical exposure is expected (e.g., breastfeeding [postnatal]), those before (pre- and peri-puberty) and at the completion of puberty. These are discussed to a greater extent elsewhere (Pryor et al., 2000; Lemasters et al., 2000). The recruitment of participants during pre-conception will ensure that exposures in the first trimester of gestation will be characterized, and data collected at this time may serve as a baseline comparison for throughout the pregnancy and the postpartum period.

The successful completion of sexual maturation is dependent on proper anatomic development of the organs and hormonal regulation leading to their function. The anatomic development of organs begins during pregnancy or the *in utero* period. Organogenesis occurs in the first trimester (fourth to eighth week) of gestation and disruption during this time can lead to disorders that may be apparent at birth or not until adulthood. Some notable examples of this include diethylstilbestrol (DES) and thalidomide. DES was used in the early 1940s to 1950s for the treatment of irregular vaginal bleeding and threatened abortions, and is associated with the occurrence of cervical or vaginal clear cell adenocarcinoma in the F1 generation when exposure was before 18 weeks of gestation. Thalidomide was used in the late 1950s to treat nausea and vomiting associated with pregnancy. It causes severe limb malformations in the newborn when exposure occurred during days 34 to 50 of gestation.

The *in utero* exposure to stimuli leading to altered pubertal development was demonstrated in animal experiments using intra-uterine artery ligation and various chemical treatments. Intra-uterine ligation during gestation was used to simulate malnutrition in a rat model, and it was shown to cause a delay in the onset of puberty in males and females (Engelbregt, 2000). Bisphenol A (Howdeshell et al., 1999) and octylphenol (Wright et al., 1999; Bogh et al., 2001) treatments in animals were demonstrated to advance puberty by shortening the time to either the first estrus or its equivalent. However, octylphenol did not alter testicular size in boars that were exposed to octylphenol as fetuses (Bogh et al., 2001). Atrazine delayed mammary gland development in female rats when they were treated with atrazine either as fetuses during gestational days 15 to 19 or during lactation (Rayner et al., 2004). In the same model, vaginal opening was delayed only in rat pups that were exposed to atrazine by lactation. Phytoestrogens, such as genistein (Casanova et al., 1999) and the lignan containing flaxseed (Tou et al., 1998), advanced the time of onset of puberty in female rats when high doses were initiated during the gestational period and continued postnatally. Of note, when flaxseed was administered at a lower dose, it delayed the onset of puberty (Tou et al., 1998). There were no observed pubertal effects (ie., time of onset, testicular weight) in males in these phytoestrogen models. The effects of *in utero* exposure to environmental chemicals on pubertal development were evaluated in several epidemiological studies, and for the PCBs

and DDE, this route of exposure was compared to that from breast-feeding in one study (Gladen et al., 2000). The significant difference between these two exposure periods is that the amount of chemical delivered to the infant from lactation is usually greater than the amount of chemical delivered to the fetus by the placenta.

During the in utero period, the fetus should be assessed for exposures that coincide with the developmental windows of the primary and secondary sexual organs (Pryor et al., 2000) (see *Tables 2-6a* to *2-6d*). For example, mammary gland development begins at about the 4th week and continues through the 10th week of gestation. The primary mammary bud is formed in this period. From the 10th through 15th week of gestation there is the formation of secondary buds, the nipple, and the areola. The critical window of development for the external genitalia is from the 7th through 12th week of gestation. Because the diagnosis of pregnancy is uncommonly made early in the first trimester, it is recommended to recruit study participants prior to conception to ensure that this critical period during gestation is not missed.

Once the sexual organs are formed, their activation for reproduction is determined by hormonal (gonadotropins, estrogens, androgens) regulation. In females, this is largely determined by estrogenic activity and in males by androgens. In the prepubertal female, estrogen levels are low and stable until puberty, when there is a surge in estrogen levels, resulting from gonadotropin stimulation. The pituitary gland begins to secrete leuteinizing hormone at about 1 to 3 years before the onset of puberty. The prepubertal period is an important time to assess for environmental chemical exposure because there have been several reported cases of girls developing premature thelarche after being exposed to estrogen compounds (Teilmann et al., 2002). Thus, the sampling periods would include in utero (gestation), postnatal (for breast-feeding infants), before puberty (pre- and peri-), and post-puberty (or the completion). It is important to monitor puberty to its completion because the time of onset of puberty and the duration for its completion may vary independently. Further definitions of the frequency for monitoring during the prepubescent period may be gained from reviewing the longitudinal studies on puberty (Lee et al., 1980; Roche et al., 1995; Biro et al., 1995).

### *Susceptible/Vulnerable Populations*

Certain populations may be susceptible or vulnerable to environmental chemical exposure and they will need to be considered in the study design. The reasons are variable and include genetic pre-disposition, nutrition, and socioeconomic factors. For example, people frequently ingesting fish or whale meals are likely to have elevated levels of PCBs, PCDDs, and PCDFs. This was observed in people both residing near the Great Lakes region of the U.S. (Gerstenberger et al., 2000), and who are members of the Inuit tribe in Canada (Ayotte et al., 1997). Children residing in homes built before 1946 were found to have approximately a 5 fold increased incidence of blood lead levels greater than or equal to 10ug/dL compared to children residing in homes built after 1973 (Pirkle et al., 1998). Low-income status and African-American race/ethnicity were associated with elevated blood lead levels ( $\geq 10\text{ug/dL}$ ) in families living in older housing.

### *Environmental Chemicals*

There are many environmental chemicals observed to have endocrine or hormonal activity (NRC, 1999); however, only a few have been associated with reproductive health effects in people. These include lead (Selevan et al., 2003; Wu et al., 2003), polybrominated biphenyls (Blanck et al., 2000), polychlorinated biphenyls (Den Hond et al., 2002; Gladen et al., 2000), PCDDs/PCDFs (Den Hond et al., 2002), DDT (Krstevska-Konstantinova et al., 2001; Gladen et al., 2000), endosulfan (Saiyed et al., 2003), phthalates (Colon et al., 2000), and phytoestrogen (Freni-Titulaer et al., 1996). To encompass all of these chemicals into the assessment strategy, the chemicals can be categorized as either persistent or non-persistent. Generally, persistent chemicals have long half-lives (months to years) and are measured in the

**Table 2-6a. Pre-conception, Pregnancy, and Peri-Natal Sampling Periods for Persistent Chemical Exposure Assessment**

	Pre –Conception <sup>3</sup>	In utero	Peri-natal Period
Urine <sup>*, ++</sup>	M/F <sup>1</sup>	M (10-15wks <sup>2</sup> )	M
Serum <sup>*</sup>	M/F	M (10-15wks <sup>2</sup> )	M
Whole blood <sup>*</sup>	M/F	M (10-15wks <sup>2</sup> )	M
Hair <sup>*</sup>			M
Human milk <sup>*</sup>			2wks – 2mos post-partum
Cord serum <sup>*</sup>			•
Cord whole blood <sup>*</sup>			•
Meconium <sup>*</sup>			•
Dietary assessment <sup>+</sup>	•	M (10-15wks <sup>2</sup> )	•
Home air sample <sup>*, +</sup>	•	•	•
Home composite dust sample <sup>*, +</sup>	•	•	•
Other environmental samples <sup>*, +</sup>	Special studies	Special studies	Special studies
Questionnaire <sup>+</sup>	•	•	•
Ecologic analysis (e.g., GIS) <sup>+</sup>	•	•	•

Notes:

1. Mother (M), Father (F).
  2. E.g., the 10wk to 15 wk period is a critical period for breast development.
  3. At the time of enrollment.
- \* Media with extant laboratory methods for likely target chemical.
- + Environmental sampling, dietary assessment, questionnaires, and ecological analysis are necessary for persistent chemicals to determine either route or pathway of exposure; otherwise, exposure can be established through the analysis of biological specimens (see text). Information should be obtained proximate to the sampling period for biological specimens.
- ++ Timed specimen collection. Morning void specimen with creatinine measurement.

**Table 2-6b. Children Sampling Periods for Persistent Chemical Exposure Assessment**

	Post-Partum (18 to 24mos.)	Pre-puberty (5 to 6 yrs.)	Mid-puberty	Post-puberty
Urine <sup>1, *, 3</sup>	•	•	•	•
Whole Blood <sup>2, *</sup>	•	•	•	•
Serum <sup>2, *</sup>	•	•	•	•
Dietary assessment <sup>++</sup>	•	•	•	•
Home air sample <sup>++, *</sup>	•	•	•	•
Home dust sample <sup>++, *</sup>	•	•	•	•
Other environmental samples <sup>++, *</sup>	Special studies	Special studies	Special studies	Special studies
Questionnaire <sup>++</sup>	At 6, 12, 24 and 36 months, and then annually until puberty.			
Ecologic analysis (e.g., GIS) <sup>++</sup>	•	•	•	•

Note:

1. Pediatric urine bag or diaper sample for non-toilet trained children. If not diaper, spot samples or multiple spots.
  2. When blood collection is at a young age, piggy-back it on CDC recommended lead screen at 12 and 24 months.
  3. Timed specimen collection. Morning voided specimen with creatinine measurement.
- ++ Environmental sampling, dietary assessment, questionnaires, and ecological analysis are necessary for persistent chemicals to determine either route or pathway of exposure; otherwise, exposure can be established through the analysis of biological specimens (see text). Information should be obtained proximate to the sampling period for biological specimens.
- \* Media with extant laboratory methods for likely target chemical.

**Table 2-6c. Pre-conception, Pregnancy, and Peri-natal Sampling Period for Nonpersistent Chemical Exposure Assessment**

	Pre –Conception <sup>3</sup>	In utero	Peri-natal Period
Urine <sup>*, ++</sup>	M/F <sup>1</sup>	M (10-15wks <sup>2</sup> )	M
Serum <sup>*</sup>	M/F	M (10-15wks <sup>2</sup> )	M
Human milk <sup>*</sup>			2wks – 2mos post-partum
Cord serum <sup>*</sup>			x
Meconium <sup>*</sup>			x
Dietary assessment <sup>+</sup>	x	M (10-15wks <sup>2</sup> )	x
Home air sample <sup>*, +</sup>	x	x	x
Home composite dust sample <sup>*, +</sup>	x	x	x
Other environmental samples <sup>*, +</sup>	Special studies	Special studies	Special studies
Questionnaire <sup>+</sup>	x	x	x
Ecologic analysis (e.g., GIS) <sup>+</sup>	x	x	x

Notes:

1. Mother (M), Father (F).
  2. E.g., The 10wk to 15 wk period is consistent with breast development.
  3. At the time of enrollment.
- \* Media with extant laboratory methods for likely target chemical agent. Researchers may need multiple samples of biological specimens during this time because of possible variability in biological exposure level due to the short half-life of the chemical. This would be particularly important if a critical exposure period was being considered. Alternatively, pilot data may demonstrate the lack of need for frequent sampling if there is constant exposure in a stable environment.
- + Environmental sampling, dietary assessment, questionnaires, and ecological analysis are necessary (in addition to biological monitoring) for nonpersistent chemicals to determine exposure. Such information can be used to determine either route or pathway of exposure as well. Information should be obtained proximate to the sampling period for biological specimens.
- ++ Timed specimen collection. Morning voided specimen with creatinine measurement.

**Table 2-6d. Sampling Periods for Children for Nonpersistent Chemical Exposure Assessment**

	Post-partum (18 to 24mos.)	Pre-puberty (5 to 6 yrs.)	Mid-puberty	Post-puberty
Urine <sup>1, *, 3</sup>	•	•	•	•
Serum <sup>2, *</sup>	•	•	•	•
Dietary assessment <sup>++</sup>	•	•	•	•
Home air sample <sup>++, *</sup>	•	•	•	•
Home dust sample <sup>++, *</sup>	•	•	•	•
Other environmental samples <sup>++, *</sup>	Special studies	Special studies	Special studies	Special studies
Questionnaire <sup>++</sup>	At 6, 12, 24 and 36 months, and then annually until puberty.			
Ecologic analysis (e.g., GIS) <sup>++</sup>	•	•	•	•

Note:

1. Pediatric urine bag or diaper sample for non-toilet trained children. If not diaper, spot samples or multiple spots.
  2. When blood collection is at a young age, piggy-back it on CDC recommended lead screen at 12 and 24 months.
  3. Timed specimen collection. Morning voided specimen with creatinine measurement.
- ++ Environmental sampling, dietary assessment, questionnaires, and ecological analysis are necessary (in addition to biological monitoring) for nonpersistent chemicals to determine exposure. Such information can be used to determine either route or pathway of exposure as well. Information should be obtained proximate to the sampling period for biological specimens.
- \* Media with extant laboratory methods for likely target chemical. Researchers may need multiple samples of biological specimens during this time because of possible variability in biological exposure level due to the short half-life of the chemical. This would be particularly important if a critical exposure period was being considered. Alternatively, pilot data may demonstrate the lack of need for frequent sampling if there is constant exposure in a stable environment.

blood. Although this may seem advantageous from a sampling perspective (i.e., wide window of opportunity), it does pose a problem when critical windows of exposure are of concern and subacute exposures may have occurred. Thus, if there are critical periods during a child’s development, then a specimen should be obtained proximate to that time. Questionnaires and environmental assessment would be necessary if the pathways of exposure are important because biological monitoring would not be able to identify this or the exposure location. For example, the presence of a persistent chemical in the blood of a person who just moved to a new location would most likely represent an exposure from the former

location. However, this would not be known by just the blood test. If documentation of exposure of a persistent chemical is all that is necessary, then biological monitoring should be adequate. Non-persistent chemicals have short half-lives (hours to days) and are measured in the urine (see *Chapter 4*). For the non-persistent organic chemicals, increased reliance is needed on questionnaires and environmental samples to document their exposure. This is because of the relatively short duration of time these chemicals exist in the body.

### 2.5.3 Measurements

#### *Exposure Assessment*

The choice of the biological matrix to measure for the chemicals is largely determined by the properties of the chemical (e.g., lipid solubility, protein binding capacity, and ionic charge), which govern the distribution of the chemical in the body as well. Blood and urine are the standard specimens used for the measurement of chemicals, and water soluble chemicals are commonly quantified in urine. In addition, the target organ or organism needs to be considered. For example, if the interest is in fetal exposure, then cord blood is the preferred specimen. If the route of exposure is important, say to determine either the source of exposure, or the degree to which one source may be more important in determining a health outcome if two are involved, then several matrices will have to be sampled. For example, an infant's exposure can come from either during the in utero period through the placenta or from breastfeeding. However, neither the amount of chemical exposure nor the determined health effect is consistent by either of these routes of exposure. In the case of PCB exposure, although it was determined that breastfeeding contributed to a larger amount of exposure to the infant than by placental transfer, it was the latter that was deemed more consequential to delayed neurodevelopment (Jacobson et al., 1996). If the extent to which a chemical partitions into various biological matrices is known, then alternative specimens may be used if the desired specimen is not adequate. For example, maternal blood, cord blood, and maternal milk may be used to assess for fetal exposure to persistent organic chemicals. Meconium is a biological matrix that is currently under investigation as an indicator of chemical exposure. The interest in this matrix is that it has the potential to reflect chemical exposures to the fetus from as early as the third trimester.

Researchers may need to customize questionnaires and environmental sampling based on the usage patterns of particular classes of chemicals. Phthalates, for example, are commonly found in personal care products (e.g., cosmetics, shampoos); therefore, researchers should ask specific questions regarding the use of these products (Tiwary, 1998; Tiwary and Ward, 2003; Li et al., 2002). Similarly, changes in location throughout the life stages (home, school, work site) may warrant adjustments of these assessment tools.

#### *Biological Markers of Health Effect*

Biological markers should be used to monitor for the onset and completion of puberty. Various tools can be used; including biochemical (e.g., hormones), physical examination, and diagnostic scans (see *Table 2-7*). Dual energy x-ray absorptiometry (DXA) is a noninvasive method to assess body fat mass, body fat-free mass, and bone mineral mass and density for skeletal maturity. These items were discussed at the NCS inter-workgroup meeting on "Obesity and physical development" are listed in tabular format. This meeting was co-chaired by the Nutrition, Growth, and Pubertal Development and Exposure to Chemical Agents workgroups at Baltimore, MD, on December 17-18, 2002.

**Table 2-7. Health Outcome Measures to Assess for Pubertal Development**

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<b>1. Onset of puberty</b>
Sexual maturity rating (Tanner staging)
a. Performed by observer, by self-reporting
b. Frequency—annually
Stature
Hormones (LH, estradiol, testosterone, dehydroepiandrosterone-sulphate [DHEA-S])
a. Salivary hormone levels (-need validation)
b. Thyroid stimulating hormone
<b>2. Later stages of puberty</b>
a. Voice pattern change (males)
b. Menarche
c. Menstrual history
d. DXA (dual energy X-ray absorptiometry)-bone density analysis (hand, wrist, forearm) for skeletal maturity. At age 6 and 12 years for females, and at 6 and 14 years for males.

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#### 2.5.4 Confounders

There are several factors that either are known to cause or are associated with altered pubertal development, and they need to be considered in the design of such studies. These items are listed in **Table 2-8**.

#### 2.5.5 Conclusion

There is evidence that nutritional status, genetic predisposition (race/ethnicity), and environmental chemical exposure are associated with altered age at puberty. The assessment for exposure to environmental chemicals that may affect the attainment of puberty in the developing child is challenged by several factors, including the various time frames when an exposure can affect the maturation process, the limited access to certain biological specimens during select periods of human development, and the many chemicals with varying properties. The recommended approach is to conduct the assessment by lifestages (i.e., in utero, postnatal, peri-pubertal). Study participants need to be recruited at pre-conception to ensure that the “critical window” of gestation or the first trimester is included in the exposure assessment process. Chemicals should be categorized by biological persistence, and biological specimens that are most likely to yield meaningful information collected and stored. The latter will allow for the judicious use of specimens of limited quantity for special investigations (e.g., nested case-control studies), and time to develop new and improved analytical methods. For chemicals that either can not be measured in biological specimens or have short biological half-lives, the analysis of environmental samples and use of questionnaire data are necessary to complete the assessment for their exposure. Food and dietary data may assist in determining the extent to which nutrients and chemicals from this pathway contribute to the variance in the timing of puberty. Factors that are either known causes of or associated with altered pubertal development need to be controlled for during the assessment of health outcomes. The National Children’s Study is uniquely poised to evaluate the effects of environmental chemicals on the age at puberty and the above approach will allow it to accomplish this task.

**Table 2-8. Confounders to Pubertal Development**

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1.	Precocious Gonadotropin-dependent (central precocious puberty) <ul style="list-style-type: none"> <li>– Brain pathology (e.g., hypothalamic hamartoma, tumors, hydrocephalus, severe head trauma)</li> <li>– Hypothyroidism, untreated</li> </ul> Gonadotropin-independent precocious puberty <ul style="list-style-type: none"> <li>– McCune-Albright syndrome in girls</li> <li>– Familial male precocious puberty (Testotoxicosis)</li> <li>– Tumors (ovarian, adrenocortical, Leydig cell, chorionic gonadotropin-secreting tumors)</li> <li>– Exogenous pharmaceutical estrogen or androgen use</li> </ul> Isolated pre-mature thelarche, pre-mature pubarche/adrenarche, pre-mature menarche.
2.	Delayed Poor nutrition Acute illness Chronic illness Constitutional growth delay Intense physical training
3.	General categories associated with altered pubertal development General nutrition General health Brain injury Obesity Social economic status Immigration / adoption Race/ethnicity Pharmaceuticals (estrogenic, androgenic, estrogen blocker [i.e., aromatase inhibitors], androgen blocker [e.g., finasteride, flutamide]) Genetics Gestational age

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## 2.6 References for Chapter 2

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### 3. Methods for Measurement of Exposures and Candidate Environmental Chemicals and Agents

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#### 3.1 Measurement of Airborne Particulate Matter, Gaseous Pollutants and Air Toxics

##### 3.1.1 Particulate Matter

Overviews of measurement techniques for the purpose of exposure assessment are provided in two WHO guidance documents (WHO/IPCS, 2000; WHO/SDE, 2001) and in Rodes and Wiener (2003) and Rodes and Thornburg (2004). ACGIH (2001) provides comprehensive but concise descriptions of approaches to sampling and analysis of air contaminants in general and particulate matter sampling methods specifically. Willike and Baron (2003) present a more exhaustive treatment of particulate matter sampling and analysis. At present, active sampling is the most proven way to perform exposure assessments of particulate matter with accurate size cut. Active particle samplers operate by drawing aerosols into a sensor or on to a collection surface (e.g., a filter) by means of a pump (Hinds, 1982; Lehtimäki and Willeke, 1993). Large stationary samplers that operate with a standard flow rate of approximately 16.7 liter/min are available commercially and are useful for collecting large sample volumes. Small stationary samplers that operate with flow rates in the range of 1-10 liter/min are also commercially available. Both sampler sizes are available in configurations that allow for sampling of specific size fractions (e.g., PM<sub>2.5</sub> or PM<sub>10</sub>). Low-flow coarse particle (2.5-10 mm) monitors should also be investigated. Personal aerosol samplers that allow collection of particulate matter of specific size fractions are also available.

Impaction is the most commonly used method of collecting particles. Impactors rely on inertial forces to separate particles based on aerodynamic diameter. Air is accelerated through a nozzle or jet and then forced to make a 90° turn around an impaction plate before passing through a filter and exiting the sampler. Depending on their size, particles suspended in the air stream pass through the acceleration nozzle and then either remain entrained in the flow or collide and are retained on the impaction plate. The cut-point of an impactor is determined by the flow rate, jet size and shape (e.g., the distance between the jet and the impaction surface) (Pastuska, 1988; Lehtimäki and Willeke, 1993). The airflow rate must be calibrated carefully because correct size selection depends largely on precise flow rates.

Filters are made either from fiber mats of glass, cellulose, quartz or mixed fibers, or from synthetic membranes (e.g., Teflon). The selection of appropriate filters depends on the pump, filter static pressure, collection efficiency, extraction and analytical requirements, and the potential for sampling artifacts. Filter mass is determined by weighing the filter under controlled temperature and humidity conditions before and after use following a conditioning period of at least 24 h at those same conditions. The collected mass can be extracted and analyzed for chemical composition. The extraction and analysis procedures used depend on the analytes of interest. A recent summary of methods for extraction and analysis of components of particulate matter may be found in Koutrakis and Sioutas (1996).

Certain gases present in air may react with chemicals present in particles. For example, ammonia gas present in the sample or air stream can neutralize sulfuric acid particles collected on filters. The preferred sampling approach to avoid such artifacts is to use a denuder to remove the reactive gas before it reaches the downstream filter. In the case of sulfuric acid monitoring, a citric-acid-coated denuder is used to remove the ammonia gas. Small denuder systems are commercially available for personal monitors. Denuder technologies are described in Lodge (1988) and Koutrakis and Sioutas (1996). Another artifact

occurs when the target air contaminant is present in both the vapor- and particle-bound phases (for example, airborne PAHs with 5 phenyl rings or less), so that a significant portion of the semi-volatile compound is lost when using only a filter for collecting particles. This artifact is addressed by placing a sorbent bed downstream from the filter to collect the vapor phase. Typical sorbent beds include materials such as polyurethane foam and XAD-2.

Passive PM monitoring has been made possible based on the light scattering properties of particles. The nephelometers integrate the scattered light in such a way as to respond to the mass median aerodynamic diameter ( $d_{p50}$ ) primarily between 0.1 and 1  $\mu\text{m}$ , with a sharp decrease in light scattering response for  $d_p$  beyond this range. The nephelometer measures the light scattering coefficient,  $B_{sp}$ , with an optical filter defined at a specific wavelength (e.g., 530 nm for the Radiance nephelometer and 880 nm for the Thermo-MIE DataRAM) and a variable-rate flashlamp. Commercially available light scattering instruments have data logging capabilities to store  $B_{sp}$  measurements at a rate of 1  $\text{s}^{-1}$ . In the past several years, many exposure assessment studies have evaluated the passive light devices against the aforementioned gravimetric monitors and demonstrated the advantages and disadvantages of these real-time devices (Brauer, 1995; Chang et al., 2000; Quintana et al., 2000, 2001; Williams et al., 2000; Muraleedharan and Radojevic, 2000; Rea et al., 2001; Liu et al., 2002).

Methods for measuring particles are summarized in *Table 3-1* and *Table 3-2* (see also *Appendix A*). Stationary indoor-outdoor monitoring has minimal burden but lacks the specificity of personal monitoring. Personal monitoring may be inconvenient and/or require an unacceptable (or unaffordable) number of technician visits. The issue of child compliance with personal monitors is also important. Although the acceptable burden level changes with child age, most systems may be too heavy and cumbersome for use on children and require technicians to deploy/collect. Similarly, integrated measurements provide a physical sample for speciation of particulate matter, but do not provide estimates of temporal variability. Continuous measurement will provide temporal variability, but no physical sample. Active MIE nephelometer with final filter is the only available combination system but the burden is too high for child personal exposure use. This system could be used as an indoor monitor or as an outdoor monitor although this application would be limited due to the relative humidity artifact unless an upstream desiccant system were installed.

### 3.1.2 Other Criteria Pollutants: Gases

#### *Passive Samplers*

Commercial passive samplers are available for a variety of air pollutants, including inorganic gases such as carbon monoxide, nitrogen dioxide, sulfur dioxide and ozone. Passive air samplers are probably the most convenient tools for conducting large-scale personal exposure assessments because they are small, inexpensive, and can be used, with proper instruction to participants, in applications across all ages of children for both ambient and indoor air monitoring. However, sampling rates are of the order of 10-50 ml/min and the limit of detection is usually high. In addition, face velocity affects the effective collection rate for most passive samplers, i.e., insufficient air movement in most indoor environments may create a starvation effect, whereas too much wind in the outdoor environment may result in over sampling (Koutrakis et al., 1990; Liu et al., 1992). To obtain a detectable signal, passive samplers must be exposed for lengthy times and thus often miss unusually high concentrations, which are key exposure events that trigger health effects. In addition, prolonged sampling may contribute to analyte losses from instability of the analytes on the collection media. Passive samplers operate on the principle of molecular diffusion. The rate of diffusion is related to the diffusion coefficient of the compound, the cross-sectional area of the absorbing surface and the length of diffusion path. Manufacturers can provide specific information on the calculation of sampling rates. The samplers for inorganic gases rely on reaction of the contaminant with a

**Table 3-1. Summary of Collection Methods For Gaseous Pollutants and Particulates in Air**

Contaminant	Method Type	Sampler Type	Range of Quantification	Sampling Location
Carbon monoxide (CO)	Gas Filter Correlation Ambient CO Analyzer	Active	0.1 - 100 ppm	Indoor/outdoor
	Personal Air Control (Pac III) Tedlar bags, GC, reduction gas detector Activated charcoal tube, color change Solid adsorbent, GC/FID Diffusion type dosimeter	Active Active Active Active Passive Passive	0.30 ppm - 30 - 1,600 ppm-h 1 mg/m <sup>3</sup> (0.87 ppm) -	Personal Outdoor Personal Personal/indoor/outdoor Personal (workplace)/indoor /outdoor
Nitrogen dioxide (NO <sub>2</sub> )	Personal Air Control (Pac III)	Active		Personal
	Sodium arsenite, colorimetry	Active	0.005 - 0.15 ppm	Indoor/outdoor
	Sodium arsenite, colorimetry, continuous	Active	0.005 - 0.15 ppm	Indoor/outdoor
	Chemiluminescence after conversion of NO <sub>2</sub> to NO	Active	0.25 (0.005 ppm - MDL) - 25 ppm	Outdoor
	UV differential optical absorption spectrometry (DOAS)	Active	0.004 - 0.5 ppm	Outdoor
	TGS-ANSA Method	Active	0.008 - 0.15 ppm	Indoor/outdoor
	Triethanolamine (TEA)-impregnated molecular sieve, IC	Active	0.19 ppm (3-L air) -	Indoor (workplace)
	Palmer-type diffusion tube, spectrophotometry	Passive	0.01 µg NO <sub>2</sub> per sample (0.150 ppm-h) -	Personal/Indoor/outdoor/workplace
	Ferm badge, FIA	Passive	0.05 - 200 ppm	Outdoor
	Ogawa badge, TEA, IC	Passive	N/A	Indoor/outdoor/personal
CSPSS badge, CHEMIX™, spectrophotometry or CFA	Passive	0.0001 - 0.050 ppm (1 month),	Outdoor/(indoor)	
Triethanolamine (TEA), IC	Passive	0.5 µg/m <sup>3</sup> (1 month), 15 µg/m <sup>3</sup> (24 hr) -	(Indoor)/outdoor	
Yanagisawa-type sampler, TEA, Spectrophotometry	Passive	N/A	(Indoor)/outdoor	
Ozone (O <sub>3</sub> )	Chemiluminescence O <sub>3</sub> analyzer, ethylene	Active	0.002 - 0.5 ppm	Outdoor
	Chemiluminescence O <sub>3</sub> analyzer, ethylene, Rhodamine B organic dye	Active	0.002 - 0.5 ppm	Outdoor
	UV Photometry	Active	0.002 - 1 ppm	Ambient (outdoor)
	UV differential optical absorption spectrometry (DOAS)	Active	0.0015 - 0.5 ppm	Outdoor
	Personal sampler using a diffusion denuder	Active	0.045 ppm -	Personal
	Active, Impregnated glass fiber filter, IC-UV	Active	0.03 ppm (90-L air) -	Personal
	Bubbler, Vis spectrophotometry	Active	N/A	Outdoor
	Filter in a plastic tube, Vis spectrophotometry	Passive	0.003 mg/m <sup>3</sup> (1 wk) -	Outdoor
	UV spectrophotometry	Passive	N/A	Outdoor
	Reflectance spectroscopy (color analyzer)	Passive	0.03 ppm (24 hr) or 0.001 ppm (1 month) -	Outdoor
	Diffusion, IC-Conductivity	Passive	0.017 ppm (12 hr) or 0.008 ppm (24 hr)	Personal, outdoor
	Diffusion, IC-UV	Passive	~ 0.017 - (12 hours)	Personal, outdoor
	Diffusion, IC-UV	Passive	0.003 - 1 ppm for 1-day exposure; 0.0001 - 0.14 ppm for 1 month exp.	Outdoor
	I <sub>2</sub> /Nylon-6 charge-transfer complex, coulometry	Passive	0.4 ppm-h - 1.4 ppm-h	Outdoor
	Rubber cracking	Passive	~0.060 ppm (24 hr)	Outdoor (forest or agricultural areas)
Personal Air Control (Pac III)	Active		Personal	

**Table 3-1. Summary of Collection Methods For Gaseous Pollutants and Particulates in Air (continued)**

Contaminant	Method Type	Sampler Type	Range of Quantification	Sampling Location
Sulfur dioxide (SO <sub>2</sub> )	Pararosaniline method, Spectrophotometry	Active	25 µg/m <sup>3</sup> (0.01 ppm) - 1,130 µg/m <sup>3</sup> (0.43 ppm) for short-term sampling 13 µg/m <sup>3</sup> (0.005 ppm) - 590 µg/m <sup>3</sup> (0.23 ppm) for long-term sampling	Indoor, outdoor
	UV Fluorescent SO <sub>2</sub> analyzer	Active	0.0004 - 1.0 ppm	Ambient
	UV differential optical absorption spectrometry (DOAS)	Active	0 - 0.5 ppm or 0 to 1.0 ppm	Outdoor
	Gas wash bottle method (H <sub>2</sub> O <sub>2</sub> , IC)	Active	0.8 µg/m <sup>3</sup> -	Outdoor
	Filter pack method (NaOH, IC)	Active	0.08 µg/m <sup>3</sup> -	Outdoor
	Sulfation measurement with lead dioxide	Passive	N/A	Outdoor
	Permeation, spectrophotometry	Passive	N/A	Outdoor
	Permeation, ion-exchange chromatography	Passive	2 µg/m <sup>3</sup> (1 month)	Outdoor
	NaHCO <sub>3</sub> + Na <sub>2</sub> CO <sub>3</sub>	Passive	N/A	Indoor (workplace)
	TEA, IC	Passive	0.7 µg/m <sup>3</sup> (1 month), 21 µg/m <sup>3</sup> (24 hr) -	Outdoor
Low dose diffusive sampler (NaOH, IC)	Passive	0.2 µg/m <sup>3</sup> (1 month)	Outdoor	
PM <sub>10</sub> /PM <sub>2.5</sub>	Gravimetric methods - exchangeable filters	Active		Ambient
	Beta Attenuator Methods	Active	~ 2 µg/m <sup>3</sup> (1 hr) -	Ambient
	Tapered Element Oscillating Microbalance (TEOM) Methods	Active	~ 5 µg/m <sup>3</sup> (5 min) -	Ambient
	Real-Time Total Ambient Mass Sampler (RAMS)	Active	~ 5 µg/m <sup>3</sup> (1 hr) -	Ambient
	Continuous Ambient Mass Monitor System (CAMMS)	Active	~ 2 µg/m <sup>3</sup> (1 hr) -	Ambient
	Piezoelectric Microbalance	Active	10 µg/m <sup>3</sup> (1 min) -	Ambient
	Personal Microenvironmental Aerosol Speciation Sampler (PMASS)	Active		Personal/Microenvironment
	ChemPass Personal Sampling System	Active		Personal
	Triplex CycloneSCC1.062 Triplex Cyclone	Active		Personal/Ambient
	GK2.05 (KTL)/GK2.05 SH (KTL) Cyclone	Active		Personal/Ambient
Personal Microenvironmental Monitor (PEM)	Active		Personal/Ambient (Indoor)	
URG's Personal Sampler for Particulates/Pesticides	Active		Personal/(Ambient)	

**Table 3-2. Summary of Collection Methods for Metals in Air**

Contaminant	Size Cut	Sampler Type	Sampler Type	Collection Media	Suitable Sampling Location
Total Metals	TSP	Filter based sampler	Active	Quartz filter	Outdoor
Total Metals	TSP	Filter based sampler	Active	same as above	Outdoor
Total Metals	TSP	Filter based sampler	Active	Whatman EPM 2000 borosilicate glass microfiber filter	Outdoor
Total Metals	TSP	Filter based sampler	Active	Whatman-41 filter (12.5 cm diameter)	Outdoor
Total Metals	TSP	Filter based sampler	Active	25-mm filters: Glass fiber, 1.0 um, PVC, 5.0 um, or Teflon w/ PMP support, 3.0 um	Outdoor, Indoor, Personal
Total Metals	TSP	Biomonitor	Passive	plants	Outdoor
Total Metals	TSP	Filter based sampler	Active	8"x10" quartz filter	Outdoor
Hg	TSP	Filter based sampler	Active	6-mm quartz fiber filter disc supported by Ni-screen	Outdoor, Indoor, Personal
Pb	PM <sub>10</sub>	Immunoassay	Active	fiberglass filter	analytical alternative
As, Pb, Cd, Cr	PM <sub>10</sub>	Filter based sampler	Active	Teflon	Outdoor, Indoor, Personal
Pb, As, Cd, Cr, Ni	PM <sub>10</sub>	Filter based sampler	Active	Teflon	Outdoor, Indoor, Personal
Total Metals, esp. Pb	PM <sub>c</sub> and PM <sub>2.5</sub>	Filter based sampler	Active	?	Outdoor
Total metals	PM <sub>2.5</sub>	Filter based sampler	Active	25-MM Teflon filter	Outdoor
Total metals	PM <sub>2.5</sub>	Filter based sampler	Active	46.2-mm Teflon filter	Outdoor
Total metals	PM <sub>2.5</sub> , PM <sub>1</sub> , PM <sub>10</sub> , or TSP	Filter based sampler	Active	Partisol filter	Outdoor
Total metals	PM <sub>2.5</sub> , PM <sub>1</sub> , PM <sub>10</sub> , or TSP	Filter based sampler	Active	Series 1400a filters	Outdoor
Mn, Al, Ca, Mg	PM <sub>2.5</sub> and PM <sub>10</sub>	Filter based sampler	Active	N/A	Outdoor, Indoor, Personal
Mn, Al, Ca, Mg	PM <sub>2.5</sub> and PM <sub>10</sub>	Filter based sampler	Active	N/A	Outdoor

chemical coating on the collection surface. Selection and use of passive samplers should take into consideration potential sources of error such as wind effects, temperature, humidity and interfering gases.

In practical applications, personal monitoring is performed by mounting the passive sampler on a participant's collar to estimate air pollution concentrations in the breathing zone. For exposure of infants to indoor air, a passive sampler could be mounted in the sleeping room or near the crib and in other areas most frequented. For exposure of infants in ambient air, the sampler could be mounted on a stroller and capped off after each outing. A questionnaire would accompany each passive sampler to document events. After collection, the adsorbent is removed from the sampler and extracted with the recommended solvent. The extract is then analyzed by a suitable method (e.g., ion chromatography, spectrophotometry, gas chromatography with specific or unspecific detectors, HPLC, etc.). As with any monitoring procedure, measures should be taken to evaluate sample preservation and integrity. These procedures should be described as part of the quality assurance (QA) protocol and the standard operation procedures (SOPs).

Although meteorological and other ambient air conditions cannot be considered pollutants or chemicals, their influence on a subject's general health and degree of response to air pollutant exposures should be considered in the NCS. It is recommended that data on several of the following potential stressors be recorded: temperature, relative humidity, pressure, solar radiation (including ultraviolet exposure), radon, noise, and possibly radio-frequency waves.

### *Active Samplers*

There are many commercially available liquid-media samplers for reactive and soluble gases, such as liquid-containing bottles, and solid-sorbent tubes for insoluble and non-reactive gases and vapors, such as activated charcoal, silica gel, porous polymers or other materials. Pollutants are transported with the carrier gas (air), and are captured by collecting media. The most frequently applied mechanisms in the collection of air pollutants in these media are chemical reactions (e.g., acid-base and color-forming), and absorption/adsorption of the pollutant molecules on collecting media. Solid sorbent collection efficiency depends on contacting surface area, airflow rate, temperature, humidity and presence of interfering compounds.

The sampling rate, breakthrough volume and method limit of detection are important parameters, which need to be considered for an accurate exposure assessment by active samplers. The identification and quantification of collected air pollutants are usually performed by analytical instruments, such as spectrophotometers, gas chromatographs with specific or non-specific detectors, HPLC, etc. Analysis is typically done by gas chromatography following thermal desorption. Although not yet used extensively, small, evacuated canister samplers have been developed for personal monitoring (Pleil and Lindstrom, 1995). These have the advantage of not using sorbents.

### *Direct Reading Instruments*

The concentration of gases and vapors (e.g., carbon monoxide, sulfur dioxide) in an individual's breathing zone can also be determined with the use of portable direct-reading instruments. Commercially available direct-reading instruments have data logging capabilities to store measurements at a rate of  $1 \text{ s}^{-1}$ . Depending on the frequency of measurements, these instruments can operate up to 2 weeks continuously. Instrument software allows for direct calculation of concentrations with different averaging times and statistical analysis of the data.

Methods for measuring criteria pollutants are summarized in **Table 3-1** (see also **Appendix A**)

### 3.1.3 Air Toxics

#### VOCs

There are two basic approaches for sampling VOCs: active sampling and passive sampling. Active sampling requires the use of air-moving devices or pumps to draw a sample of air from the atmosphere. Passive sampling approaches use the principle of diffusion as described by Fick's law (Palms and Lindenboom, 1979), so that vapor or gas-phase chemicals can be trapped in a sorbent material as a result of a concentration gradient; therefore, passive methods do not require the use of air pumps. Each of these approaches has inherent advantages and disadvantages, as **Table 3-3** summarizes. The limitations of active methods are mainly associated with the use of air pumps, which limits their use for personal sampling. Passive devices are limited by the range of VOCs they can collect effectively and their sampling rates. The current method of analysis for VOCs involve separation by gas chromatography and detection by either multiple detectors (e.g., FID/ECD) or by mass spectrometry (MS).

**Table 3-3. Advantages and Disadvantages of Active and Passive Sampling Methods (VOCs)**

Characteristic	Active	Passive
Environments where suitable	Central site; microenvironmental residential indoor and outdoor; personal only for individuals > 12	Central site; microenvironmental residential indoor and outdoor; personal only for individuals > 6
Range of VOCs	Broad for whole air sampling Depends on collection medium for active sorbent sampling.	Limited (depending on device/sorbent)
Sensitivity	High for whole air sampling Depends on collection medium and method of desorption for active sorbent sampling. Presence of VOC background in the sorbent may increase detection limits.	Limited by sampling rate and mode of desorption of the sorbent prior to analysis. Lower sampling rates can be compensated by longer sampling periods. Presence of VOC background in the sorbent may increase detection limits.
Specificity	High (depends on analytical method)	High (depends on analytical method)
Participant burden	High because of use of sampling pumps that the participant has to carry over long periods of time. High potential for impact on recruitment and retention. Not suitable for personal monitoring for use by children <12	Low. Participant burden is limited to replacing the device when clothes are changed. Low impact on recruitment and retention. Suitable for personal monitoring of children > 6.
Ease of operation/personnel training/level of field activity	Moderate to high. Samplers require careful calibration prior to and after sampling. There is an increase risk of loss of samples due to pump malfunction.	Low. Do not require calibration. Minimal personnel training is necessary. Have the potential for being provided to the participants with minimal training so they can place and collect them.
Level of prior experience for VOC monitoring	EPA reference methods are active and there are detailed SOPs	Moderate. Experience has been developed over the last 10 years.
Cost	Higher	Lower

The use of mass spectroscopy as the preferred method of analysis offers multiple advantages, including lower detection limits for some compounds, unique identification of each VOC, and the potential for identification of VOCs not initially included in the sampling and analysis protocol. Active sampling methods can be classified into two main categories: whole air sampling and sorbent collection (ACGIH, 2001). Whole air sampling consists of the collection of a volume of air inside a vessel that can later be transported to a laboratory, where the air sample can be run through a trap in which the VOCs can be condensed by cryogenic cooling. The condensed VOCs can then be injected directly into a gas chromatogram. Thus, there is little dilution of the sample and little impact from background contamination. The nature of the collection vessel affects the volume to be collected as well as the integrity of the sample. The state of the art collection devices are made of stainless steel and the inside walls are either glass or have been treated to minimize wall reactions. The volume can vary from relatively large (6 liters) to 500cc, with the latter being suitable for personal monitoring. These vessels are available commercially.

Sorbent sampling involves drawing a sample of air through a bed of a granular material that adsorbs the VOCs present in the air stream. Sorbents vary by chemical composition and available surface area with these variables affecting the range of VOCs that can be sampled as well as the amount that is absorbed. Harper (2000) and Dettmer and Engewald (2002) contain excellent and concise summaries of sorbent sampling methods. **Table 3-4** presents a summary of different VOC classes and the appropriate sorbent materials (see also Appendix A). Typically, a sorbent is packed within a glass tube through which air is drawn at a known rate to allow for adsorption of the VOC on the sorbent. A tube can be packed with separate beds of different sorbents to allow the measuring of a broader range of VOCs within a single sample. There is a broad range of commercially available single and multiple sorbent sampling tubes on the market (see for example, SKC Inc., PA). The sensitivity of sorbent methods can be affected by the presence of one or more target chemical in the sorbent material or the release of a VOC from the material during sampling or analysis. A well known example of the latter is the release of benzene from TENAX during thermal desorption, which increases the limit of detection for benzene significantly when using this sorbent. In addition, reactions can occur between the target compounds and other pollutants present in air, oxidants in particular. Some sorbents, specifically the activated carbons, have to be desorbed with the use of solvents which dilutes the sample and impacts the method detection limit. Thus, selection of the appropriate sorbent(s) and the sampling rate and duration are critical considerations when using these devices. Active sorbent approaches are more suitable for personal monitoring than whole air sampling with evacuated vessels or cylinders because they are small and light and less burdensome to the participant.

Passive sampling methods for VOCs are the most suitable for personal monitoring from the participant burden perspective, and they can also be used for microenvironmental and central site monitoring. They are small and very light in weight, do not require calibration, and most individuals can place and collect the samplers with minimal training. Some of these samplers have been evaluated and used in exposure studies (Chung et al., 1999a; Chung et al., 1999b; Wiesel et al., 2004). The main limitations are the relatively low sampling rates compared to active devices, so that longer sampling times may be required. Beyond sampling rates and because the VOCs are trapped in a sorbent, passive samplers have comparable limitations to sorbents for active sampling. Passive monitoring is generally less expensive than active monitoring.

Recoveries of analytes of interest from both active and passive samplers may be of concern particularly when the sampling duration is long (e.g., over 12 hours) or the vapor pressure of the compound of interest is large (e.g., 1,3-butadiene). Spiking with deuterated species of the same compounds helps quantify the recoveries and determine potential sampling problems. In addition, VOC collection may be affected by the presence of water vapor in the sampling environments for certain sorbents (e.g., activated charcoals) because water molecules tend to occlude adsorption sites.

### *Aldehydes*

Although airborne aldehydes are considered members of the VOC class from a technical standpoint, they are usually considered separately because they require different sampling and analysis methods from other hydrocarbons. Similar to VOCs, there are active and passive sampling approaches for carbonyl compounds such as aldehydes. The traditional methods, both active and passive, involve the well known classical reaction between the carbonyl moiety and dinitrophenyl hydrazine (DNPH), which produces hydrazone derivatives of each specific carbonyl compound. Unlike sorbent methods, capture of the aldehyde is mediated through a chemical reaction rather than physical sorption or adsorption. These derivatives can be separated by high-performance liquid chromatography (HPLC) and detected by UV/VIS absorption. Both active and passive samplers currently being sold (e.g., SKC, Inc.; GMD, Inc.) coat the DNPH on a granular support such as C<sub>18</sub> (active sampling) or on a fibrous or Teflon pad (passive sampling). The limitations of the method include the background of hydrazones present in the DNPH-

**Table 3-4. Summary of Collection Methods for Volatile Organics in Air**

Contaminant	Method Type	Sample Type	Range of Quantification	Sampling Location
Volatile Organic Compounds	Passivated canister sampling (EPA ref. methods TO-12, TO-14 and TO-15)	Active	0.01ppb to < 1 ppm level (with injection dilution) MDL varies by compound	Indoor/outdoor/personal (with MiniVac)
	VOCs condense as they flow through a trap cooled to $\approx$ - 50 C.	Active	0.1-200ppb	indoor/outdoor
	Granular sorbent material in a tube or cartridge adsorbs VOCs.	Active	MDL varies by compound and is strongly dependent of sorbent background.	Indoor/outdoor/personal
	Granular sorbent materials in a tube or cartridge adsorb VOCs.	Active	MDL varies by compound and is strongly dependent of sorbent background.	Indoor/outdoor/personal
	VOCs diffuse as a function of concentration gradient and are adsorbed on a sorbent.	Passive	ppb to ppm levels	Personal/indoor/outdoor
Carbonyl compounds	Compounds with a carbonyl moiety such as aldehydes and ketones react with DNPH in an impinger solution to produce compound-specific derivatives amenable to separations by HPLC and detection by UV/VIS.	Active	1-50ppbv	Indoor/outdoor
	Compounds such as aldehydes and ketones react with DNPH coated on a polymeric support (typically C18) contained within a tube or cartridge.	Active	0.5 to 100 ppbv	Indoor/outdoor/personal
	Compounds with a carbonyl moiety such as aldehydes and ketones react with DNPH coated on a fiber or filter support glass support	Passive	0.05 - 100 ppb	Indoor/outdoor/personal
	Compounds with a carbonyl moiety such as aldehydes and ketones react with DNSH coated on a C18 support	Passive	0.01 - 100 ppb	Indoor/outdoor/personal
Nicotine	Airborne nicotine diffuses to a filter coated with sodium bisulfate	Passive	0.01 - 100 ppb	Indoor
PAHs	Air is drawn through a filter followed by a sorbent bed to collect particle-bound and gas phase PAHs.	Active	0.5 - 500 ug/m3	Indoor/outdoor

coated materials which impact the detection limits of the method, potential interference from reactions with ozone present in air (a KI containing cartridge can be placed in front of an active sampling cartridge to remove this artifact), and the unstable nature of DNPH-based methods to sample acrolein (Liu et al., 2001). More recently, the reaction with dansylhydrazine (DNSH) has been proposed as an alternative approach (Zhang et al., 2000). DNSH also reacts with the carbonyl moiety to produce specific hydrazones that can be separated by HPLC but detected by fluorescence rather than UV/VIS absorption. The advantage of this method is that it is more sensitive because of the use of fluorescence detection, and that is more suitable for monitoring acrolein. The disadvantage is that the method is still considered a research tool and there are no commercially available samplers, so the active or sampling devices have to be prepared in the laboratory. The advantages and disadvantages with respect to passive or active approaches are similar to those described for VOCs.

### *PAHs*

PAHs can be present in air in either or both the particle-bound and gas phases, depending mainly on their molecular weight/saturated vapor pressure and, to a lesser extent, on environmental conditions. For this reason, appropriate sampling devices require the collection of both airborne particles and gas-phase compounds using an active approach. The current state-of-the-art method involves collection of the particle-bound PAHs on a pre-fired quartz filter, followed by a pre-cleaned sorbent plug, commonly polyurethane foam (PUF) placed downstream from the filter. The sorbent collects PAHs that were present originally in the air in the gas phase, as well as the portion of PAHs in the particle-bound phase collected on the filter that are re-entrained in the flow during sampling. The filter and sorbent are extracted and processed separately, and analysis is typically performed by gas chromatography with MS detection. The limitations of the method are the time and resources required for pre-treatment of filters and sorbent plugs, and the extensive sample extraction and cleanup prior to analysis. Analyte recoveries and breakthrough problems are also negative considerations. The method is suitable for microenvironmental sampling both indoors and outdoors. There are microscale cassettes/sorbent cartridges that can be used for personal monitoring, but these have not been used to any extent to date.

## 3.2 Measurement of Indoor Allergens and Endotoxin

Sampling for environmental agents linked with either cause of asthma or involved in the trigger of asthma requires sampling of indoor air at home and collecting floor and surface dust samples for analyses of dust mites, mold, endotoxin and other arthropod or rodent antigens. Furniture, mattress and stuffed toy samples should also be collected at routine intervals to assess potential sources of greater exposures to a young child. The subjects for these investigations are either assumed to be susceptible based on genetics or blood/serum/skin test specific IgE screening. Most of the samples require collection by a technician using active pump sampling techniques. Unfortunately, some of these methods lack uniform protocols and depend on the skill level of the technician. Inhalation is the most important route for exposure for indoor bioaerosols. Floor and surface dust measurements are useful when these media are possible sources of dust mites. Likewise, furniture, pillows, mattress and stuffed toys could also be reservoirs for dust mites. Because young children contact these surfaces extensively, they need to be screened frequently over the first 3-5 years of life. Analysis of the samples collected may involve immunochemical assays. Biological samples, such as blood, serum, and breast milk, from the mother or the child are considered to be important biomarkers of either susceptibility or exposure. **Tables 3-5** and **3-6** summarize the types of measurements for assessing exposures to dust mites, endotoxin, arthropod and rodent bioallergens, by person of interest (mother, father or child), by life stage (pre-conception, conception to birth, birth to 3 months, 3 months to 3 years, 3 years and above), and by media and route of exposure. Limitations of available measurement methods and other caveats or concerns are provided in these tables and their footnotes.

**Table 3-5. Measurement Methods by Life Stage, Media, and Route for House Dust Mites and Endotoxin Exposures**

Age Class	Person of Interest	Media	Importance of Media for Exposure	Route	Importance of Route for Exposure	Importance of Route to Outcome	Limitations of Measurements	Collection Method	Concerns*	Refs		
Prior to Conception	Mother	Air	High- stimulates maternal immune system response	inhalation	High	Possible*	Point in time (PIT)—varies seasonally	Actively pumped sample: Glass liquid impinger	a, b			
		Water	n/a					Possible-media containing mites			PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count Arlian 2—Immuno-chemical Assay Chapman
		Food-Solid	n/a									
	Food-Liquid	n/a	Possible-media containing mites	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count Arlian 2—Immuno-chemical Assay Chapman							
	Surface Dust											
	Floor Dust	Possible-media containing mites	Dermal exposure is not reported to be an issue— source of resuspended material	Possible*	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count Arlian 2—Immuno-chemical Assay Chapman						
Soil	n/a											
Father	only ↓ Blood/serum	DUST	Possible	Dermal exposure is not reported to be an issue— source of resuspended material	Possible*	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immuno-chemical Assay	b, c, d				
		Mattress	Possible									
		Furniture	Possible									
		All media containing mites										
		Blood/serum	Genes/Genetic polymorph? Specific IgE				Venous draw			e		
		Blood/serum	Genes/Genetic polymorph? Specific IgE							f		
		Blood/serum	Genes/Genetic polymorph? Specific IgE						e			
		Blood/serum	Genes/Genetic polymorph? Specific IgE						f			

**Table 3-5. Measurement Methods by Life Stage, Media, and Route for House Dust Mites and Endotoxin Exposures (continued)**

Age Class	Person of Interest	Media	Importance of Media for Exposure	Route	Importance of Route for Exposure	Importance of Route to Outcome	Limitations of Measurements	Collection Method	Concerns*	Refs
Conception to Birth	Mother	Air	High- stimulates maternal immune system response n/a	inhalation	High	Possible*	PIT—varies seasonally	Liquid glass impinger	a, b	
		Water Food-Solid Food-Liquid	n/a n/a							
		Surface Dust	Possible-media containing mites				PIT—varies seasonally PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immunochemical Assay		
		Floor Dust	Possible-media containing mites		Dermal exposure is not reported to be an issue—source of resuspended material	Possible*	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immunochemical Assay	b, c, d	
		Soil <b>OTHER:</b> DUST Mattress Furniture Pillow	n/a  Possible Possible Possible All media containing mites		Dermal exposure is not reported to be an issue—source of resuspended material	Possible*	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immunochemical Assay	b, c, d	
		Blood/serum Amniotic Fluid? Cord Blood Placenta? Colostrum?	Specific IgE Specific IgE				Venous draw	f	Thor	

**Table 3-5. Measurement Methods by Life Stage, Media, and Route for House Dust Mites and Endotoxin Exposures (continued)**

Age Class	Person of Interest	Media	Importance of Media for Exposure	Route	Importance of Route for Exposure	Importance of Route to Outcome	Limitations of Measurements	Collection Method	Concerns*	Refs
Conception to Birth	Child	Air Water Food-Solid Food-Liquid Surface Dust Floor Dust Soil <b>OTHER:</b> Amniotic fluid transfer of specific IgE to gut of fetus Blood/serum Cord Blood	n/a n/a n/a n/a n/a n/a n/a n/a ingestion? osmosis?  Specific IgE	possible/ postulated	possible/ postulated	appears constant through time in mother and child	Venous draw		Thorton  f	
Birth to 3 months	Child	Air  Water Food-Solid Food-Liquid  Surface Dust	High  n/a n/a Unknown/ Possible Breast milk Specific IgE? undefined Possible-media containing mites	inhalation  ingestion  inhalation	High*  possible/unconfirmed  unknown	Unknown: May be Critical  unknown: may be critical  possible* source	PIT—varies seasonally  PIT—varies seasonally	Liquid glass impinger  Heat Capture, or Vacuumed Sample Analysis 1—Whole Body Count 2—Immuno-chemical Assay	a, b	Thor  Kauff
		Floor Dust	undefined Possible-media containing mites		Dermal exposure is not reported to be an issue—source of resuspended material	Possible	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immuno-chemical Assay	b, c, d	

**Table 3-5. Measurement Methods by Life Stage, Media, and Route for House Dust Mites and Endotoxin Exposures (continued)**

Age Class	Person of Interest	Media	Importance of Media for Exposure	Route	Importance of Route for Exposure	Importance of Route to Outcome	Limitations of Measurements	Collection Method	Concerns*	Refs
		Soil <b>OTHER:</b> DUST Mattress Furniture Stuffed Toys Pillow	n/a  Possible Possible Possible All media containing mites		Dermal exposure is not reported to be an issue—source of resuspended material	Possible	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count arian 2—Immuno-chemical Assay Chapman		Bisch Arlia
		Blood/serum	Specific IgE				Venous draw		f	
3 months to 3 years		Air  Water Food-Solid  Food-Liquid  Surface Dust     Floor Dust	High- stimulates immune system response n/a unknown (other mites) unknown Breast milk Specific IgE? unknown Possible-media containing mites   unknown/ Possible-media containing mites	inhalation  ingestion ingestion  inhalation	High*  unknown possible/unconfirmed  source   Dermal exposure is not reported to be an issue—source of resuspended material	Unknown-probably high*  unknown unknown: may be critical  possible   Possible	PIT—varies seasonally  PIT—varies seasonally PIT—varies seasonally  PIT—varies seasonally   PIT—varies seasonally	Liquid glass impinger  body counts    Heat Capture, or Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immuno-chemical Assay Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immuno-chemical Assay		Thor

**Table 3-5. Measurement Methods by Life Stage, Media, and Route for House Dust Mites and Endotoxin Exposures (continued)**

Age Class	Person of Interest	Media	Importance of Media for Exposure	Route	Importance of Route for Exposure	Importance of Route to Outcome	Limitations of Measurements	Collection Method	Concerns*	Refs
		Soil <b>OTHER:</b> DUST Mattress Furniture Stuffed Toys Pillow	n/a  Possible Possible Possible All media containing mites	n/a	n/a  Dermal exposure is not reported to be an issue—source of resuspended material	n/a  Possible	n/a  PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immunochemical Assay		
		Blood/serum	Specific IgE				Venous draw			
3 years and above		Air	High- stimulates immune system response	inhalation	High*	Unknown- probably high*	PIT—varies seasonally	Liquid glass impinger		
		Water Food-Solid	n/a (other mites)	ingestion	unknown	unknown	PIT—varies seasonally	body counts		
		Food-Liquid	unknown/unlikely	ingestion	unknown/unlikely	unknown: may be critical possible	PIT—varies seasonally PIT—varies seasonally	RAST		
		Surface Dust	Possible-media containing mites	inhalation	source			Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immunochemical Assay		
		Floor Dust	Possible-media containing mites		Dermal exposure is not reported to be an issue—source of resuspended material	Possible	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immunochemical Assay		
		Soil <b>OTHER:</b>	n/a	n/a	n/a	n/a	n/a			

**Table 3-5. Measurement Methods by Life Stage, Media, and Route for House Dust Mites and Endotoxin Exposures (continued)**

Age Class	Person of Interest	Media	Importance of Media for Exposure	Route	Importance of Route for Exposure	Importance of Route to Outcome	Limitations of Measurements	Collection Method	Concerns*	Refs
		DUST Mattress Furniture Stuffed Toys Pillow	Possible Possible Possible All media containing mites		Dermal exposure is not reported to be an issue—source of resuspended material	Possible	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immunochemical Assay		
		Blood/serum Skin Test	Specific IgE Specific IgE				Venous draw			

Notes:

- \* Assumes the individual is susceptible based on genetics
- a. Technician collected pumped samples necessary.
- b. Indoor microenvironmental sampling necessary.
- c. Lack of uniform methodology.
- d. Lab technician skill level essential to accurate outcome.
- e. Biomarker of susceptibility
- f. Biomarker of exposure

**Table 3-6. Measurement Methods by Life Stage, Media and Route for Arthropod and Rodent Allergen Exposures**

Age Class	Person of Interest	Media	Importance of Media for Exposure	Route	Importance of Route for Exposure	Importance of Route to Outcome	Limitations of Measurements	Collection Method	Concerns*	Refs
Prior to Conception	Mother	Air	High- stimulates maternal immune system response n/a	inhalation	High	Possible*	PIT—varies seasonally	Actively pumped sample: Glass liquid impinger	a, b	
		Water Food-Solid	Possible-media containing arthropods, rodent feces/ urine/saliva n/a	ingestion	Possible	Possible		— Immunochemical Assay Chapman		
		Food-Liquid Surface Dust	Possible-media containing arthropods, rodent feces/ urine/saliva n/a	inhalation	Possible	Possible	PIT—varies seasonally	Vacuumed Sample Analysis- — Immunochemical Assay Chapman		
		Floor Dust	Possible-media containing arthropods, rodent feces/ urine/saliva n/a	inhalation	Dermal exposure is not reported to be an issue—source of resuspended material	Possible*	PIT—varies seasonally	Vacuumed Sample Analysis- — Immunochemical Assay Chapman	b, c, d	
		Soil OTHER: Blood/serum	n/a Genes/Genetic polymorph? Specific IgE					Venous draw		e f
Prior to Conception	Father	only ↓ Blood/serum	Genes/Genetic polymorph? Specific IgE						e f	
Conception to Birth	Mother	Air	High- stimulates maternal immune system response n/a	inhalation	High	Possible*	PIT—varies seasonally	Actively pumped sample: Glass liquid impinger	a, b	
		Water Food-Solid	Possible-media containing arthropods, rodent feces/ urine/saliva n/a	ingestion	Possible	Possible		— Immunochemical Assay Chapman		

**Table 3-6. Measurement Methods by Life Stage, Media and Route for Arthropod and Rodent Allergen Exposures (continued)**

Age Class	Person of Interest	Media	Importance of Media for Exposure	Route	Importance of Route for Exposure	Importance of Route to Outcome	Limitations of Measurements	Collection Method	Concerns*	Refs
		Food-Liquid	Possible-media containing arthropods, rodent feces/ urine/saliva	ingestion	Possible	Possible		— Immunochemical Assay Chapman		
		Surface Dust	Possible-media containing arthropods, rodent feces/ urine/saliva	inhalation	Possible	Possible	PIT—varies seasonally	Vacuumed Sample Analysis- — Immunochemical Assay Chapman		
		Floor Dust	Possible-media containing arthropods, rodent feces/ urine/saliva	inhalation	Dermal exposure is not reported to be an issue—source of resuspended material	Possible*	PIT—varies seasonally	Vacuumed Sample Analysis- — Immunochemical Assay Chapman	b, c, d	
		Soil Other: Blood/serum Amniotic Fluid? Cord Blood Placenta? Colostrum?	n/a Specific IgE Specific IgE				Venous draw		f	Thor
Conception to Birth	Child	Air Water Food-Solid Food-Liquid Surface Dust Floor Dust Soil OTHER: Amniotic fluid transfer of specific IgE to gut of fetus Blood/serum Cord Blood	n/a n/a n/a n/a n/a n/a n/a ingestion? osmosis? Specific IgE	possible/ postulated	possible/ postulated	appears constant through time in mother and child	Venous draw		Thorton f	

**Table 3-6. Measurement Methods by Life Stage, Media and Route for Arthropod and Rodent Allergen Exposures (continued)**

Age Class	Person of Interest	Media	Importance of Media for Exposure	Route	Importance of Route for Exposure	Importance of Route to Outcome	Limitations of Measurements	Collection Method	Concerns*	Refs
Birth to 3 months	Child	Air	High- stimulates maternal immune system response n/a	inhalation	High	Possible*	PIT—varies seasonally	Actively pumped sample: Glass liquid impinger	a, b	
		Water Food-Solid Food-Liquid	n/a Possible-media containing arthropods, rodent feces/ urine/saliva	ingestion	Possible	Possible		— Immunochemical Assay Chapman		
		Surface Dust	protein through maternal Breast Milk Possible-media containing arthropods, rodent feces/ urine/saliva	inhalation	Possible	Possible	PIT—varies seasonally	Vacuumed Sample Analysis- — Immunochemical Assay Chapman		
		Floor Dust	Possible-media containing arthropods, rodent feces/ urine/saliva	inhalation	Dermal exposure is not reported to be an issue—source of resuspended material	Possible*	PIT—varies seasonally	Vacuumed Sample Analysis- — Immunochemical Assay Chapman	b, c, d	
		Soil Other: Blood/serum Cord Blood Placenta? Colostrum?	n/a Specific IgE				Venous draw		f	
3 months to 3 years		Air	High- stimulates immune system response n/a	inhalation	High*	Unknown- probably high*	PIT—varies seasonally	Liquid glass impinger		
		Water Food-Solid	unknown (other mites)	ingestion	unknown	unknown	PIT—varies seasonally	body counts		

**Table 3-6. Measurement Methods by Life Stage, Media and Route for Arthropod and Rodent Allergen Exposures (continued)**

Age Class	Person of Interest	Media	Importance of Media for Exposure	Route	Importance of Route for Exposure	Importance of Route to Outcome	Limitations of Measurements	Collection Method	Concerns*	Refs
		Food-Liquid	unknown Breast milk Specific IgE?	ingestion	possible/unconfirmed	unknown: may be critical	PIT—varies seasonally			Thor
		Surface Dust	unknown Possible-media containing mites	inhalation	source	possible	PIT—varies seasonally	Heat Capture, or Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2— Immunochemical Assay		
		Floor Dust	unknown/ Possible-media containing mites		Dermal exposure is not reported to be an issue—source of resuspended material	Possible	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2— Immunochemical Assay		
		Soil OTHER: DUST Mattress Furniture StuffedToys Pillow	n/a  Possible Possible Possible Possible All media containing mites	n/a	n/a  Dermal exposure is not reported to be an issue—source of resuspended material	n/a  Possible	n/a  PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2— Immunochemical Assay		
		Blood/serum	Specific IgE				Venous draw			
3 years and above		Air	High- stimulates immune system response	inhalation	High*	Unknown- probably high*	PIT—varies seasonally	Liquid glass impinger		
		Water	n/a							
		Food-Solid	(other mites)	ingestion	unknown	unknown	PIT—varies seasonally	body counts		
		Food-Liquid	unknown/unlikely	ingestion	unknown/unlikely	unknown: may be critical	PIT—varies seasonally	RAST		

**Table 3-6. Measurement Methods by Life Stage, Media and Route for Arthropod and Rodent Allergen Exposures (continued)**

Age Class	Person of Interest	Media	Importance of Media for Exposure	Route	Importance of Route for Exposure	Importance of Route to Outcome	Limitations of Measurements	Collection Method	Concerns*	Refs
		Surface Dust	Possible-media containing mites	inhalation	source	possible	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immunochemical Assay		
		Floor Dust	Possible-media containing mites		Dermal exposure is not reported to be an issue—source of resuspended material	Possible	PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immunochemical Assay		
		Soil OTHER: DUST Mattress Furniture StuffedToys Pillow	n/a  Possible Possible Possible All media containing mites	n/a	n/a  Dermal exposure is not reported to be an issue—source of resuspended material	n/a  Possible	n/a  PIT—varies seasonally	Heat Capture, or Vacuumed Sample Analysis- 1—Whole Body Count 2—Immunochemical Assay		
		Blood/serum Skin Test	Specific IgE Specific IgE				Venous draw			

Notes:

- \* Assumes the individual is susceptible based on genetics
- a. Technician collected pumped samples necessary.
- b. Indoor microenvironmental sampling necessary.
- c. Lack of uniform methodology.
- d. Lab technician skill level essential to accurate outcome.
- e. Biomarker of susceptibility
- f. Biomarker of exposure

### 3.3 Measurement of Exposures to Metals

#### 3.3.1 Considerations for Assessing Exposures to Metals

Hypotheses proposed for the NCS include the examination of adverse neurocognitive, neurodevelopmental, reproductive and other developmental outcomes that may result from multiple stressors in the environments of mothers and children. Assessing exposures to metals will also be important for both their direct effects on outcomes as well as their potential confounding for outcomes related to other stressors. Recent review articles describe important issues in our current understanding in exposures to toxic metals, bioavailability, toxicity, and health outcomes for children and women of childbearing age (Bellinger, 2004; Davidson et al., 2004; Calderon et al., 2003; Jarup, 2003; Robson, 2003; Tchounwou et al., 2003; Vahter et al., 2002; Wigg, 2001). In some cases it may also be necessary to assess exposures to metals not normally associated with adverse health outcomes because they may play a role in the uptake and biological levels of more toxic metals. For example, biological levels of lead may be related to environmental levels of zinc and dietary intake of calcium. Toxicity, bioavailability, and health outcomes associated with metals may be highly dependent on the metallic species. Valence state and association of metals with organic molecules or particulates are extremely important determinants of the effects of metals on mothers and children. Sampling and analysis methods must be tailored to the species of interest.

Sources and exposure pathways for some of the important metals are discussed briefly here. For more information on metals speciation, sources, analytical methods, and health effects refer to the individual toxicological profiles developed by the Agency for Toxic Substances and Disease Registry (ATSDR, 2004). These profiles provide detailed information for many metals that can be used to determine whether it is likely to be of concern or interest for exposure assessment in the NCS, and if so, the important forms of the metal and likely routes of exposure. The U.S. Environmental Protection Agency is in the process of developing a Framework for Metals Risk Assessment (U.S. EPA, 2002). The process and components for development of these guidelines have been published (Sappington, et al., 2003). Five key areas for development of this guidance are discussed in external draft issue papers and include a) bioavailability and bioaccumulation of metals; b) ecological effects of metals; c) metal exposure assessment; d) environmental chemistry of metals; e) human health effects of metals (U.S. EPA, 2003).

Metals may be found in the air, drinking water, food, dust, and soils in many of the environments in which children and adults spend their time. The recent National Human Exposure and Assessment Study characterized total exposures to several metals in three different U.S. populations (Clayton et al., 1999; Clayton et al., 2002; O'Rourke et al., 1999; Ryan et al., 2001). Other studies have examined exposures in European populations (Seifer et al., 2002). Metals may be found in a number of residential and consumer products and structural components (i.e., lead in paint in older homes). Exposure may occur through inhalation, dietary ingestion of food and water, dermal absorption, and ingestion of contaminated soil and dust. In the absence of occupational exposures, the greatest mass intake of metals results primarily from the dietary and non-dietary ingestion pathways. Inhalation may be an important component of total exposure for people living in some locations. In addition, as mentioned earlier, the route of exposure may have a significant impact on outcome, probably due to pharmacokinetic factors responsible for dose to the target organ or tissue.

A preliminary evaluation of the relative importance of exposure to metals from various media/pathway combinations for different age groups is shown in **Table 3-7**. Dietary ingestion is likely to be an important exposure route for most metals and as such, foods are shown as a highly significant route of exposure at all ages. Other pathways, including ingestion of water, ingestion of dusts and soils, and inhalation of airborne particles may be important for some metals at some ages, but not for others. The

**Table 3-7. Preliminary Assessment of the Important Exposure Routes and Media at Different Life Stages for Metals**

Medium	Route	Location	Pre-natal	Fetal*	Months				Years			
					0 - <3	3 - <6	6 - <12	1 - <2	2 - <3	3 - <6	6 - <11	11 - <21
Air	inhalation	personal	2	2	NA	NA	NA	NA	NA	2	2	2
	inhalation	indoor home	2	2	2	2	2	2	2	2	2	2
	inhalation	indoor day care	NA	NA	2	2	2	2	2	2	2	2
	inhalation	indoor schools	NA	NA	NA	NA	NA	NA	NA	2	2	2
	inhalation	indoor work	2	2	NA	NA	NA	NA	NA	NA	NA	2
Water	ingestion/dermal/inhalation	home	2	2	2	2	2	2	2	2	2	2
	ingestion/dermal/inhalation	day care	2	2	2	2	2	2	2	2	2	2
	ingestion/dermal/inhalation	school	2	2	NA	NA	NA	NA	NA	2	2	2
	ingestion/dermal/inhalation	work	2	2	2	NA	NA	NA	NA	NA	NA	2
Food	ingestion	liquid	2	2	2	2	2	2	2	2	2	2
	ingestion	solid	1	1	NA	NA	1	1	1	1	1	1
	ingestion	breast milk	NA	NA	1	1	1	1	NA	NA	NA	NA
Surface residues	dermal/ingestion	home	2	2	2	2	2	2	2	2	2	2
	dermal/ingestion	day care	NA	NA	2	2	2	2	2	2	2	2
	dermal/ingestion	school	NA	NA	NA	NA	NA	NA	NA	NA	2	2
	dermal/ingestion	work	2	2	NA	NA	NA	NA	NA	NA	NA	2
Floor dust	ingestion/dermal	home	2	2	2	2	2	2	2	2	2	2
	ingestion/dermal	day care	NA	NA	2	2	2	2	2	2	2	2
	ingestion/dermal	school	NA	NA	NA	NA	NA	NA	NA	NA	2	2
	ingestion/dermal	work	2	2	NA	NA	NA	NA	NA	NA	NA	2
Soil	ingestion/dermal	home	2	2	2	2	2	2	2	2	2	2
	ingestion/dermal	day care	NA	NA	2	2	2	2	2	2	2	2
	ingestion/dermal	school	NA	NA	NA	NA	NA	NA	NA	NA	2	2
	ingestion/dermal	work	2	2	NA	NA	NA	NA	NA	NA	NA	2
Paint	ingestion	home	2	2	2	2	2	2	2	2	3	
Fish/Seafood	ingestion	solid	2	2	2	2	2	2	2	2	2	

\* Maternal samples as a surrogate for fetal samples

NA = sample cannot be practically collected

1 = important route for exposure for all chemicals in this class

2 = may be important for some chemicals in the class or some life stages; refer to text for more details

3 = not an important route for exposure for any chemical in this class

metals lead, cadmium, and mercury may be considered to be bioaccumulative in that they have long excretion half-lives, and are likely to persist in environmental and biological media. A range of other metals that are considered to be non-bioaccumulative may be of interest for exposure assessment in the NCS. These include, but are not limited to arsenic, beryllium, chromium, cobalt, manganese, nickel, and vanadium.

Lead is the metal of most significant nationwide public health concern due to its role in reduced cognitive function in children. Although it was removed from gasoline many years ago, lead is still prevalent in the general environment and remains a significant risk to young children living in older homes with lead-based paint. Children are still exposed to lead in the environment through ingestion and dermal contact with contaminated dust and soil. In some instances adults working in manufacturing or battery processing have created potential exposures to their children by bringing contaminated clothing and shoes into the residential environment. And in some locations there may be locally high levels of lead in the outdoor environment resulting from mining and smelting, industrial, or agricultural activities. Lead is found in the food consumed by most people in the United States, and dietary intake is probably the most important exposure pathway for people that do not have lead-based paint in their homes or highly localized exposures from industrial activities. Lead is still found in tap water, usually as a result of contamination in home plumbing systems. Exposure to lead in tap water may contribute to the exposure resulting from dietary intake. Some older homes may still have lead pipes and many homes have lead-containing solder in copper plumbing joints. These may contribute to lead exposure through consumption of tap water for drinking and cooking. The amount of lead in tap water can be affected by residence time in the pipes and by the pH of the water. Lead is still prevalent in association with airborne particles in most of the United States, but air concentrations are low in most locations and the inhalation route is likely to contribute only a fraction of the exposure received from other routes and pathways.

Cadmium is usually present at much lower levels than lead in environmental media. Cadmium is naturally occurring and may also be released into the environment through burning of coal, mining activities, as a trace component of fertilizers, and from waste disposal activities. In the general population, most exposure to cadmium likely occurs through ingestion of solid foods, and to a lesser extent through consumption of drinking water. However, the highest exposures to mothers and children may be found in localized areas due to mining or smelting operations where cadmium may contaminate dust and soil with subsequent potential for young children's exposures. Airborne levels of cadmium are low in most places distant from specific industrial emission sources. Cadmium is also usually found at low concentrations in tap water.

Mercury is of significant concern due to its role in adverse neurological development in fetuses and potential for impaired cognitive function in children and adults. Mercury contamination is widespread due to industrial uses of the metal and its wide release during combustion of coal in power plants. Mercury accumulates in the food chain and biological processes can lead to formation of the more toxic methylmercury. Thus, dietary intake of fish and shellfish can be an important pathway for exposure for some people. Inhalation may play a contributing role in some locations, and ingestion of contaminated dusts and soils may be important at some local areas due to industrial contamination. As with many of the metals, toxicity is highly dependent upon the species to which people are exposed. In the case of mercury, the metallic and some organic compounds, particularly methylmercury, are of most concern, whereas inorganic mercury compounds are thought to have lower risks. Properly assessing health outcomes often requires determining the particular form or species of metals. In any study of human exposure it will be important to identify the specific species of concern and to employ collection, storage, and analytical methods that allow accurate measurement of the species of interest. Maternal exposures shortly before and during gestation, and exposures to very young children will likely be the most significant concern in the NCS due to the potential susceptibility to neurological damage at those life stages.

Arsenic is present in most environments and can be found in most exposure media. Arsenic is a naturally occurring compound in many locations and can also be released from human activities. In locations with high levels in soils and rocks, arsenic may be released due to weathering activities and can be found in the dust, soil, and water at high concentrations. Releases from human activities include industrial processes and commercial products, smelting of ores for many metals, use in pesticides and wood preservatives, and by-products of the smelting process for many metal ores. Arsenic exists in three common valence states: As(0), As(III), and As(V). Inorganic arsenic is generally more toxic than organic arsenic. Methyl and phenyl arsenate compounds have been shown to produce adverse health effects in some animal studies. As(III) is more toxic to organisms than As(V) largely due to its much higher uptake at the cellular level. Chemical speciation during the analysis of exposure media is important to provide more accurate assessments of the toxicological effects of the exposure. Exposure to arsenic occurs through all pathways and from multiple media. Significant exposures to organic arsenic may result from consumption of some saltwater fish species. Exposures to more toxic inorganic species may occur from dietary intake, drinking water, non-dietary ingestion of soil and dust, and through inhalation. EPA's Consumer Safety Information Sheet on Inorganic Arsenical Pressure-Treated Wood provides the following note on potential for dermal exposure: "Inorganic arsenic penetrates deeply into and remains in the pressure-treated wood for a long time. However, some chemical may migrate from treated wood into surrounding soil over time and may also be dislodged from the wood surface upon contact with skin. Exposure to inorganic arsenic may present certain hazards" ([http://www.epa.gov/pesticides/factsheets/chemicals/cca\\_consumer\\_safety.htm](http://www.epa.gov/pesticides/factsheets/chemicals/cca_consumer_safety.htm)).

Other non-bioaccumulative metals (e.g., chromium, copper, nickel, manganese) may be of interest in the NCS because in some forms and through some exposure pathways they may either cause toxic effects themselves, or have effects on other agents of interest. Most of these metals will have both natural and anthropogenic sources. Exposures may be higher in local areas with specific industrial activities or that have high naturally occurring levels in the environment. In some cases it will be important to measure the specific species of the metal or metal compounds. For example, chromium (III) is an essential dietary metal, whereas chromium (VI) is more toxic and may affect reproductive health and cause lung cancer in highly exposed individuals. In other cases, the specific exposure pathway may be important. For example, manganese is an essential dietary nutrient, but inhalation of high concentrations may have neurological effects that could confound assessments of other stressors. The need to assess exposures of specific metals in the NCS will depend on their potential importance as direct or confounding stressors for health effects addressed in the study hypotheses. Selection of media and methods for measurement will depend on the important exposure pathways and media, as well as the species of most concern.

### 3.3.2 Methods for Measuring Metals in Dust and Soil

Because measurements of dermal and dietary ingestion exposures to metals employ similar methods to those for measuring dermal and non-dietary exposures to pesticides, the discussion presented in **Section 3.4** provides information on the approach for assessing dermal and non-dietary exposures that is applicable to both metals and pesticides. This section will provide references to a) methods that have been used to measure metals in environmental media, b) studies of levels in environmental media and relationships between environmental levels and biomarkers of exposure, c) some factors affecting dermal exposures to metals, and d) examinations of approaches for mitigating residential levels and exposures, primarily for children and lead. A majority of the research studies cited here examine exposures or outcomes for lead because it has been a pervasive and ongoing public health issue across the country. However, other studies have examined exposures and environmental levels of arsenic, cadmium, chromium, and other metals that may be of interest in the NCS.

Several comparisons of methods for measurement of lead and other metals in house dust have been performed (Bai et al., 2003; Lanphear et al., 1995; Liroy et al., 1993; Rich et al., 1999; U.S. EPA

1991,1995a,b,c,d; Yu et al., 2001). Procedures for direct measurement of metals in environmental media have been described and may have use in screening-level assessments in studies such as the NCS (Clark et al., 1999). Many studies have been performed in recent years that describe both the methodology and the results for measurement of lead and other metals in house dust and other dust or soil related media in the United States (Adgate et al., 1995; U.S. Department of Housing and Urban Development 2001; Wolz et al., 2003). The National Human Exposure Assessment Survey (NHEXAS) examined exposures to lead, arsenic, and several other metals in residential exposure media in two population-based studies (Clayton et al., 1999; Clayton et al., 2002; Hysong et al., 2003; O'Rourke et al., 1999a,b). Research performed in other nations may provide additional information on measurement methods (Banerjee, 2003; Chattopadhyay et al., 2003; Keegan et al., 2002; Kim and Fergusson, 1993; Lambert and Lane, 2004; Massadeh and Snook, 2002; Meyer et al., 1999; Ng et al., 2003; Ordonez et al., 2003; Polissar et al., 1990; Rasmussen et al., 2001; Seifert et al., 2000).

Several studies have examined the exposure of children to lead in residential environments (Lanphear and Roghmann, 1997; Lanphear et al., 2002; Yiin et al., 2000). Another study examined lead in house dust, soil, and water and examined the performance of a lead exposure questionnaire completed by teenagers (Hoppin et al., 1997). Child exposure to arsenic from CCA-treated wooden decks and playground structures has been examined (Hemmond and Solo-Gabriele, 2004). Other studies have examined dermal exposures, soil loadings, and their role in exposures to metals and other pollutants (Holmes et al., 1999; Karita et al., 1997; Kissel et al., 1996; Rodes et al., 2001; Stauber et al., 1994; Sun et al., 2002). A number of studies have reported on the relationship between environmental levels of metals and biomarker levels in blood or urine (Emond et al., 1997; Lanphear et al., 1996; Lanphear et al., 1998a; Lanphear et al., 1998b; National Center for Healthy Housing, 2001; Stern et al., 1998; U.S. Department of Housing and Urban Development, 1994). The bioavailability of metals from dust and soil media is an important factor in understanding the dose resulting from environmental exposures. Bioavailability studies of arsenic and lead have been performed in the laboratory (Ellickson et al., 2001; Rodriguez et al., 2003). A number of methods have been proposed or tested for mitigation of exposure to lead in residential environments (ASTM International, 2003; Galke et al., 2001; Lioy et al., 1998; U.S. EPA, 1995a, b, c, d; U.S. EPA, 1997; Yiin et al., 2003). Finally, procedures for assessing health effects of metal in chronic low level exposures and from household dusts have been described (Nordberg, 1988; Lemus et al., 1996).

### 3.3.3 Methods for Assessing Dietary Exposures to Metals

Assessing dietary exposure to metals will be important in the NCS because some of the metals may directly or indirectly affect outcomes of interest. There are several approaches and methods for assessing dietary exposures that may be considered for use in the NCS. These approaches are discussed below along with their advantages, disadvantages, and limitations. Specific methodology for conducting dietary exposure assessments is not extensively discussed here, but details can be found in references shown at the end of this section.

Direct and indirect approaches have been used to characterize dietary exposures to metals for individuals and for larger population groups. Direct approaches require physical collection and analysis of food, beverage, and drinking water samples. Indirect approaches for individuals require collection of food intake data, which is combined with extant residue data to estimate exposure for a specific time period. Indirect approaches for estimating intake, or a distribution of intakes in the population use food consumption data in combination with extant residue data, or a market-basket survey approach in which representative samples of foods are collected in a region and analyzed for chemical content.

Each of these approaches has advantages and disadvantages for cost, complexity, burden, accuracy, and representativeness. Approaches are typically selected based on the specific study objectives. For

example, several approaches and methodologies were considered in preparation for the National Human Exposure Assessment Survey (NEHXAS) (Cronin et al., 1993). Some dietary exposure assessment approaches that might be considered for metals (as well as other chemical contaminants) in the NCS, and their strengths and limitations, are briefly discussed in the subsections below.

### *Direct Measurement of Individual Dietary Intake*

The most widely used approach for accurate characterization of an individual's chemical contaminant intake over short time periods is through collection and analysis of foods prepared and eaten by the study participant. A duplicate diet procedure has been the most widely used approach for collecting foods (WHO, 1985; Berry, 1996; Thomas et al., 1997; Vahter et al., 1991). In this method, a second portion of all foods and beverage is collected, as prepared for consumption, over a specified time period, usually ranging from one to seven days. The advantage of this approach is that it can measure the actual metal contaminant intake for an individual, and it includes any contamination of the foods from handling and cooking. The duplicate diet method can be adapted to collect only foods likely to be contaminated (e.g., fish for mercury).

Many duplicate diet studies have been performed to measure human dietary intake of metals over short time periods. Some studies examined multiple elements in the diets of adults (Berry et al., 1997a ; Berry et al., 1997b; Bro et al., 1990; Buchet et al., 1983; Dabeka and McKenzie, 1995; Ellen et al., 1990; Guthrie et al., 1977; Ikebe et al., 1988a; Ikebe et al., 1988b; Morgan et al., 1988; Ohgane et al., 1989; Sherlock et al., 1983a; Sherlock et al., 1983b; Takahata et al., 1989; Vahter et al., 1990; Vahter et al., 1991; Walters et al., 1998; Wilson, 1983). More recently in the United States, the NHEXAS studies examined aggregate exposure to metals including exposures from dietary and drinking water intake (Clayton et al., 1999; Clayton et al., 2001; O'Rourke et al., 1999; Ryan et al., 2001; Scanlon et al., 1999; Thomas et al., 1999). Some studies have examined adult dietary intake of single metals including lead (Ikebe et al., 1989b; Sherlock et al., 1982a), cadmium (Louekari et al., 1987), arsenic (Mohri et al., 1990), mercury (Haxton et al., 1979; Sherlock et al., 1982b), selenium (Reis et al., 1990), and cesium radioisotopes ( Walker et al., 1991). And several studies have focused on the dietary intake of lead in infants and children (Freeman et al., 2001; Lacey et al., 1985; Manton et al., 1991; Melnyk et al., 2000; Sherlock et al., 1986; Sherlock and Quinn, 1986; Smart et al., 1987; Stanek et al., 1998).

There are a number of disadvantages for a measurement approach to assessing dietary intake. Collection, transport, and analysis of dietary samples are costly and have a relatively high study participant burden (although successful dietary collections have been implemented in several exposure measurement studies as described by the referenced studies). Duplicate diet samples can only be collected for a few days at a time. Accurate characterization of the long-term intake of some metals may require sample collection across multiple seasons and years (Basiotis et al., 1987; Buck et al., 1995; Sempas et al., 1991; Tarasuk and Beaton, 1992a; Tarasuk and Beaton, 1992b; Thomas et al., 1997). Finally, some people may alter their food consumption during the collection period or may not provide a complete sample of the foods they eat, resulting in bias in the dietary exposure assessment (Bro et al., 1990; Barrett-Conner, 1991; Kim et al., 1984; Mertz et al., 1991; Pennington, 1991; Stockley, 1985).

There are important chemical analysis considerations in dietary metals exposure measurements. A wide range of analytical methods is available, including some that allow simultaneous determination of many different metals. For example, inductively coupled plasma/mass spectrometry (ICP/MS) allows determination of many elements with adequate sensitivity in food samples (U.S. FDA, 1997; Melnyk et al., 2003). However, methods such as ICP/MS do not allow identification of particular species of metals. Because the toxicological effects of metals often depend on the particular species—different oxidation states or inorganic compounds—performing separate and specialized analyses, and ensuring that samples

are collected and handled in ways that preserve the original species, may be necessary. For example, chromium (VI) is typically less stable in samples than the less toxic chromium (III).

The cost of making direct measurements is likely to limit the number of participants for which direct dietary contaminant intake data can be obtained. Direct measurements could be used in subsets of NCS study participants to assess the accuracy of intake estimates made by other approaches or to examine specific exposure scenarios or health outcomes.

### *Individual Intake Estimation Approach*

Extant food residue data can be combined with an individual's food intake to estimate dietary exposure to metals. Food consumption data are obtained from individuals using one of several approaches, including 24-h recall questionnaires, food diary records, or food frequency questionnaires that gather "usual" food consumption data for periods up to a year. This approach might target specific food consumption when it is likely that the food(s) contain relatively high contaminant concentrations (e.g., fresh and saltwater fish species consumption for mercury intake). An advantage of this approach for the NCS is that food consumption data are likely to be collected as part of nutritional assessments. Thus, characterization of dietary metals intake could be performed for every individual in the study for a relatively low cost with little added participant burden.

However, there are important limitations to this approach that may create significant potential for exposure misclassification. Extant food residue data may not be applicable to the contamination on the foods eaten by individuals in the study. Individuals in the study are likely to eat many foods for which no residue measurement data are available. This approach does not account for the potential contamination of foods in the home, which can be substantial for lead in some residential environments. This approach is limited to the time period(s) for which reliable food consumption data can be collected for the individual. The intake for an individual may be highly variable over days, seasons, or years, requiring frequent data collection to adequately characterize long-term exposures for risk assessment analyses. Finally, food consumption records must be coded and combined with food residue data to prepare intake estimates.

Dietary intake and chemical residue data can be combined to estimate dietary exposures in populations and some sub-populations. The results of such data compilations would not provide the information on an individual's dietary intake that is likely to be needed for health outcome and risk analysis in the NCS. But the data can be used to inform estimations of contaminant intake from individual dietary intake records. However, there are certain cautions in using residue data for estimating exposures. The residue data are usually not collected from a statistically representative sample of foods, and are limited by the number, types, and frequencies of residue measurement. Estimates would not account for contamination of the food in the home. Regional or local differences in foods, such as those from home or community gardens would not be included. Finally, food intake and residue data may need to be updated periodically, relying on external data collection activities.

Food intake information has been collected in the Continuing Survey of Food Intake for Individuals (CFSII) and in the National Health and Nutrition Examination Surveys (NHANES) performed in the past 20 years. (In future years, the CFSII and NHANES collections will be combined into the NHANES). These national population-based surveys provided detailed information on food intake and distributions of intake in the general population and for some sub-populations (by age, region, etc.).

Food residue databases can provide limited information about chemical residues measured in food items. In the United States, the FDA Total Diet Study provides information on the residues of selected metals for a group of over 200 food items that represents over 85% of the average diet (Pennington, 1992; Gunderson, 1995; Pennington et al., 1996; Egan et al., 2002). A list of over 300 core foods in the U.S.

diet has recently been identified and proposed for assessment of nutrient and contaminant intakes in the population and sub-populations (Pennington and Hernandez, 2002).

An example of a regional market-basket approach for assessing dietary intake of metals is the FDA Total Diet Study (TDS). A list of approximately 200 different foods are collected from multiple grocery stores within a region on a periodic basis. Different samples of individual foods are combined and analyzed for metal residue content. Using population-based food intake data, an estimation of the metal intake for that region's population is estimated. The approach is best suited for regional population-level surveys. There is no individual study participant burden using this approach. Results cannot be directly applied to an individual, and thus are of limited use in the NCS, which will rely on individual exposure estimates. However, the results for analysis of the individual foods can be used in an estimate of individual exposure.

Food residue and food intake data can be combined to estimate the distribution of dietary intakes for the U.S. population and selected subpopulations. A Dietary Exposure Potential Matrix (DEPM) has been developed by the U.S. EPA to combine different food intake and residue databases to estimate dietary intake of metals and some other contaminants (Tomerlin et al., 1997). The DEPM can be used to identify foods likely to contain the chemical residue of interest and the ranges of concentrations in individual foods and dietary intake. This information can inform researchers on collection of dietary intake information and collection of dietary samples.

### 3.4 Measurements of Dermal and Non-Dietary Ingestion Exposures to Metals and Pesticides

Infants and young children may be particularly vulnerable to exposure to metals, pesticides, and persistent organic chemicals by the dermal and non-dietary ingestion routes because of their activities, which may include ingestion of soil and housedust, frequent contacts with surfaces contaminated with chemicals, their unique mouthing behaviors, and their eating patterns (Cohen Hubal et al., 2000). Young children may have frequent contacts indoors with flooring materials, such as carpet, contaminated with metals and/or persistent chemicals. They may also contact and mouth other surfaces, woodwork or furnishings that may be contaminated with residues of persistent organic pollutants or metals such as lead. Outdoors, young children may have frequent contact of extended duration with soils contaminated with persistent pesticides and metals such as lead and arsenic.

This section discusses approaches and methods for quantifying children's dermal and non-dietary exposures. The approaches and methods are applicable to metals, pesticides, and persistent organic pollutants. The material in this section supplements material presented in other sections of this paper by providing more detail on the approach for exposure assessments for these routes. The discussion is combined for dermal and non-dietary ingestion exposure because many of the measurement methods are similar, although the algorithms for estimating the exposure differ. Dermal and non-dietary ingestion exposures are also related because loading of chemicals on the hands can be ingested when children mouth their hands.

In this section, "children" refers to young children who exhibit frequent crawling and mouthing behaviors (e.g., up to age 5 or 6 years) rather than individuals through age 21 years, as typically defined in the NCS. The activities most likely to result in significant dermal and non-dietary ingestion exposures are likely to vary with the developmental stage of the child. Very young children may be particularly susceptible to pesticide exposures as a result of the microenvironments in which they spend time (e.g., kitchen floor), and the activities in which they are involved (e.g., mouthing of hands and toys and handling foods). It is important to understand that physiological characteristics and behavioral patterns

will result not only in different exposures for children and adults, but also for children of different developmental stages. Thus, exposure assessments are required for children in each age group, with age group being defined by developmental stage.

Developing a classification scheme for children by age group has been the subject of significant debate. The EPA Risk Assessment Forum (RAF) held a workshop on this topic in July 2000 (U.S. EPA, 2000). Some examples associated with relevant age-related developments for several exposure pathways are presented in **Table 3-8**. The age bins recommended by the RAF workshop for classifying children based on behavior are presented in **Table 3-9**. These age bins were considered when identifying the important routes of exposure and needs for environmental measurements in the NCS.

**Table 3-8. Relevant Age-Related Developments (From U.S. EPA, 2000)**

Exposure Pathway	Examples of Relevant Age-Related Developments
Mouth-Hand Contact	Prevalence of hand-to-mouth behaviors, such as thumb-sucking. Gross motor skills determine access to areas where the hand can become contaminated. Succession of gross motor milestones: rolling (4 months), creeping (6 months), crawling (8 months), walking (12 months), and climbing (18 months).
Mouth-Object Contact	The ability to interact with objects is a major factor. The ability to grasp an object to one's mouth begins roughly at 3 to 5 months. A pincer grasp and moderate strength are achieved by 9 months. Children become aware that objects exist even when covered around 6 months but generally do not understand the meaning of the word "no" until 12 months.

**Table 3-9. Behavioral Age Bins (From U.S. EPA, 2000b).**

Age Bin	Characteristics Relevant to Oral and Dermal Exposure
0 to 2 months	Breast and bottle feeding. Hand-to-mouth activities. Rapid growth makes children particularly vulnerable to chemicals.
3 to 5 months	Solid food is introduced. Contact with surfaces increases. Object-to-mouth activities increase.
6 to 11 months	Food consumption expands. Children's floor mobility increases. Children are increasingly likely to mouth non-food items.
12 to 23 months	Children consume a full range of foods. They participate in increased play activities, are extremely curious, and exercise poor judgment. Breast and bottle feeding cease.
2 to 5 years	Children begin wearing adult-style clothing. Hand-to-mouth activities begin to approximate adult patterns.
6 to 10 years	There is decreased oral contact with hands and non-food items, as well as decreased dermal contact with surfaces.
11 to 15 years	Smoking may begin. There is an increased rate of food consumption.
16 to 20 years	High rate of food consumption continues.

Dermal exposure refers to skin contact with concentrations or residues in various media (e.g., residential surfaces, soil, house dust). Children's unique activity patterns can bring their skin and clothes into frequent and extensive contact with potentially contaminated surfaces. The processes of chemical loading and removal at the skin surface over time are complex, making quantification of dermal exposure challenging (Cohen Hubal et al., 2000; Zartarian and Leckie, 1998).

Dermal exposure can be measured directly for either metals or pesticides using skin rinse, skin wipe/wash, or cloth dosimeter methods. Current techniques, however, may not accurately reflect loadings or losses at the skin surface that occur prior or subsequent to sampling. An alternate, indirect way to estimate an individual's dermal exposure for both metals and pesticides involves aggregating the mass transferred from a contaminated medium to the skin surface, associated with a series of contacts. The approach typically used involves combining residues (dust, soil, and/or surface residues) contacted in each microenvironment/macroactivity combination reported in a person's daily activity diary with dermal exposure factors specific to the medium of interest. Exposure for each microenvironment/macroactivity combination over a 24-hour period can either be tracked as a time series, or a time-averaged value over all events can be reported.

Non-dietary ingestion refers to ingestion exposure (contact between a chemical and a human at the mouth or lining of the GI tract) by indirect non-dietary pathways that include the following:

- chemical residues ingested while mouthing contaminated hands and/or objects (e.g., toys or surfaces in the home such as painted woodwork); and
- ingestion of contaminated soils or contaminated house dust found in residential or other environments.

In addition to these pathways, another potential pathway for indirect ingestion is ingestion of foods that have been inadvertently contaminated with chemicals as the result of food contact with contaminated hands and/or surfaces (e.g., during food preparation and/or consumption) (Melnyk et al., 2000; Akland et al., 2000). Small children are less likely than adults to consume food in a structured environment, and eat most food with their hands. They may sit on the floor or lawn to eat and often pick up and eat foods that have fallen on the floor. In many cases, non-dietary ingestion may occur after repeated contacts of the same object (food or any other object that enters the mouth) with multiple contaminated media, and from multiple contacts with the mouth. For example, a food item may contact several surfaces, hands, and utensils, before it is partially or completely ingested. Exposure through this pathway is not normally included in estimates of dietary exposure. Non-dietary exposure is also referred to as “indirect ingestion” exposure.

Sucking and mouthing hands, objects, and surfaces are natural behaviors in childhood development. Infants are born with a sucking reflex, providing them with both nutrition and a sense of comfort or security. If infants do not receive unrestricted breastfeeding, they will suck on a pacifier, thumb (or other finger), or other object like a blanket or stuffed animal. As infants develop, they begin to explore their world through mouthing (Groot et al., 1998). During this stage of development, children put almost everything that they contact into their mouths. Young children may also begin to use the mouth as a third hand, placing some objects in the mouth to manage them. Teething is another important stimulus for mouthing activities. Biting and chewing on fingers and objects to relieve the discomfort of teething may be extensive. Teething usually begins between 4 and 7 months of age, but may start several months earlier or later. As with all childhood behaviors, mouthing activities vary significantly from child to child and, therefore, the impact on exposure will also be highly variable.

Non-dietary ingestion exposures are difficult to quantify and assess because there are no methods for directly measuring contaminants that are ingested by these pathways. The typical approach for estimating non-dietary ingestion exposure is to combine activity information (collected by questionnaires and/or videography) with residue concentrations (measured or modeled) on/in hands, objects, and foods that are commonly handled, mouthed, and/or ingested as identified and measured in the field and with available exposure factor information (e.g., soil ingestion rate, saliva removal efficiency). Because no measure of non-dietary ingestion exposure analogous to a dermal dosimeter exists currently, measurements of residues or concentrations collected at specific time points are used to estimate ingestion exposures over the time frame of interest. However, because contaminant loadings over time can vary significantly, measurements collected at a single point in time may not reflect changes that occur prior to, or subsequent to, sampling (e.g., evaporation or removal by hand washing or mouthing). Thus, to the extent possible, exposure media concentrations need to be linked to contact activities in space and time. It is possible that in the future, controlled studies could be conducted using a nontoxic tracer that could be tracked in biological samples such as urine. Such a tracer would need to be applied as a surrogate for the environmental contaminants of interest in a setting where children could interact with the items of interest and exposures could be limited to non-dietary ingestion pathways.

### 3.4.1 Approaches and Data Needs for Assessing Dermal and Non-Dietary Exposure

The approaches and methods used for estimating dermal exposures for children are based on methods for measuring dermal exposures in workplaces. Conceptual and methodological advances in workplace dermal exposure assessment were presented in a series of manuscripts in the *Annals of Occupational Hygiene* in 2000 (e.g., Schneider et al., 2000). The authors of this series of manuscripts discuss the advantages and limitations of the available measurement methods and the more difficult problem of how to interpret the measurement data. Cohen Hubal et al. (2000) also discussed dermal exposure methods in their manuscript on the challenge of assessing children's residential exposure to pesticides. They discussed a conceptual model for children's exposures, the exposure algorithms, factors, and data requirements. The discussions in the manuscript were further developed in EPA's *Draft Protocol for Measuring Children's Non-Occupational Exposure to Pesticides by all Relevant Pathways*" (Berry et al., 2003). The Draft Protocol also presents algorithms, data requirements, and methods for estimating children's exposures to pesticides by the non-dietary ingestion route, which in the Draft Protocol was identified as the "indirect ingestion" route of exposure. The following discussion draws heavily from the Draft Protocol document.

Dermal exposure may be estimated directly using measurements made with gloves, body patches, or body garments worn by study participants. This direct measurement approach has been used extensively to measure occupational exposure for pesticide applicators in agricultural operations. It has had limited application for non-occupational exposure measurements. Ross et al. (1990) used body suits worn by adults to estimate exposures to pesticide residues from indoor fogger use. Researchers in EPA have used full body cotton garments (Fortmann et al., 2002) in studies of children's pesticides exposures, but performance of the method has not been fully evaluated. Use of full-body cotton garments or socks worn by children for one to two hours in the home is a relatively low-burden, low-cost method for collecting surface residues for the purposes of estimating dermal exposure to pesticides and other semi-volatile organic chemicals.

Exposure models for assessments of dermal or non-dietary ingestion exposure use one of two general approaches: a time-series approach that estimates microenvironmental exposures sequentially as individuals go through time, or a time-averaged macroactivity approach that estimates microenvironmental exposures using average microenvironmental concentrations and the total time spent in each microenvironment/macroactivity. The time-series approach to modeling personal exposures provides the appropriate structure for accurately estimating personal exposures (Esmen and Hall, 2000; Mihlan et al., 2000). In addition, the time-varying dose profile of an exposed individual can be modeled only by using the time-series approach (McCurdy, 1997; 2000). However, a time-series approach requires additional data, such as sequential time-location-activity diaries, that can be collected in a field study. Because most environmental monitoring provides either an integrated 24-hour concentration (as in air or duplicate diet samples) or a single time-point concentration (as in transferable residue samples), the time-averaged approach is often used. Equations for the time-averaged approach are given below. Zartarian et al. (2003) presents equations using the time-series approach.

#### *Dermal Exposure Assessment*

Dermal exposure should be estimated individually for each of the microenvironments (me) where a child spends time and each macroactivity (ma) that the child conducts within that microenvironment (Cohen Hubal et al., 2000).

Exposure over a 24-hour period is the sum of all the microenvironment/macroactivity exposures, expressed as:

$$E_{derm24} = \sum E_{dme/ma}$$

where

- $E_{derm24}$  = dermal exposure over a 24-h period for all microenvironments and macroactivities ( $\mu\text{g/d}$ )  
 $E_{dme/ma}$  = dermal exposure for a given microenvironment/macroactivity combination ( $\mu\text{g/d}$ )

For each microenvironment/macroactivity combination, dermal exposure is defined as:

$$E_{dme/ma} = (C_{surf})(TC_{me/ma})(AD_{me/ma})$$

where

- $C_{surf}$  = surface loading (total or transferable) measured in the microenvironment ( $\mu\text{g/cm}^2$ )  
 $TC_{me/ma}$  = transfer coefficient for the microenvironment/macroactivity ( $\text{cm}^2/\text{h}$ )  
 $AD_{me/ma}$  = activity duration that represents the time spent in each

As shown by the equation, the estimate of dermal exposure requires measurements of the transferable residue concentrations from the surface contacted, an estimate of the transfer coefficient, and a measure of the activity time. The transfer coefficient provides a measure of dermal exposure resulting from contact with a contaminated microenvironmental surface while engaged in a specific macroactivity (e.g., playing, sitting, lying on a contaminated surface). The transfer coefficient takes into account the fraction of the transferable surface residue that is transferred from a surface to skin, the character of the microenvironmental surface that is contacted, and the area of the microenvironmental surface that is contacted during a time increment for a given activity. Transfer coefficients are empirically derived in laboratory tests or controlled field experiments.

### *Hand-to-Mouth or Object-to-Mouth Non-Dietary Exposure*

Several modeling approaches have been used to estimate non-dietary hand-to-mouth or object-to-mouth exposure. The microactivity approach has been used most extensively in the past. To assess indirect ingestion exposure using the microactivity approach, exposure is estimated individually for all of the microactivities (e.g., hand-to-mouth, object-to-mouth, food-to-mouth, hand-to-food-to-mouth contacts) in which indirect ingestion occurs. Because it would be too burdensome and costly to collect all the data required to apply the microactivity approach in a large study, a macroactivity approach is presented here to provide a simplified assessment of indirect ingestion exposure to an individual based on measurement data collected in the field. In this approach, objects (including hands and food) that are commonly handled, mouthed, and/or ingested are identified in the field. The residue loadings on these objects are measured directly or estimated from surface concentration measurements. General information relating to the frequency and nature of these mouthing and ingestion activities is also collected. Data on the fraction of residues that may be removed from an object during mouthing that has been obtained in the laboratory experiments is then required to complete the assessment.

Indirect ingestion exposure during the 24-h period can be defined as:

$$E_{ing/mi} = (C_{surfx})(TE_x)(SA_{xm})(EF)$$

where

- $E_{ing/mi}$  = indirect ingestion exposure for each microactivity over a 24-h period ( $\mu\text{g/d}$ )  
 $x$  = hand, object, food item or anything else that enters the mouth  
 $C_{surfx}$  = surface loading (total or transferable) on  $x$  ( $\mu\text{g/cm}^2$ )

$TE_x$  = transfer efficiency of contaminant from x to mouth (unitless)  
 $SA_{xm}$  = area of x contacted by mouth ( $cm^2/event$ )  
 $EF$  = frequency of indirect ingestion events over a 24-h period (event/d)

The above equations can be modified slightly for use in a time-series approach, by estimating exposures for each sequential microenvironment/macroactivity event over the course of a day or longer.

### 3.4.2 Measurement Methods

To estimate dermal and non-dietary ingestion exposure, information must be collected to describe the child's activities associated with mouthing and ingestion of objects, residues on hands, surfaces, objects, or foods; surface residue, soil, and dust concentrations; and exposure factors relating to transfer of residues from those media to the mouth.

#### *Activity Information*

A questionnaire should be used to collect information on the objects that are mouthed or eaten most often by a child, and the characteristics of the activities that potentially result in dermal exposure or non-dietary ingestion of the contaminant of interest. The information on the important objects and locations of child activity should be used to determine what samples to collect to measure surface residue loadings. Information on mouthing characteristics (e.g., frequency of mouthing, surface area mouthed, teething), hand washing practices, eating environment, and the likelihood of the child handling food items should be linked to the sampled items to facilitate assessment of non-dietary ingestion exposure.

An activity timeline survey instrument can be used to collect information on the child's activities, the location of the child throughout the exposure period, surfaces contacted, and clothing worn during the exposure period. Use of a timeline that is completed by the caregiver during the exposure measurement period will generally be more accurate and less burdensome to the participant than the use of a recall questionnaire at the end of the measurement period.

The activities most likely to result in significant exposures are likely to vary with the developmental stage of the child. Very young children may be particularly susceptible to pesticide and metals exposures as the result of the microenvironments in which they spend time (e.g., kitchen floor), and the activities in which they are involved (e.g., pica, mouthing of hands and toys and handling foods). It is important to understand that physiological characteristics and behavioral patterns will result not only in different exposures for children and adults, but also for children of different developmental stages. Thus, exposure assessments are required for children in each age group, with age group being defined by developmental stage, as recommended by the Risk Assessment Forum (U.S. EPA, 2000).

#### *Residue Loading and Concentration Measurements of Objects and Surfaces*

The objects to be sampled should be representative of the objects that the child frequently comes in contact with and amenable to the designated methods of sampling. This information can be obtained through discussions with the child's caregiver and/or by observation of the child's activities. Questionnaires can also be developed to provide a systematic approach to defining the objects appropriate for sampling. All samples should be collected such that the measurements can be related in time to the activity data in the questionnaires.

Methods for collection of dust samples for assessing dermal and non-dietary exposure have been described by Lewis (2000) and Liroy et al. (2002). There are a number of different methods that have been used to collect dust from floors and other surfaces (e.g., upholstered furniture). They are either vacuum or wipe methods. A high volume surface sampler (HVS3) was developed specifically for quantitative

collection of dust from floors. The sampler has been used to determine dust loading and chemical concentrations in a number of studies of persistent and non-persistent organic chemicals and metals (e.g., lead). The ASTM Standard Practice for *Collection of Floor Dust for Chemical Analysis* (ASTM, 2003a) describes use of HVS3. Similar but lower-cost vacuum samplers have been used to collect metals, pesticides, and POPs from surfaces indoors (summarized by Lewis, 2000). The simplest method, one used in many studies, is to analyze dust samples collected by study participants using their own household vacuum cleaners. This approach provides data on the concentration of chemicals in the dust, but does not provide information on loading. Low volume air sampling pumps have also been used to collect dust on filters for lead analysis, as described in an ASTM standard practice (ASTM, 2003b).

One of the limitations of the vacuum methods for dust collection is that the methods do not quantitatively measure the amount of the dust that is actually available for transfer to the skin. Vacuum samples may draw dust from deep within carpet fibers, for example. To address this problem, methods have been developed to measure the “transferable residue” by attempting to simulate a child’s contact with a surface (based on pressure that would be applied by a child’s hand). Lewis (2000) summarized the available methods, their advantages, and their limitations. The three methods used most extensively during the last ten years include the PUF roller, the drag slide, and the California roller. All of these methods are applicable for use on carpet and hard surface floors. But the methods are complex and need to be performed by a trained technician.

For collection of metals, pesticides, and persistent organic pollutants in residues on hard surfaces, wipe sampling methods have been used most extensively. The wipe sampling methods involve use of media such as a filter, cotton, or gauze that may, or may not, be wetted with water or a solvent such as isopropanol. The performance of wipe sampling methods has been evaluated for a number of pesticides (Lewis, 2000). ASTM has published standard practices for collection of settled dust using wipe sampling methods for lead (ASTM, 2003c; 2003d) and organic compounds, such as PCBs, dioxins, and pesticides (ASTM, 2003e). Although wipe sampling methods are applicable to a wide range of chemicals, both non-persistent and persistent, the performance of these residue collection methods (collection efficiency and precision) has been reported for only a select set of chemicals, predominately the current use pesticides and metals such as lead. If the methods are to be applied in the NCS, method validation tests need to be performed for the chemicals of interest.

### *Dermal Exposure Measurements*

Dermal wipe methods have been used extensively in studies of lead exposure, in pesticide exposure studies, and for workplace measurements. The methods, originally developed for occupational exposure measurements, have been applied in children’s exposure studies. The dermal wipe consists of wiping the child’s hand with a commercial “baby” wipe or with cotton or gauze that has been wetted with water or a solvent such as isopropanol. A variation of the wipe is to rinse the hand with isopropanol by passing a stream of the solvent over the hand or by immersing the hand in the solvent. Sampling efficiency varies for hand rinses and hand wipes, as discussed by Brouwer et al. (2000) for workplace sampling and by Fenske and others (as reviewed by Lewis, 2000).

Hand wipe and hand wash techniques assess contamination adhering to an individual’s skin at the time of sample collection. Measurements of skin loading do not reflect losses that occur prior to sampling; e.g., volatilization or removal by hand washing. The protocol for sample collection is critical because timing of sample collection with respect to recent exposure or recent hand washing events has a significant impact on the measurement results. Skin loading over time may vary significantly and may be the result of many discrete events. Current sampling integrates dermal loading over extended time periods. Therefore, variations in time cannot be characterized with available measurement methods. One approach to address this issue is to use modeled estimates of dermal hand exposure using a time series

approach, by combining surface residues with dermal transfer coefficients, and reducing dermal hand loadings based on mouthing frequency and hand washing information.

### *Soil Measurements*

Soil may represent a significant route of exposure for some children. Methods for collection of surface soil samples are well developed (e.g., see Appendix 13.3 of the HUD Guidelines [U.S. HUD, 1995]).

### *Residue Measurements of Foods from Contacting Hands/ Surfaces*

Individual samples of foods that have been handled by a child prior to eating and samples of foods that have contacted surfaces during eating (e.g., cheese that has been placed on counter tops, floors, or high chair trays) can be collected to estimate the chemical loading on the food items caused by contact with contaminated surfaces and hands. These residues are not normally measured in dietary exposure studies that use duplicate diet sampling methods. Melynk et al. (2000) describe approaches that have been used to assess dietary exposure of children in a lead-laden environment. One approach to collect this data is to identify food items that a child in the study is known to handle when eating. During the monitoring period, the caregiver for the child collects one set of the individual food items that were not handled by the child and a second set that were handled by the child. Analyses of the two sets of the individual food items can provide the amount of pesticides transferred onto the foods. Another approach is for the monitoring technician to directly measure the transfer potential for samples of foods that have contacted surfaces. Food items should be collected that are of sufficient quantity such that there will be leftovers for collection, both handled and not handled. Another approach is to use standardized food items that can be contacted with contaminated surfaces so that sources of contamination can be identified, both within and among exposure scenarios.

#### 3.4.3 Exposure Factors

As described above, estimates of dermal and non-dietary exposures require use of a number of exposure factors. These are developed in laboratory tests and field studies. The Child-Specific Exposure Factors Handbook (U.S. EPA, 2002) contains exposure factors and the supporting information used to develop these factors. The following subsections describe recent work on exposure factors.

#### 3.4.4 Hand-to-Mouth, Object-to-Mouth, and Surface-to-Food Transfer Efficiencies

Transfer efficiencies (TE) are a function of:

- the properties and form of the chemical (residue, particle bound, formulation, age, physicochemical properties),
- characteristics of surfaces (hard, plush, porous, moisture, oil, content, age, loading, previous transfer),
- characteristics of skin (moisture, oil or fat content, age, loading, previous transfer),
- contact mechanics (sucking, licking, pressure, duration, smudge, repetition), and
- environmental conditions (temperature, relative humidity, air exchange, redeposition rate).

Transfer efficiencies are developed in laboratory studies. Available transfer efficiency data for chlorpyrifos were summarized by Zartarian et al. (2000). Zartarian et al. (2003) summarized transfer efficiency data for arsenic to skin from contact with treated wood.

### *Soil and Dust Ingestion Rates*

Based on a comprehensive review of soil ingestion studies, Zartarian et al. (2003) used a lognormal soil ingestion rate variability distribution with geometric mean of 31 mg/day, geometric standard deviation of 4, arithmetic mean of 81mg/day, and 95<sup>th</sup> percentile value of 303 mg/day, based primarily on Stanek and Calabrese (2000) and Stanek et al. (2001). These values are also consistent with the recommended values in EPA Children's Exposure Factors Handbook (U.S. EPA, 2002).

### *Surface Area of Hands Mouthed*

Zartarian et al. (2003) fit a beta (3.7,25) distribution with mean of 0.129 cm<sup>2</sup>, standard deviation of 0.0615 cm<sup>2</sup>, median of 0.120 cm<sup>2</sup>, 25<sup>th</sup> percentile of 0.0834 cm<sup>2</sup>, and 75<sup>th</sup> percentile of 0.165 cm<sup>2</sup>, based on a videography data set for 20 suburban children in which information on number of fingers mouthed and surface area categories (partial fingers, full fingers, palm with fingers, palm without fingers) was collected (Leckie et al., 2000). This is consistent with the EPA 2001 draft SOPs value of 20 cm<sup>2</sup> (central value) as the surface area of 3 fingers for oral hand-mouth contact, based on a 3 year-old child.

### *Surface Area of Objects Mouthed*

Leckie et al. (2000) presented data for the surface areas of objects mouthed by children based on a videography study. Additional information could be obtained from questionnaires during the exposure measurement period.

### *Frequency of Hand-to-Mouth Activity*

The frequency of hand-to-mouth activity has been reported in several studies (Leckie et al., 2000; Zartarian et al., 1998; Reed, 1998; Reed et al., 1999; Tolve et al., 2002). Data from these studies were used by Zartarian et al. (2003) to fit a Weibull (0.73,6.93) distribution with a mean of 8.45 contacts per hour, standard deviation of 11.75, median of 4.21, 25<sup>th</sup> percentile of 1.27, and 75<sup>th</sup> percentile of 10.86. Leckie et al. (2000) obtained frequency of hands actually inserted into mouth based on videography analyses for 20 children outdoors. Reed (1998) and Reed et al. (1999) reported hourly frequency counts of hand mouthing behaviors for 30 daycare and residential NJ children ages 2-5 years. Zartarian et al. (1998) reported skin-mouth contacts for each hour of the study day for 4 children in the Salinas Valley of California. Reed et al. (1999) provided the basis for EPA OPP Draft SOP 2000 values of 9.5/hr (central) and 20/hr (high end). Tolve et al. (2002) reported indoor hourly mouthing frequencies for 69 children less than 24 months old and 117 children greater than 24 months. Two other studies with summary statistics were used for the uncertainty distributions. Freeman et al. (2001) reported hand-to-mouth contacts/hr (mean +/-std) based on 4 hrs observation per child for 19 children in MN. Black et al., (2003) reported data for 4 hrs of videotaping for 6 children ages 7-12 months, 10 children 13-24 months, 13 children 25-36 months, and 7 children >36 months.

### *Hand-to-Mouth Dermal Transfer Fraction (Saliva Removal Efficiency)*

Camann et al. (1995) reported approximately 50% removal efficiency by human saliva for chlorpyrifos on freshly spiked human hands. This is consistent with OPP standard operating procedures presented in EPA (2001). For arsenic on skin, Zartarian et al. (2003) fit a beta (14.5,4.1) distribution with mean of 0.780, standard deviation of 0.0935, median of 0.790, 25<sup>th</sup> percentile of 0.721, and 75<sup>th</sup> percentile of 0.849. This was based on a triangular distribution using a lower bound of 0.5 from Camann et al. (1995), a mode of 75%, upper bound assumed as 100%.

### *Frequency of Food-to-Surface-to-Mouth Contact*

Data are currently inadequate to estimate the frequency of food-to-surface-to-mouth contacts. This information needs to be collected during the exposure measurement period.

## 3.5 Measurements of Exposures to Persistent Organic Pollutants

### 3.5.1 Introduction

Many organic chemicals are persistent in the environment, are not readily metabolized in the human body, and therefore bioaccumulate, often in fatty tissues. The classes of chemicals that are persistent organic pollutants (POPs) include certain congeners of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, polybrominated diphenyl ethers (PBDEs), and other halogenated flame retardants as well as other persistent organochlorine compounds (including pesticides) and certain perfluorinated compounds (e.g., perfluorooctanoic acid and perfluorooctyl sulfonate). Potential human health concerns, including adverse neurodevelopment in children, have been associated with exposure to select POPs. Several POPs have been identified as potential endocrine disrupting chemicals (EDCs) that may affect normal functioning of the endocrine system in humans and wildlife. Measurements of exposures to a number of different POPs will enable the NCS to address hypotheses focusing on neurobehavioral outcomes and early onset of puberty. **Table 1-1** lists some of the chemicals considered to be POPs.

Humans are exposed to POPs in a number of different environmental media. Researchers need to perform aggregate exposure assessments for POPs to assess exposure and relate it to health outcomes. This approach involves estimation of exposures by all routes and pathways in all microenvironments that the individual occupies. This type of exposure assessment is also referred to as “total” exposure. This is the same approach as is required for the non-persistent chemicals described in **Section 3.6**. To estimate exposures via all routes (inhalation, dietary ingestion, indirect (non-dietary) ingestion, and dermal) by environmental measurements would require measurements of concentrations of the chemicals in air, water, food, soil, house dust, surface dust, and other media, and measurements or estimates of the duration and frequency of contact with the media. However, because of cost limitations, the media to be collected and analyzed should be prioritized based on the chemical(s) to be measured, the age (life stage) at which exposure occurs, and the microenvironments that the study participants occupy. For example, children’s exposures by the dermal and indirect ingestion routes may be highest during the life stage when they crawl, spend large amounts of time in contact with floors and other contaminated surfaces, and have high mouthing activity. However, for an adolescent, dietary ingestion may be the most important route of exposure. To relate exposure to health outcomes, exposures must be measured during the developmental periods that are critical to the health effect.

For children eating solid food, especially meat and dairy products, and for adults, diet is generally the most important route of exposure for most POPs. Methods for collection of duplicate diet samples are well developed and have been implemented in a number of studies, as described in **Section 3.3**. Therefore, fetal exposures to most POPs occur primarily from the mother’s consumption of food containing these POPs. Breastfeeding infants are exposed to most POPs by ingesting mother’s milk, which again are present due to their mothers’ diets. For example, it has been estimated that diet contributes more than 90% of the total dioxin intake in adults.

For young children, exposures to POPs may occur due to dermal exposure or ingestion of dust containing the chemicals. The dusts may be in carpet, on hard flooring surfaces, or on other indoor

surfaces. Past studies of children's pesticide exposures have focused on measurements of dust and residues primarily from floor surfaces. Some studies also collected residue samples from other surfaces that a child may contact such as tables and furniture. In a limited number of studies, residues were collected from surfaces that the child's food might contact such as high chairs and countertops. For some persistent organics, such as brominated flame retardants, exposure may occur due to dermal contact with surfaces such as upholstered furniture and mouthing of treated furnishings. The sampling protocols need to assess all relevant sources of exposure when selecting the sampling locations and media to be collected. However, sampling methods are not well developed for these more complex surfaces and there are inadequate data on contact frequency and transfer efficiency for use in algorithms to estimate exposure by the dermal or indirect ingestion routes.

Soil may represent a significant route of exposure for some children. Methods for collection of surface soil samples are well developed. Methods developed for collection of surface samples of soil for metals determinations are applicable to measurements for POPs. However, sampling protocols need to specifically address the chemicals of interest with regard to the sampling equipment, storage containers, and storage methods to avoid contamination of the samples.

As discussed in the following sub-sections, the costs and burden for collection of environmental media for measurements of POPs are high. Sample analyses costs are also high. As a result, exposure estimates based on measurements of the POPs in environmental media may not be practical for the general study, but may need to be limited to special studies conducted for subsets of the study population. As discussed in other sections of this document, collection of biological samples, or more preferably some combination of biological and environmental samples, may be more appropriate for estimating exposure to both persistent and non-persistent organic chemicals.

### 3.5.2 Exposure Assessment Methods for POPs

Because POPs are a diverse set of chemicals, there is no single method that can be applied for their measurement in the NCS. Specific chemicals will need to be identified before selecting the appropriate sample collection, sample storage, sample processing, and analysis methods. However, the methods for collection of samples and analyses of POPs in environmental media are very similar for many of the classes of chemicals. They are also very similar to the methods used to collect and analyze matrices for the non-persistent chemicals described in *Section 3.6*. The following subsections, therefore, provide a general description of the methods used for sampling and analyses of various media for POPs; the discussion does not address either specific classes of chemicals or individual chemicals. Detailed descriptions of methods are available in the scientific literature. *Table 3-10* summarizes the general multi-media sampling and analysis methods that may be used to measure POPs in environmental media.

#### *Measurement Methods for Assessing Inhalation Exposures*

POPs are normally semi-volatile compounds that may exist in air in the gas phase or as particulate matter. Therefore, air samples are generally collected with a sampler that is a combination of a filter for collection of particulate and a sorbent such as XAD or PUF for collection of the gas-phase. Samples are collected using vacuum pumps. The volume of air sampled depends on the pump sampling rate and the duration of the sampling period. For collection of samples indoors, the sampling rates are low (liters per minute) so that quiet pumps can be used and so that the collection of the sample does not substantially increase infiltration of outdoor air into the structure. A number of studies are cited in *Section 3.6* that used low-volume sampling methods (0.5 to 4 lpm) for collection of pesticides with these filter/sorbent methods. Inhalation may be measured by collection of personal exposure samples with pumps and filter/sorbents worn by the study participant. Personal samples have been collected in some exposure studies. But, the pumps are relatively heavy, the sampling duration is long (generally 24 to 48 hours), and the participant burden is high for collection of personal samples. Due to the heavy burden on participants,

personal samples are generally not collected for POPs. If they are collected, personal samples can only be collected for adults because the pumps are too large and heavy for children to wear. Therefore, air samples for measurements of POPs are usually collected with samplers placed at fixed sites indoors and outdoors. This limits the ability to measure the “total” exposure by inhalation in all microenvironments occupied by study participants. Collection of samples by active pumping methods is complex and must be performed by trained technicians. The costs for sample collection, therefore, are high.

**Table 3-10. General Sample Collection and Analysis Methods for POPs**

Media	Collection Method	Analysis Method	Detection Limit	Analysis Costs (per sample)	Comments
Air	Personal – Pump/ Filter/ Sorbent	GC/MS; LC/HRMS; LC/MS/MS	Low ng/m <sup>3</sup>	\$300-600	a, b, c, d, e
Air	Indoor or Outdoor – Pump/Filter/Sorbent		Low ng/m <sup>3</sup>	\$300-600	
Air	Passive -SPMD		Low ng/m <sup>3</sup>	\$300-600	f
House dust	Vacuum samplers		Low µg/g	\$300-600	g
Surface residues	Wipe samples		< 1 µg/m <sup>2</sup>	\$300-600	g
Hand residues	Wipe or rinse		< 1 µg/m <sup>2</sup>	\$300-600	g
Soil	Surface scraping		Low µg/g	\$300-600	g
Diet	Duplicate diet		Low µg/kg	\$300-600	
Water	Tap sample		Low ppt	\$300-600	

Comments:

- (a) For all media, samples generally must be shipped and stored frozen
- (b) Sample processing involves solvent extraction/cleanup/concentration, then analysis by chromatography/mass spectrometry method for most POPs in all media
- (c) Personal samples can not be collected for young children,
- (d) Costs are high for both collection and analysis
- (e) Long-term storage stabilities need to be assessed
- (f) Long-term passive sampling – the method is not validated for many chemicals; sampler preparation, extraction, and analysis costs are high
- (g) Time/activity and mouthing activity data need to be collected to estimate exposure by dermal and indirect ingestion routes

Samples collected on filters and sorbent media are returned to the laboratory for extraction, clean-up, concentration, and analysis. Because of the high cost of sample analysis, the gas-phase and particulate fractions are usually combined for analysis. Analysis is generally performed by gas chromatography (GC) or high performance liquid chromatography (HPLC) with mass spectrometer (MS) detectors, which are selective and provide positive identification of the chemicals of interest. Analysis may be performed at a slightly lower cost with non-selective detectors, such as an electron capture detector (ECD) for halogenated compounds, if the analysis is performed for only a few select analytes and their identification is verified by a selective detection method. Costs for sample analysis are relatively high. Depending on the class of chemicals being analyzed and the number of analytes being analyzed in the sample, costs will generally range from \$300 to \$600 per sample.

An alternative to the use of pumps to collect the air sample is the use of passive sampling methods. There are few passive methods available for the semi-volatile POPs. A semi-permeable membrane device (SPMD) has been developed and evaluated for select POPs. The method has been demonstrated to be a suitable method for long-term (one to 12 months) collection of a number of POPs including organochlorine pesticides and PCBs (e.g., Ockenden et al., 1998). The SPMD is deployed for measurements indoors by hanging it in a suitable area for extended periods. The SPMD gives an average concentration for the selected analytes for the total period in which it is deployed. Sampling rates must be determined for each chemical in laboratory tests prior to use of the SPMD. Although the labor requirement for deploying and retrieving the sampler are lower than that for active (vacuum pump)

samples, costs for preparation of the SPMD are relatively high. Costs for extraction and analysis of the SPMD are comparable to that for filter/sorbent samples.

### *House Dust, Surface Residues, Soil, and Dermal Exposure Measurement Methods*

Methods for collection of dust and wipe samples for analysis for non-persistent chemicals are described in **Section 3.6**. The same sampling methods are applicable for collection of soil, dust, and surface residues for measurements of POPs, and are only briefly summarized in this section.

Methods for collection of dust samples for exposure assessment and source characterization have been reviewed recently by Lewis (2000) and Liroy et al. (2002). There are three primary approaches for collecting surface dusts and chemical residues to estimate children's exposures to POPs by the dermal route or by indirect ingestion. They include the following:

- Vacuum methods – A number of different vacuum methods have been used to collect dust from carpets and hard surfaces (see Lewis, 2000). A high volume surface sampler (HVS3) was developed specifically for quantitative collection of dust from floors. The sampler has been used to determine dust and chemical loading in a number of studies of persistent and non-persistent chemicals. Similar, but lower cost vacuum samplers have been used to collect metals, pesticides, and POPs from surfaces indoors. In a number of studies, participants have collected dust samples using their own household vacuum cleaners. This approach provides data on the concentration of chemicals in the dust, but does not provide information on loading. House dust collected from floors has been shown to contain a large number of POPs. Measurements of the concentrations of chemicals in floor dust are difficult to use to estimate exposure without information on loading and children's activities. To estimate exposure by the dermal and indirect ingestion routes, additional information is needed on the frequency of contact with contaminated surfaces, the efficiency of transfer from the surface to the skin, dermal transfer rates, and ingestion rates (from contaminated skin to the mouth).
- Surface residue methods – One of the limitations of the vacuum methods for dust collection is that the method does not actually determine the amount of the dust available for transfer to the skin. Surface residues that may be available for transfer to the skin have been measured in numerous studies of children's exposure to pesticides. Many of the "transferable residue" sampling methods attempt to simulate a child's contact with a surface (based on pressure that would be applied by a child's hand) to estimate the amount of residue that may transfer to a child's skin. Lewis (2000) summarized the available methods, their advantages, and their limitations. The three methods used most extensively during the last ten years include the PUF roller, the drag slide, and the California roller. All of these methods are applicable for use on carpet and hard surface floors. But the methods are complex and need to be performed by a trained technician. For hard surfaces, wipe sampling methods are also available. The wipe sampling methods involve use of media such as a filter, cotton, or gauze that may, or may not, be wetted with water or a solvent such as isopropanol. The residue collection methods are applicable to a wide range of chemicals, both non-persistent and persistent. But the performance of these residue collection methods (collection efficiency and precision) has been reported for only a select set of chemicals, predominately the current use pesticides. If the methods are to be applied in the NCS, method validation tests need to be performed for the chemicals of interest.
- Dermal wipe and rinse methods – Dermal wipe methods have been used extensively in pesticide exposure studies to measure chemical residues on the skin. The methods, originally developed for occupational exposure measurements, have been applied in children's exposure studies. The dermal wipe consists of wiping the child's hand with a commercial "baby" wipe or with cotton or gauze that has been wetted with water or a solvent such as isopropanol. A variation of the wipe is

to rinse the hand with isopropanol by passing a stream of the solvent over the hand or by immersing the hand in the solvent. Hand wipes or rinses are useful for determining the chemicals on the skin that are available for absorption into the body or for ingestion. However, the protocol for sample collection is critical because timing of sample collection with respect to recent exposure or recent hand washing events has a significant impact on the measurement results. Many studies have failed to show a relationship between hand wipe or rinse measurement results and exposure based on biomarker measurements.

### *Dietary Exposure*

The general methods for estimating dietary exposure that were described in **Section 3.3** for metals are applicable for the POPs with modifications that address handling and storage that are specific to the chemical class of interest.

#### 3.5.3 Analytical Methods

The methods used to analyze environmental samples for POPs are complex, time-consuming, and relatively expensive. Analysis generally involves extraction of the media with a solvent, clean-up of the extract to remove interfering compounds, concentration of the extract, then analysis by a chromatography system with a selective detector, usually gas chromatography (GC) with mass spectrometry (MS) or high performance liquid chromatography (HPLC) with MS or MS/MS. Because the extraction, clean-up, and analysis methods differ for the different classes of compounds, and for different compounds within the classes, detailed discussion of the methods is beyond the scope of this paper. Multi-residue methods that allow quantitation of many chemicals in the same analysis have been developed for all of the classes of POPs. Rudel et al. (2001), for example, reported multi-residue methods for analyses of selected PAHs, PCBs, pesticides and phthalates in air and dust. There are numerous publications in the scientific literature describing analytical methods. The methods need to be selected, adapted, and optimized for the specific compounds selected for the study. In all cases, laboratories must demonstrate successful method performance prior to their use in a study of this size and significance.

Because of the complexity of the analyses, costs for analyses of POPs are high. In general, costs will be \$300 to \$600 per sample, depending on the media and the compounds being measured.

#### 3.5.4 Issues Related to Exposure Assessments for POPs

To assess children's aggregate exposure to POPs, researchers need data that allow estimation of exposure by each route. Measurements of chemicals in house dust, surface residues, diet, and air will provide an indication of potential exposure, but measurements alone are inadequate for estimating exposure. For example, to estimate dermal exposure, information must be collected to estimate the frequency and duration of contact with the contaminated surfaces as well as the transfer efficiency (surface to skin). For estimates of exposure by indirect ingestion, information that needs to be collected includes (1) the amount of contact with the contaminated surface (number of contacts and the area of skin contacted), (2) the transfer efficiency (surface to skin), (3) the frequency of mouthing (hand to mouth), and (4) an estimate of the amount of chemical ingested (number of mouthing events, area of hand placed in mouth, and transfer efficiency for hand to mouth). Although this information is difficult to collect accurately, it is required to estimate exposure if this is an important route of exposure. If the data cannot be collected within the constraints of the study, researchers should collect surrogate measures (e.g., estimates of the magnitude of the child's mouthing activity) to estimate exposures. Researchers in academia and government continue to develop protocols for collecting this information more effectively and more efficiently (U.S. EPA, 2003).

The sample collection protocols for POPs must address all potential sources to determine the locations and media to sample. For example, if PBDEs are targeted for analysis, upholstered furniture that contains treated foam should be considered as a potential location that the child may contact or mouth.

Although sampling and analysis methods are generally available for the chemicals that are classified as POPs, the methods are complex and expensive to implement. Sample collection generally must be performed by trained technical staff. Collection of samples by participants is not a viable option. The chemical analysis of the samples is also complex and expensive, precluding analyses of large numbers of samples. Measurements of POPs in environmental media likely will need to be restricted to a subset of the study cohort. Measurement data from a subset of the cohort, used in combination of source information, time/activity data, and other exposure information collected with questionnaires may then be used for exposure classification in the larger cohort.

### 3.6 Measurement of Exposures to Non-Persistent Pesticides

Quantifying child exposures to non-persistent pesticides is necessary to address NCS hypotheses on neurodevelopment. Non-persistent pesticides are chemicals that are metabolized and excreted from the body in hours or days and that do not accumulate in body tissues such as fat, in contrast to the older organochlorine pesticides. These insecticides also often degrade rapidly in the ambient environment. However, persistence in the indoor environment is likely to be longer due to the lack of degradation by microorganisms, hydrolysis and ultraviolet light. Non-persistent pesticides are routinely used in homes and agriculture and are found in dust, on surfaces, and in air, drinking water, food, and soils in many of the environments in which children and adults spend their time. Measurements of pesticides in environmental media for key routes of child exposures, e.g., dietary and non-dietary ingestion, inhalation, and dermal absorption, augment exposure measurements based on biomonitoring. In many cases, for example the organophosphate oxydemeton methyl, laboratory methods are available for parent pesticide compounds in the environment whereas no laboratory methods are available for the parent compound or pesticide-specific metabolites in biological media. Thus, environmental monitoring can provide information about exposure that cannot be quantified with biomonitoring. Utilizing measurements of pesticides in environmental media in concert with biomonitoring will improve our ability to accurately classify exposure. As discussed above (see *Section 2.1.2*) the NCS Exposure and Neurobehavioral Outcome Inter-Working group has recommended that the NCS focus principally on neurobehavior effects following pre-natal exposures to the OP, carbamate, pyrethroid and neo-nicotinoid insecticides.

**Table 1-1** provides a list of insecticides that fall into these classes. This list is not comprehensive and will need to be updated prior to finalizing the NCS study design. NCS study planners should track pesticide use patterns and build in flexibility to evaluate and measure new pesticides as use and market patterns change. In this section we review strategies to measure pesticides in environmental media and estimate exposures to children.

#### 3.6.1 Personal and Indoor Air Sampling

Many pesticides are semi-volatile (Lewis et al., 2001) and are readily detectable in indoor and personal air samples. These include the organophosphate and carbamate insecticides, many of the older organochlorine compounds, herbicides such as alachlor, atrazine, 2,4-D and dicamba, and several fungicides (e.g., folpet and ortho-phenylphenol) (Geno et al., 1995). The pyrethroids are less volatile and some of the newer insecticides (e.g., abamectin) are basically non-volatile. Air sampling may thus not be the best protocol for these less volatile compounds; however, both semi- and non-volatile pesticides can be resuspended into air on particles by human and pet activity (Lewis et al., 2001). There have been numerous prior studies of pesticide levels in indoor air (Lewis, 2001; Whitmore, 1994; Andrew et al., 2003) and personal air (Whitmore, 1994; Andrew et al., 2003; Whyatt et al., 2002; Whyatt et al., 2003).

Indoor air samplings have been conducted over hours to weeks at flow rates ranging from 0.5 to 4 liters per minute (LPM). Sampler height needs to be considered as prior research indicates that residential pesticide air concentrations vary with height, being greatest near the floor (Fenske et al., 1991; Albertini, 2001). Due to participant burden, personal air samples have generally been collected over shorter time periods (24 to 48 hours) at the higher flow rates (e.g., 4 LPM). However, a recent study collected 6-day integrated average personal air sample from 60 children in Minnesota (Andrew et al., 2003). Indoor air pesticides levels have been shown to be considerably higher than outdoor air levels.

Most prior air monitoring studies have used the EPA Compendium Method TO-10A, with a URG 2500-25A model self-contained air sampling cartridge comprised of a 2.5- $\mu\text{m}$  inlet, a 2.2-cm quartz-fiber particle filter and a 2.2-cm X 7.6 cm polyurethane foam (PUF) vapor trap (Whitmore, 1994; Pang et al., 2002; Whyatt et al., 2002). The choice of sampler inlet may not affect monitored pesticide levels; a 1994 study on chlorpyrifos and lindane showed that similar ambient air concentration were determined using an open-faced compared to 2.5  $\mu\text{m}$  inlet sampler (Camann et al., 1994). It is important that sampling pumps be quiet to reduce participant burden. Pesticide detection limits depend on the analytical technique and amount of air sampled but are generally in the low  $\text{ng}/\text{m}^3$  range. Samplers need to be frozen upon collection ( $-12^\circ\text{C}$ ) and shipped to participating analytical laboratories on dry ice. A storage stability study of 10 pesticides (including chlorpyrifos and diazinon) found that the compounds were generally stable in the sampler at  $-12^\circ\text{C}$  over 90 days (Camann and Wyatt, 2001). After the pesticides are extracted from the filters and PUF plugs, they appear to be very stable in the frozen extract, but this has currently been assessed only for up to 240 days (Ortiz et al., 2000). Dual-column gas chromatography with electron capture detection (GC/ECD) is a sensitive analytical technique for determination of chlorinated pesticides (Geno et al., 1995; Whitmore et al., 1994). A wide variety of neutral and acid pesticides, including the organochlorines and organophosphates, can be detected by a multiresidue GC/MS screen operating in a selected-ion monitoring mode, which is the method of choice for these compounds (Geno et al., 1995). LC/MS is needed for analysis of the more polar and non-volatile pesticides (e.g., hydramethylnon, fenoxycarb, sulfluramid, imidacloprid, and abamectin). Analytic costs range around \$400-\$500 per sample.

Prior studies have shown that inhalation exposure to semi-volatile pesticides in indoor air can be substantial and may be a primary route of exposure following residential use of insecticides (Whitmore et al., 1994; Fenske et al., 1990; Whyatt et al., 2002; Whyatt et al., 2003). However, for any given pesticide/exposure scenario, the primary route of exposure (inhalation versus ingestion) will depend both on use patterns and on the volatility of the pesticides. For example, an aggregate exposure assessment of chlorpyrifos among residents from Baltimore, Maryland, found that inhalation exposures accounted for approximately 85% of total daily dose (Pang et al., 2002). Similarly, results from the U.S. EPA Non-occupational Pesticides Exposure Study (NOPES) indicate that 85% of the total daily exposure of adults to airborne pesticides is from breathing air inside the home (Whitmore et al., 1994). By contrast, a recent assessment of pesticide exposures among children in Minnesota found that exposure to the four pesticides assessed (chlorpyrifos, diazinon, malathion and atrazine) came principally from ingestion rather than inhalation (Andrew et al., 2003).

Studies with chlorpyrifos, which is similar to many other semi-volatile pesticides, indicate that following residential application, indoor levels peak and then decline fairly rapidly in a two-phase process: an aerosolized particle phase (with residue concentration on surface areas peaking after 36 hours) and a gas phase during which chlorpyrifos gradually volatilizes into the room. This gas phase begins 12 hours after application and continues for a least two weeks (with residue concentrations on surface areas peaking after 72 hours at levels similar to those in the initial particle phase) (Gurunathan et al., 1998). Although levels of semi-volatile pesticides decline substantially after use, evidence indicates that they are more persistent in the indoor than outdoor environment because there is less degradation by microorganisms, hydrolysis and UV light. For example, chlorpyrifos was detected in 100% of two-week

integrated indoor air samples collected continuously over 8 weeks from 49 apartments in New York City between March, 2001 and August, 2002 after residential use of the insecticide had been banned (Whyatt et al., in preparation). There was little variation in the indoor air levels of the insecticide and the correlation between chlorpyrifos levels in each of the two-week integrated indoor air samples was highly significant ( $r=0.7-0.96$ ,  $p<0.001$ ).

Studies have reported a high degree of correlation ( $r>0.7$  to  $r=0.99$ ) between pesticide levels in personal air and indoor air and between indoor air levels and levels in carpet dust, handwipes (including from mothers and children), and surfaces in the home (Whitmore et al., 1994; Pang et al., 2002; Andrew et al., 2003; Camann et al., 1995; Gordon et al., 1999). Dermal exposure and non-intentional ingestion may thus also be important routes of exposure to pesticides following residential use, particularly for children (Fenske et al., 1990; Gurunathan et al., 1998; Simcox et al., 1995). However, uncertainty remains over the extent of exposure from these sources (Lu and Fenske, 1999). One prior study reported significant associations between maternal self-reported use of can sprays, pest bomb and exterminators during pregnancy and levels of diazinon and propoxur in personal air samples collected over 48-hours during the 3<sup>rd</sup> trimester (Whyatt et al., 2003).

Low-level exposures to organochlorine pesticides are still possible even though these insecticides have been banned for decades. For example, Whyatt et al. (9) detected DDT and chlordane in the majority of personal air samples (68%-78%, respectively) collected over 48-hours during pregnancy from 72 women residing in minority communities in New York City. However, levels were generally low (averaging 0.2-0.4 ng/m<sup>3</sup>). Data from NHANES indicate the exposures to DDE, a persistent metabolite of DDT, are widespread, with higher exposure among some ethnic groups such as Mexican-Americans (<http://www.cdc.gov/exposurereport/2nd/pdf/dichlorodiphenyltrichloroethane.pdf>). The organochlorine insecticides should therefore be considered among the potential confounders in hypotheses evaluating health effects of current-use insecticides.

### 3.6.2 Dust, Surface, and Dermal Wipe Sampling

Multiple organic chemicals (both persistent and non-persistent) can be measured in a single house dust sample. For example, laboratory methods are available for pesticides (both semi-volatile and non-volatile), PCBs and other organochlorine compounds, dioxin, dibenzofurans, polycyclic aromatic hydrocarbons and phthalates (Rudel et al., 2001; Butte and Heinzow, 2002). Studies designed to characterize children's exposure to pesticides indicate that the largest number of pesticides and the highest concentrations are found in household dust compared to air, soil and food (Lewis et al., 1994). House dust samples in which no pesticides are found are rare (Butte and Heinzow, 2002). In addition, whereas air levels of semi-volatile pesticides decline rapidly after use, levels remain more constant in house dust and can still be detected for months or years after use (Rudel et al., 2001; Roinestad et al., 1993). Because of hand-to-mouth activities, dust may thus be a significant medium of contaminant exposure for young children in the home environment. This has led some researchers to conclude that the majority of household pesticides are better detected by dust sampling than by air sampling (Butte and Heinzow, 2002).

Chlorpyrifos appears to be the organophosphate pesticide found most often in house dust samples in the U.S. (Whitmore et al., 1994; Lewis et al., 1994; Butte and Heinzow, 2002; Roinestad et al., 1993; Camann and Buckley, 1994). However, diazinon has also been detected (Bradman et al., 1997). Permethrin is the pyrethroid that has been detected most often (Butte and Heinzow, 2002; Lewis et al., 1999). The older organochlorines such as DDT and chlordane are also often found in house dust samples. Camann et al. (2002) measured four herbicides and pentachlorophenol in dust samples collected from 622 homes in various parts of the U.S. The pesticide profile varied by region. Levels of pentachlorophenol and the herbicides 2,4-D and dicamba were highest in Iowa and smallest in Los Angeles; the herbicide MCPA

was more prevalent in Detroit, and 2,4,5-T was detected most frequently in Seattle (Camann, 2002). Fenske et al. recently reported that significantly higher levels of chlorpyrifos and parathion were found in dust samples from homes in close proximity to pesticide-treated farmland (Fenske et al., 2002).

Most prior studies have collected a sample of house dust from carpets or rugs with the high-volume, small-surface HVS-3 sampler. The HVS-3 is a cyclone-equipped vacuum sampler developed for U. S. EPA which collects small particles in a Teflon catch bottle (Roinestad et al., 1993; Roberts et al., 1995). Dust has also been collected using other vacuuming devices (Thompson et al., 2003) and several studies have sampled non-carpeted areas, although dust levels are much lower. In all cases, the protocols are labor intensive because they require that the sample be collected by the study team. Dust is collected from a pre-defined area using standardized protocols. Dust samples are sieved to remove large non-dust debris. Results are expressed in concentrations of pesticide per gram of dust or per area sampled (loadings). In one study, comparison of replicate carpet dust samples taken weekly over two weeks from the living areas of two homes showed pesticide concentrations generally differed by a factor of 2, whereas loadings differed by a factor of 2-5 (Lewis et al., 1994).

Studies have also collected dust samples by asking the participants themselves to save the bag from a vacuum cleaner (Roinestad et al., 1993; Colt et al., 1998) compared levels of pesticides and other compounds in dust obtained from used vacuum cleaner bags to those collected by the HVS3 among 15 homes and found reasonably comparable results. Advantages of this method include the relatively low cost of sample collection. Disadvantages include the fact that participation is limited to those subjects who own a vacuum cleaner. Further, although the protocol allows determination of contaminant concentrations/gram dust, concentration loadings cannot be assessed.

Prior research protocols have generally involved extraction of contaminants from the dust within several months of collection. Contaminants appear reasonably stable in the extracts over long periods of storage. However, stability of contaminants in the dust samples themselves has not been determined and would need to be determined if the extraction step was eliminated. Pesticides can be measured in dust samples as small as 0.1-0.5 grams of sieved dust or less. However, detection limits are a function of dust sample size and larger samples (2-5 grams sieved dust) are preferred. Analytic costs are approximately \$500-\$600 per dust sample.

Dust samples have also been collected using the Edward and Lioy (EL) hand press sampler and the Lioy, Wainman and Weisel (LWW) surface wipe sampler (Lioy et al., 2000). The EL sampler has been designed to collect surface concentrations of dust and pesticides that are representative of those adhering to the human hand. A prior validation study concluded that compared to hand presses, cotton gloves, synthetic skin, adhesive labels and soft-wick sponge, the EL sampler most accurately mimicked uptake of particles by the human hand (Edwards and Lioy, 1999). A significant correlation was seen between chlorpyrifos levels in EL surface and carpet samplers (Lioy et al., 2000). The LWW sampler has been used to obtain dust samples from smooth surfaces in the home (Lioy et al., 2000). A protocol that is currently being validated by NIEHS researchers involves mailing study participants an alcohol wipe with instruction for wiping dust on the top of a specified door frame. The sample is then placed in a zip-top bag and mailed back to the study team. Advantages include low cost of sample collection and low participant burden. However, research is currently ongoing to determine detection limits and detection frequencies using this method.

Several protocols have been used or are under development to define the relationship between home environmental pesticide measures and actual pesticide exposure to children. A limitation of dust sampling is that the timing of application is not known and levels in the dust may reflect use months to years prior to the sampling. The type of dust sample may also yield different results and may be difficult to interpret for exposure. For example, dust on hard surfaces may be readily available to transfer to children's skin

and result in non-dietary ingestion or dermal exposures, whereas pesticide contaminated dust lodged deeply in carpets may not be available to children. Carpet and other dusts may function as a reservoir for household pesticide contamination, re-contaminating surfaces and air after cleaning depending on the physical and chemical properties (“fugacity”) of the specific compounds. As noted in *Section 3.7.1*, air and dust pesticide levels are often correlated. However, studies on the inter-relationships of environmental and personal exposure measures can be difficult to interpret. Initial attempts to look at direct child exposures have included the use of handwipes to collect pesticides directly off of children’s hands. These methods include wiping the child’s hand with a sterile gauze dressing pads that have been moistened with propanol (Bradman et al., 1997) or asking the child to place wash his/her hand in a bag containing propanol (Lioy, 2000). In 1999, Gorden et al. (1999) found excellent correlations between chlorpyrifos in indoor air and corresponding dermal wipes but poor correlations between chlorpyrifos in dust and dermal wipes. Another study reported “a weak association between concentrations of OP pesticides in housedust, loadings in housedust, and concentration on hands, hand surface area, and urinary levels of OP metabolites” (Shalat et al., 2003). However, hand loadings of OP pesticides were more strongly associated with urinary OP metabolite levels. This finding suggests that on a cross-sectional basis, pesticides on hands may be more strongly correlated with exposure biomarkers. On a longitudinal basis, however, the dust measure may provide better classification of potential and actual exposure. EPA pilot studies, in part to support the NCS, are also investigating other methods (surface wipe, surface press, and clothing dosimeters such as union suits and socks) combined with housedust concentrations to define transfer coefficients and thereby quantify the relationship between environmental pesticide concentrations and children’s exposures. Results of these studies are expected in 2004.

### 3.6.3 Estimating Dietary Exposures

Diet is a potentially significant pathway of exposure to pesticides for children (National Research Council; Fenske et al., 2002). Market-basket survey by the USDA indicate that most foods types contain some pesticides residues (Agricultural Marketing Service, 2000). However, pesticide residues vary significantly across foods. For example, more than 50% of the foods (domestic and imported) analyzed as part of the FDA surveillance program for 1992 contained no pesticide (FDA Monitoring Program, 1993). Of the 4914 food items analyzed for the FDA Total Diet Study (1986-1991), 9% had residues of chlorpyrifos and 11% had residues of diazinon (Gunderson, 1995). Therefore, it has been difficult to estimate individual dietary exposures with any reliability using food consumption questionnaires (MacIntosh et al., 2001). Instead, studies have generally estimated dietary exposures by measuring pesticides in duplicate diet samples, in which study participants prepare and collect duplicate portions of all foods and beverages consumed.

#### *Duplicate Diet Sampling*

For example, MacIntosh et al., collected 4-day composite duplicate plate samples from a stratified random samples of 75 individuals in Maryland between 1995 and 1996. Chlorpyrifos was detected in 38% of solid food samples, malathion in 75% and p,p'-DDE in 21% (MacIntosh et al., 2001). Dietary intake was estimated to account for 13% of the aggregate exposure to chlorpyrifos (Fenske et al., 1990) and 7% of chlorpyrifos metabolites in urine (MacIntosh et al., 2001). In a study of aggregate exposures to four pesticides (chlorpyrifos, malation, diazinon and atrazine) among 102 children from Minnesota, Clayton et al., concluded that pesticide intakes came principally from ingestion of solid food rather than from inhalation. These findings are supported by results from a recent study that compared organophosphate metabolite levels in urine samples collected from children in Seattle fed organic versus conventional diets (Curl et al., 2003). Mean and medium levels of the dimethyl metabolites were 6 and 9 times lower, respectively, among children on the organic diets, suggesting that diet can significantly contribute to total pesticide exposure.

Laboratory methods for food often involve extensive clean up steps to address fatty and non-fatty foods. Some researchers recommend that acidic foods (e.g., fruit) should be collected separately from non-acidic foods (e.g., bread), potentially increasing participant burden and the possibility of error. Analytical costs are approximately \$500-\$600 per dust sample.

### *Infant formula and Breastmilk*

Solid foods are usually introduced to children at 4-6 months. Prior to this age, virtually all dietary exposures, if present, will be due to contamination of formula (powder or water) and possibly breastmilk. This section reviews information available on pesticides in these media.

**Infant Formula.** Several studies have investigated pesticide contamination in milk- or soy-based infant formula. In the U.S., Gerlardi and Mountford (Gerlardi and Mountford, 1993) reviewed tests on 2043 milk-derived samples and 1141 soy-derived samples by formula manufacturers. The 34 target pesticides included organophosphates, carbamates, herbicides, and several fungicides identified by the Committee on Pesticides in the Diets of Infants and Children of the National Academy of Sciences (National Research Council, 1993) (persistent organic pollutants were not included). No detectable results were reported. All detection limits were <1.0 ppm, with most detection limits <0.005 ppm. In Canada, Newsome et al. (Newsome et al., 2000) tested 6 composite milk-based and 6 composite soy-based formula samples for a wide array of pesticides, including organophosphates, carbamates, herbicides, and persistent organic compounds (i.e., DDT, etc). The sampling scheme was designed to represent the Canadian diet. No pesticides were detected in these composite samples. Studies in New Zealand, India, and Spain report positive detections for several pesticides including DDT and derivatives, HCB, HCH, heptachlor, aldrin, endrin, azinphos-methyl, pirimiphos-methyl, dimethoate, and malathion. In New Zealand, Cressey and Vannoort (2003) report levels 0.03-0.7 ug/kg for DDT, DDE, and dieldrin. Overall detection frequencies are low (16%), although dieldrin was found in 4 out of 5 (80%) of soy-based compounds. Azinphos-methyl and pirimiphos-methyl levels ranged from 5-22 ug/kg, with an overall detection frequency of 4%. In Spain, Pico et al. (Pico et al., 1995) report concentrations of 1-13.5 ug/L for organochlorines, which were detected in only 4 of 45 samples (8.8%). Detection frequencies in India appear higher (Mishra and Vankar, 2002), averaging 60 % for several organochlorines (range = ND-3 ug/kg) and dimethoate and malathion (range = nd-26.6 ug/kg) found in 11 samples. Overall, these studies suggest that pesticide contamination in infant formula in North American and other developed countries are low and unlikely to be a major source of infant exposure. (Contaminated water may be a direct source of pesticide contamination in reconstituted water [see review of drinking water]).

**Breastmilk.** Research in the last two decades indicate that persistent organic pollutants, including halogenated hydrocarbons such as organochlorine pesticides (e.g., DDT), PCB's, and PBDE's bioaccumulate in fat and are transferred to breastmilk, thereby exposing breastfeeding infants (Landrigan et al., 2002; Lorber and Phillips, 2002; Solomon and Weiss, 2002; Sonawane, 1995; Rogan, 1996; Meironyte et al., 1999). To date, information about levels of non-persistent pesticides, such as organophosphates, in breastmilk is limited. Many non-persistent pesticides are soluble in water, and therefore may partition to the water fraction of breastmilk. Furthermore, the log of the octanol-water coefficient ( $\log K_{ow}$ ), a measure of fat solubility, suggests that some non-persistent organophosphates such as malathion ( $\log K_{ow} = 4.5$ ) may also partition into the lipid fraction of breastmilk. As noted earlier, actual data on non-persistent pesticides in breastmilk is limited. One study of environmental contaminants in human breast milk from the Central Asian Republics reported mean concentrations of 1.9 ug/L methyl parathion, 0.8ug/L malathion, and 5ug/L fenchlorophos, but levels ranged up to 3100 ug/L for malathion, the compound with the highest detection frequency (Ledrman, 1996). Very little information on the sample collection, population, or laboratory methods are provided in the paper. Another study in Bhopal, India reported mean levels of 0.43, 0.23, and 0.001 mg/L of malathion, chlorpyrifos, and methylparathion, respectively, in breast milk (Sanghi et al., 2003). Cattle that had ingested corn contaminated

with toxic levels of fonofos had detectable residues in the milk 18 hours later, but two days later milk was free of residues. The authors wrote that “fonofos is rapidly excreted in urine and feces with almost total elimination after 96 hours, while more toxic levels resulted in delayed elimination and higher residues” (Cook and Carson, 1985). Another study of cattle with dermal exposure via diazinon impregnated ear tags showed residues of 0.020, 0.041, 0.021 and 0.012 mg/kg in the butterfat of milk at 1, 7, 29, and 58 days after treatment (Spradbery and Tozer, 1996). These data suggest that organophosphate and possibly other non-persistent pesticides may be found in breast milk, although available data are extremely limited, and the significance for exposure is unknown.

Although there has been a fair amount of research on POPs in breast milk, there are almost no data on non-persistent pesticides in breastmilk. However, Dr. Dana Barr, CDC, is leading a study to develop laboratory methods to measure non-persistent pesticides in human breastmilk. Results for several organophosphates, carbamates, pyrethroids, phthalates, fungicides, and dicarboximides are promising (D. Barr, 2004). These findings suggest the measurements of several key target analytes for characterizing infant dietary exposures may be feasible.

#### 3.6.4 Drinking Water

No federal or state agencies have completed a comprehensive assessment of pesticide exposures to the US population from drinking water. For the data that is available, pesticide concentrations generally reflect source and finished waters and not tap water. Contamination in finished water is likely to reflect contamination in tap water, however few data are available to directly assess this hypothesis. Finally, pesticides in tap water may not result in exposures to individuals who drink bottled water. The USDA Pesticide Data Program (PDP) (2001) (<http://www.ams.usda.gov/science/pdp/water.htm>) recently initiated monitoring of finished waters at drinking water treatment plants in New York and California states as a pilot program for a nationally representative drinking water assessment program. New York and California were initially chosen because they represent diverse climate, geology, and land use and also are highly populated. In the near future, monitoring sites will be expanded to include Texas, Kansas, and Colorado. The PDP screens for over 150 pesticides and metabolites, with detection limits in the part-per-trillion range. A total of 297 samples were tested in 2001, the most recent year with published data. Overall, “positive detections were reported in 145 (40%) of the samples; the detects were primarily of widely used herbicides.” Atrazine or its metabolites were detected in 42-60% of the batches tested (concentration range = 5-500 ppt). Simazine was detected in 15% of samples (concentration range = 13-93 ppt). Metalochlor, metalochlor ethanesulfonic acid (ESA), or metalochlor oxanilic acid (OA) was detected in 10-50% of samples with concentrations ranging up 4420 ppt (OA). Alachlor or metabolites was detected in 4% of samples. Detection frequencies for other detected compounds were all below 1%, including bentazon, diazinon, malaoxon, metribuzin, or propanil. EPA generally evaluates health risks chemical by chemical. Thus, human risk assessment documents for individual chemicals may contain data on potential drinking water exposures. For example, extensive review data are available for atrazine, an herbicide commonly found in drinking water (<http://www.epa.gov/oppsrrd1/reregistration/atrazine/>). However, there are no systematic risk evaluations that would guide sampling and analysis strategies for the NCS. Other potential data sources include EPA-required water monitoring by drinking water providers servicing large populations (EPA, 2001). Target analytes include a variety of pesticides and volatile organic compounds. Other US water monitoring is conducted by state environmental agencies and independent researchers. The California Department of Pesticide Regulation monitors surface and well waters state-wide. Positive detections have been reported for diazinon, dimethoate, chlorpyrifos, carbaryl, DDE, DDT, diuron, and oxamyl in surface water (concentration range = 0.1-2.8 ug/L) and for 1,2-D, 2,4-D, atrazine, DBCP, ethylene dibromide, heptachlor, simazine, bromacil, diuron, and hexazinone in well waters ([www.cdpr.ca.gov](http://www.cdpr.ca.gov)). Overall, detection frequencies are low (9%, with ultimately 0.5% verified in 2001). Melnyk et al. (1997) tested drinking water in Iowa and North Carolina for a 32 pesticides including organophosphates, carbamates, herbicides, and organochlorines (Melnyk et

al., 1997). None were detected. Zaki et al. (1982) reported aldicarb in 52% of groundwater samples collected in Suffolk County, NY (concentration range up to >75 ug/L) (Zaki et al., 1982).

In summary, available data suggest that widespread contamination of drinking water by herbicides may contribute to chronic exposures in some parts of the United States. Although other compounds have been detected in other surface and well waters, available data suggest that positive detections are widely dispersed and, although exposing isolated communities or households, do not result in population-wide exposures that are the focus of the NCS.

### 3.7 Method Literature Review

Recent work has been performed to survey relevant literature to obtain information on exposure measurement approaches and methodology to inform chemical exposure assessment in the NCS (RTI, 2004). The goal of the literature survey was to obtain and synthesize existing information about relevant human exposure measurement methods and approaches, exposure questionnaire and diary methods, and relevant exposure and environmental data. Products from the work included a brief summary report and a searchable database of information about approaches and measurements for children’s exposures that are most relevant to the NCS. Air, soil, dust, food, water media concentrations and inhalation, ingestion (dietary and non-dietary), and dermal exposure routes and pathways were addressed for 10 chemical pollutant categories (*Table 3-11*). The survey was not intended to be a comprehensive literature review, and biological monitoring methods were not included.

Initial searches were performed to identify up to 50 potentially relevant research articles and reports for the 10 chemical categories. In surveying the literature, studies considered most relevant to the NCS were those that reported on collection methods or analysis methods, or provided measurement data for routes and pathways identified as important for the age groups of interest. Studies that included children, had a larger number of participants, used longitudinal measurements, and were supported by QA/QC data were also considered more relevant. For each chemical and survey category, up to 30 articles or reports were selected for review and information extraction. Outside subject area experts were used to inform the selection process.

**Table 3-11. Chemical Classes Selected for Exposure Measurement Reviews**

General Classes of Chemical Contaminants	Example Chemicals or Chemical Groups
Criteria Air Pollutants	PM <sub>2.5</sub> , PM <sub>10</sub> , ozone, NO <sub>2</sub> , SO <sub>2</sub> , CO
Environmental Tobacco Smoke	Criteria pollutants above and cotinine
Allergens	housedust mite, rodent, cockroach, cat allergens mold spores, pollen, endotoxins
Metals	mercury (total and methyl-), lead, manganese, tin
Organophosphorus Pesticides	chlorpyrifos, diazinon, malathion
Pyrethroid Pesticides	cis- and trans-permethrin, cypermethrin, cyfluthrin, allethrin, bifenthrin, deltamethrin, esfenvalerate, cyhalothrin
Phthalates	di-2-ethylhexyl phthalate, di-isononyl phthalate, diethyl phthalate, dibutyl phthalate, butyl benzyl phthalate
VOCs	aromatic hydrocarbons (benzene, toluene, xylenes) acrylamide aldehydes (formaldehyde, acetaldehyde) aliphatic hydrocarbons (hexane) halogenated hydrocarbons (chloroform, tetrachloroethylene)
Persistent Organohalogenes	Polychlorinated biphenyls (PCBs), Polychlorinated dibenzo-furans (PCDFs) Polybrominated diphenyl ethers (PBDEs) Polychlorinated dibenzo-dioxins (PCDD Dioxins)
Organochlorine and Triazine Pesticides	DDE/DDT, chlordane, heptachlor, lindane, dieldrin atrazine

Exposure measurement specialists extracted key information to include in summary tables and the database. Information about personal exposure measurement and environmental measurement methods

for various media and pathways was extracted. Ranges of chemical concentrations measured in the reviewed studies were extracted and compiled. Other information regarding performance of the methods was extracted, where available, including sample storage, analysis methods, detection limits, and QA/QC information. The specialists classified the relative participant burden, research staff burden, collection costs, and analysis costs based on their experience. Extensive information from child or adult exposure measurement studies was identified for some chemical classes whereas relatively few relevant research reports were found for others.

Relevant exposure study survey instruments—including questionnaires, time-activity, dietary, and behavior assessment instruments—were identified and reviewed. Information about the type of instrument, the information obtained with the instrument, and the relative burden of the instrument was extracted and included in summary tables and in the database. The reviewers attempted to assess the effectiveness of the instrument when supporting information was available.

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## 4. Methods for Measurement of Chemicals in Biological Media

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### 4.1 Introduction

Following an individual's exposure to a given chemical, a proportion of the chemical may be absorbed into the bloodstream, distributed among the bodily tissues, metabolized, and/or excreted. These four complex steps of Absorption, Distribution, Metabolism, and Excretion (ADME) make up the toxicokinetic process of a chemical (in this case an "environmental" chemical) after it contacts and enters the body (Klaassen, 2003). To estimate human exposure to a given chemical, researchers can measure the chemical after the absorption step or during each of the subsequent steps of ADME. Biomonitoring measures the concentration or dose of the chemical during or after ADME, and its concentration depends on the amount of the chemical that has been absorbed into the body, the toxicokinetics (ADME) of the chemical in that body, and the exposure scenario (including the time sequence of exposure and time since last exposure). Generally, biomonitoring data are independent of the pathway of exposure. They instead reflect the amount of the chemical in the matrix sampled, which is some portion of that which actually entered the body. Researchers can estimate the internal dose by measuring the level of a chemical, its metabolite, or its reaction product (a chemical adduct) in a biological medium. The internal dose depends upon when in the ADME process the biological sample is taken (and the matrix), but generally the sample is taken following at least the distribution step (*vide infra*). To link the dose with adverse health outcomes, researchers should measure the biologically effective dose, the dose at the target site that causes an adverse health effect. Often, though, the target organ is unknown. Even when known, the target organ is sometimes unavailable for sampling (e.g., the liver). In these situations, researchers measure the level of the chemical in another biological sample to gauge the internal dose.

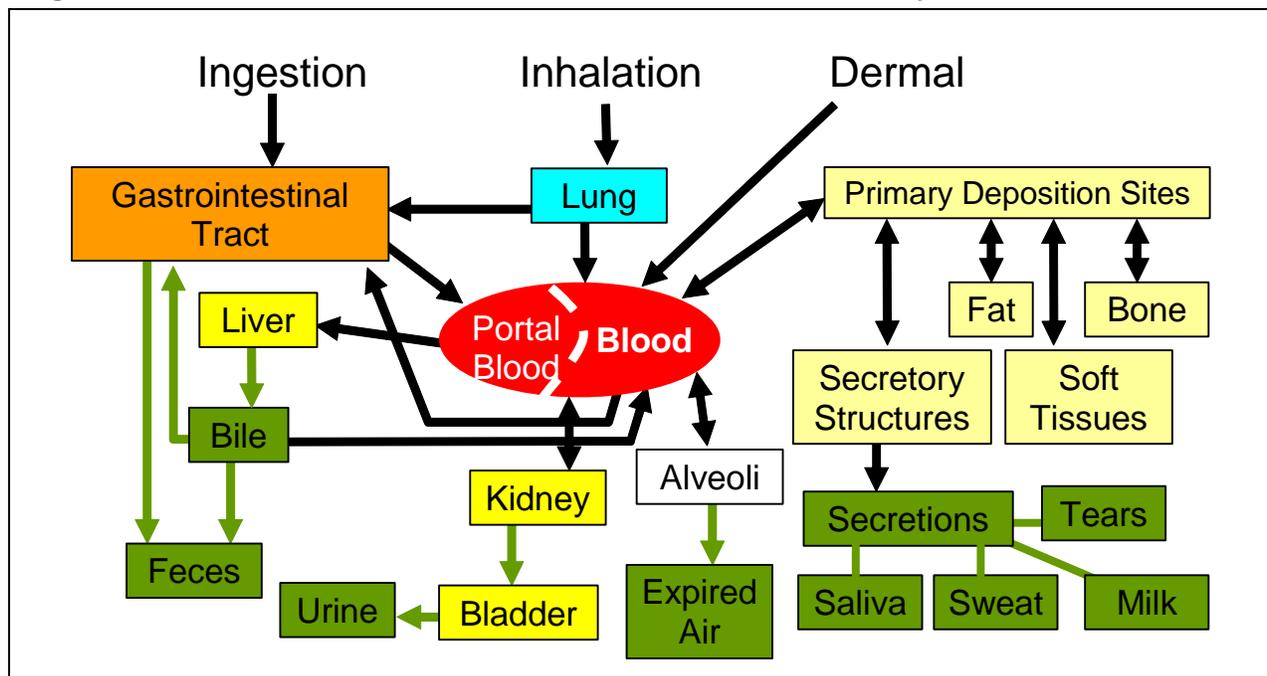
For the NCS, researcher can take the biological sample pre-conceptionally from both parents; from a pregnant woman during each of the three trimesters; during and immediately following child birth; from the mother post-natally; and from the child as he/she develops to the age of 21 years. The appropriate sample for monitoring will depend upon matrix availability and upon the different classes of environmental chemicals to be monitored. This chapter, unless otherwise stated, refers to the parents or the child and not to the fetus.

### 4.2 The Behavior of a Chemical in the Body

Absorption of a chemical into the body occurs when the chemical enters an individual's bloodstream by passing through absorption membrane barriers following exposure or contact of the chemical with an outer boundary (i.e., skin, nostrils, or mouth) of that individual. These three routes are shown in **Figure 4-1**. Following contact of the chemical with the skin (dermal route of exposure), the chemical can pass through the skin, enter the systemic blood supply, and circulate throughout the body. If, however, the chemical does not penetrate the skin, exposure (contact) still exists, but absorption does not, resulting in no toxicity unless the chemical is caustic and causes burns to the skin. Without absorption, no direct internal toxic effect can occur even if the chemical is toxic. Following contact of the chemical with the nostrils and inhalation of the chemical, the chemical penetrates into the lungs where it can pass through membranes directly into the blood supply or into the gastrointestinal (GI) tract, as a result of swallowing large particles. Following contact (exposure) with the mouth, a chemical can move through the GI tract; pass through membranes; enter the portal blood system—the body's "internal blood supply"—and be transported to the liver, which is the primary site of metabolism. Some chemicals may be metabolized in the liver and excreted into the bile, never circulating throughout the body. Any chemical handled in such

a manner (called “the first pass effect”) and that has a selective toxicity for an organ other than the liver and the GI tract would be expected to be much less toxic when ingested.

**Figure 4-1. Toxicokinetics of Environmental Chemicals in Body**



Once the chemical has been absorbed into the bloodstream, it is then distributed to the primary deposition sites, which include adipose tissue; bone; soft tissue, such as muscle; and secretory structures. Initial distribution of the chemical is generally rapid, and the site of distribution depends on the blood flow rate to through that organ and the ability of the chemical to partition into that organ. The eventual distribution site depends largely on the ability of the chemical to enter into the organ and its affinity for that chemical. Distribution is crucial to toxicity because, for example, if a target site is the kidney, but the compound is never distributed to the kidney (and is instead stored elsewhere), there may be a negligible toxic effect. Thus, the chemical must be distributed to the target organ to elicit a toxic response. However, because the concentration of the chemical in the storage depot is in equilibrium with the concentration in the blood, the chemical is slowly released from the storage depot as it is eliminated from the blood and low concentrations may reach the target organ.

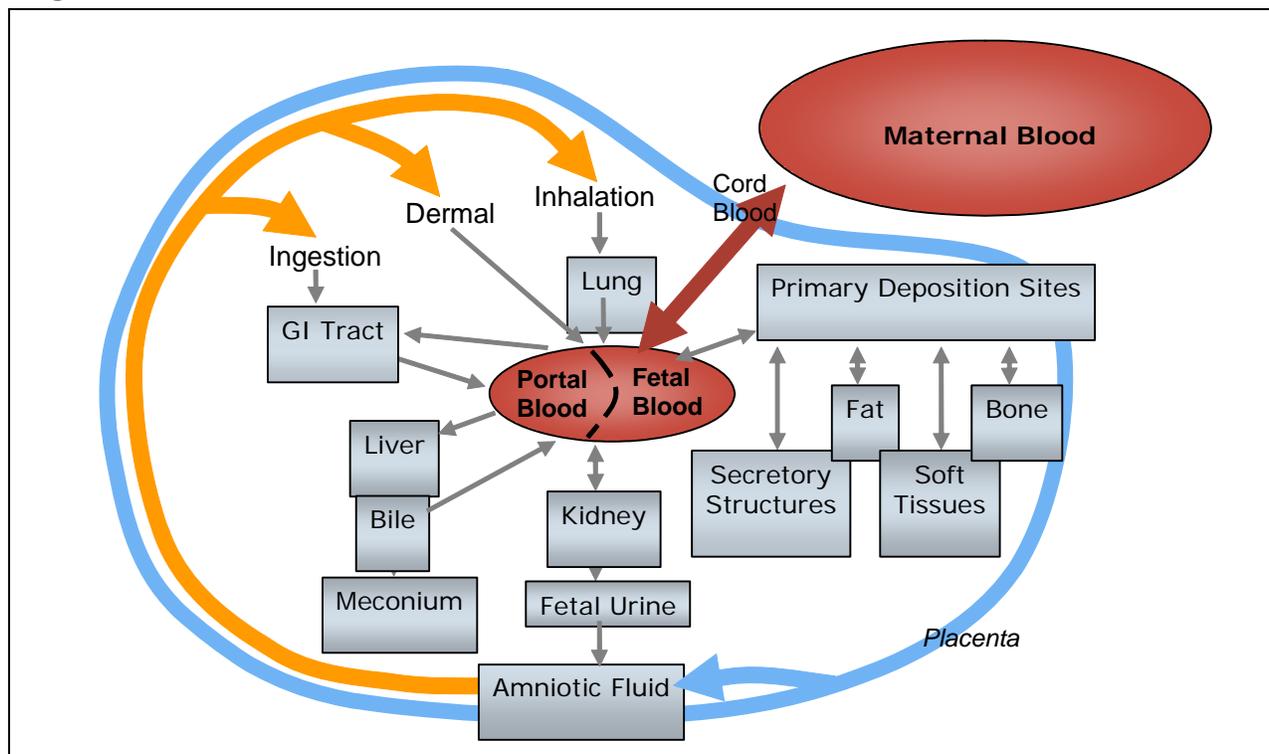
Metabolism is an enzymatic process that takes place primarily in the liver. The overall purpose of metabolism or biotransformation is to make the chemical less toxic and more hydrophilic, thus decreasing its residence time in the body via excretion through the kidneys. Metabolism has been divided into two phases. Phase 1 metabolism of the chemical typically involves inserting or substituting a functional group, making the compound more water soluble. Phase 2 metabolism usually chemically links the chemical to a glucuronide or sulfate group, which increases the water solubility and facilitates elimination of the chemical in the urine. However, metabolism does not always render a chemical less toxic.

Excretion can occur by three primary routes: urination, defecation, and exhalation. Metabolized chemicals may become hydrophilic and are excreted via urine or may be passed into the feces. Even if the chemical is not absorbed, it can go straight into the feces. Lipophilic compounds, in particular, are eliminated primarily into the feces. Volatile organic compounds (VOCs) can be excreted through the alveoli or in the expired air through exhalation. Chemicals can also be deposited in certain secretory

structures, such as tear ducts or salivary, sweat, or mammary glands, and be excreted as tears, saliva, sweat, or milk in lactating women.

In addition to the internal movement of chemicals in the body, a pregnant woman can distribute the chemicals via the bloodstream through the placenta and into the fetal blood supply (*Figure 4-2*). Biomonitoring matrices unique to the fetus include amniotic fluid and meconium. In addition, cord blood, the placenta, and the umbilical cord can be collected at birth.

**Figure 4-2. Fate of Environmental Chemicals**



### 4.3 Behavior of Specific Chemical Classes in the Body

#### Persistent Organic Chemicals

Persistent organic pollutants or chemicals (POPs; *Table 1-1*) include polychlorinated dibenzo-*para*-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls (PCBs), and organochlorine insecticides (United Nations Environment Programme, 2001; Borja-Aburto et al., 2000; 2001). Polycyclic aromatic hydrocarbons (PAHs) are also often included in this class because they persist in the environment; however, because PAHs behave more like non-persistent chemicals in the body, this white paper includes them with non-persistent organic chemicals. The primary route of exposure to POPs is ingestion. POPs are readily absorbed into the blood supply by passive diffusion. Their blood level initially decays relatively rapidly, a stage known as the alpha decay period (Flesch-Janys et al., 1996; Adgate et al., 2001). The alpha decay period represents the initial distribution of the chemical from the bloodstream into the bodily tissues. In addition, if the chemical exposure is so large that the absorbed dose overwhelms the enzyme system, a portion of the chemical may simply be eliminated. When this happens, it will also be seen in the alpha decay. Following the initial distribution and alpha decay, the POP is distributed into the fatty portions of organs and tissues (such as adipose tissue), and in lactating women, in breast milk, as well. The concentration of the POP in the fatty portions of organs and tissues is in equilibrium with the

concentration in the lipid portion of blood. The fat content of blood serum is 0.5–0.6%, milk is typically 4% lipid, and adipose tissue may be as much as 95% lipids. Thus, although the equilibrium concentrations of the chemical in the blood and fatty tissues may differ over orders of magnitude, they may be very similar when matrices are adjusted for percent lipid content.

In pregnant women, the POP may also distribute in the fetal compartment (*Figure 4-2*); therefore, other matrices such as cord blood or serum may be used for POP measurements. However, in general, the lipid content of cord blood is lower than that of an adult so the sensitivity of the analytical measurement may play a key role in obtaining a valid measurement in cord blood. Other fetal matrices, such as meconium, have not been fully explored for their potential in assessing POP exposures in the fetus. Conceivably, some of the POP circulating in the fetal bloodstream could deposit into the growing stores of meconium during fetal development; studies to evaluate this are currently underway at several academic research institutions including one of the NIEHS/EPA Children's Centers for Environmental Health and Disease Prevention. However, maternal blood or adipose tissue taken before or during pregnancy and maternal blood, milk, or adipose tissue taken soon after parturition (if the mother is breastfeeding; it can be taken later if the mother is not breastfeeding) are considered the best matrices for estimating fetal exposures to POPs.

Because metabolism and excretion of POPs are very slow, they have a long half-life in the body, usually along the order of years (Phillips et al., 1989b; Flesch-Janys et al., 1996; Michalek et al., 1996; Blanck et al., 2000; Masuda, 2001). However, because the lipophilic POPs accumulate in the breast milk of lactating women and the milk is removed from the woman's body (Galetin-Smith et al., 1990; Gonzalez et al., 1995; Schecter et al., 1998; Slorach and Vaz, 1985), the half-life of POPs in lactating women is about six months (LaKind et al., 2000). Essentially, this means that if a woman is breastfeeding, she depletes about half her store of POPs via breast milk over a six-month period, and that amount goes into the infant.

### *Non-persistent Organic Chemicals*

Non-persistent organic chemicals (*Table 1-1*)—such as current-use pesticides, phthalates, and VOCs—can be much more challenging to measure (Barr et al., 1999; Blount et al., 2000; Ashley et al., 1992; Ashley et al., 1995; Barr et al., 2003; Koch et al., 2003). Their primary routes of exposure, depending on the scenario, are generally ingestion or inhalation. These chemicals may be absorbed at rates that are similar to the absorption rates of persistent compounds, or they may vary because of their polarity; however, they differ widely in their distribution in the body. The primary reason for this difference is that they are generally rapidly metabolized and their metabolites are eliminated in urine. The deposition matrices, such as adipose tissue, are minor matrices for monitoring because only small amounts of the chemical are deposited in the body. The major matrices for assessing exposure are the excretion routes—urine for most of the semi-volatile compounds as well as expired air for some VOCs. Blood has also been used as a matrix for biomonitoring and it has the advantage of specificity because the parent chemical is generally measured as opposed to its metabolite. In fact, VOCs are often measured as the intact chemical in blood except for certain reactive chemicals such as acrylamide or vinyl chloride for which adducts or urinary metabolites may be best. Non-persistent chemicals tend to have very short half-lives in blood and the concentrations are usually about 3 orders of magnitude lower than urinary metabolite levels. Thus, if blood is used as a matrix, the sensitivity of the analytical method and the matrix volume available for analysis may become important. Blood can also be a valuable matrix for measuring biomolecular adducts such as DNA, hemoglobin or albumin adducts, such as DNA-PAH adducts. Typically when blood is used for this type of measurement, special sample collection procedures, such as washing red blood cells (hemoglobin adducts) or isolation of white cells (DNA adducts), may be required. However, if analytical requirements such as cost and speed of analysis are not critical issues, adduct measurements may provide more relevant information for relating to selected health endpoints

such as cancer. Furthermore, adducts provide a longer window for capturing an exposure because the lifetime of an adduct in the body is largely dependent upon the lifetime of the biomolecule itself. For example, hemoglobin has a lifetime of about 120 days; thus, a hemoglobin adduct could conceivably be measured weeks or months after an exposure has occurred.

Saliva has also been explored as a matrix for measuring selected non-persistent chemicals, such as atrazine (Lu et al., 1998). The existing data indicate that saliva levels can be considerably lower than blood levels of a non-persistent chemical depending upon the degree of protein binding that may occur; thus requiring a very sensitive analytical technique. Further research should be performed on additional chemicals and the relation of these measurements to more commonly used approaches before saliva levels can routinely be used for analysis.

Some VOCs have also been found at low levels in adipose tissue. Fatty matrices such as adipose tissue or maternal milk may thus provide an alternative for some VOC measurements, although these measurements are uncommon and current data on adipose tissue and milk VOCs are limited.

To evaluate fetal exposures, maternal samples collected throughout pregnancy may be used. However, because these chemicals are, by definition, non-persistent, urine or blood measurements made at a single point in time during pregnancy will only address the exposures that may have occurred in the previous few days, unless the exposure is continuous (e.g., pervasive air levels of a chemical resulting from smokers in the home) or continual (e.g., eating the same foods daily with measurable levels of pesticides). Researchers can circumvent this problem by taking multiple urine and/or blood samples every few days during pregnancy; however, taking multiple samples can be costly and unduly burdensome on the participant, and the samples may be logistically difficult to collect and store. Alternatively, researchers may collect multiple samples over particularly vulnerable stages of the pregnancy, if such stages can be appropriately identified. Another potential approach is to measure non-persistent chemicals in fetal matrices such as cord blood or meconium. Cord blood measurements have the same limitations as with maternal blood measurements in pregnancy, in that their levels are reflective of more recent exposures, unless chronic exposures are encountered. Meconium measurements have just recently been explored and require more extensive testing and validation before they prove a viable matrix.

### *Bioaccumulative Metals*

Bioaccumulative metals persist in the environment and bioaccumulate, either as the element itself or as organometallic compound, in people. This group of chemicals includes some forms of mercury, lead, and cadmium. For example, lead is readily absorbed, particularly in children, with distribution from the blood to its storage depots, bone and teeth (Aufderheide and Wittmers, Jr., 1992; Berglund et al., 2000; de, I et al., 1998; Cohen et al., 1992). Both metabolism and excretion are slow, so monitoring lead levels is straight forward. The best matrices to use would be blood, bone, and teeth. Regarding exposure to mercury, from a general population toxicity standpoint, methylmercury is the chemical of highest concern. Although its half-life is less than that of lead, cadmium, and POPs, methylmercury does bioaccumulate. Blood, hair, and nails are viable matrices for measuring methylmercury levels.

### *Non-bioaccumulative Metals*

Non-bioaccumulative metals are readily absorbed into the body, and although some proportion may distribute to various tissues including hair and nails, most will pass through the body rapidly. These metals are typically measured in urine (Bazzano and Ghersini, 1967; Campillo et al., 2000; Horng et al., 2002; Horng et al., 1999; Mandal et al., 2001). However, to gain a longer term dosimeter for exposure, arsenic can also be measured in hair (Armienta et al., 1997; Harkins and Susten, 2003; Wilhelm and Idel, 1996) and nails (Lin et al., 1998).

### *Criteria Pollutants and Bioallergens*

In general, biomonitoring has a limited role in the measurement of criteria pollutants and bioallergens. Researchers can assess exposure to carbon monoxide by measuring the carboxyhemoglobin adduct or expired CO in blood and breath, respectively. The adduct measurements provide a longer term dosimeter for the exposure than breath measurements because hemoglobin has a lifetime of about 4 months.

Researchers can measure bioallergen response by IgE in maternal, cord blood, or child blood. In addition, certain endotoxins or metabolites may be measured in blood or urine samples. Typically, the endotoxin measurements would reflect a more recent exposure similar to non-persistent chemical exposures.

### *Assessing Exposure throughout the Life Cycle (Tables 4-1 and 4-2)*

Biomonitoring measurements have been used for many years to assess exposures in adults (Pirkle et al., 1995a; Hill, Jr. et al., 1995; Kutz et al., 1992; CDC, 2001; CDC, 2003; Pirkle et al., 1995b), and to some extent, in adolescents and children (Adgate et al., 2001; Brock et al., 2002; Fenske et al., 2000; Fenske et al., 2002; Mes, 1987). Biomonitoring of fetuses, infants, and small children have been performed much less frequently, if at all. The mother or pregnant woman has generally been used as a surrogate to evaluate fetal exposures. However, for many chemicals, information on the transfer of chemicals from the mother to the fetus is not known. For example, does the chemical readily cross the placental barrier and, if so, what is the relationship between maternal and fetal blood levels? Another potential option is the use of meconium as a matrix of measurement because it begins accumulating in the bowels of the infant during the second trimester (Alano et al., 2001; Bearer et al., 1999; Ostrea, Jr. et al., 2001; Whyatt and Barr, 2001). However, there are many limitations on the use of meconium. Meconium measurements are still in their infancy of development and, to date, no reliable way to interpret most of these data exists, other than on a qualitative basis (i.e., did an exposure occur, not the degree of exposure). In addition, no information is gleaned from exposures that occurred in the first trimester.

The period from birth through one year old is also very important. During this time, the infants may be breast-feeding, so they may be exposed to chemicals via breast milk. Infants begin to crawl and their hands may come in contact with many chemicals causing dermal exposures. After crawling, the extensive hand-to-mouth activity can result in a non-dietary ingestion of a chemical as well. There may also be dermal contact with chemicals on or embedded in the carpet, so infants may have dermal exposures. Infants' breathing zone is different from older children and adults because they are often so close to the floor, a place where chemical levels are often higher. Thus, their contamination levels are different simply by breathing. At this age, researchers can likely make only urinary chemical measurements and breast milk measurements. Urine volume will likely be limited, probably to 10 mL or less. The urine collection technique may involve urine bags or collection from cloth or disposable diapers.

Once children start school, another environment with potential chemical contamination is included in the exposure scenario; however, biological sample collections become easier. At this stage in life, researchers can collect some blood, but usually only a small amount. Researchers can also collect urine and saliva samples for measurements. As children approach adolescence and adulthood, researchers can collect more biological samples and/or a greater quantity of a matrix, at levels similar to those of adults. At this life stage, researchers can collect up to 100 mL of blood for various measurements, as well as plentiful quantities of urine. **Table 4-1** shows the importance of various biological matrices for measuring exposure during the different life stages.

**Table 4-1. Importance of Various Biological Matrices for Measuring Exposure During the Different Life Stages**

Matrices	Adult Pre-con- ception	Fetal			Years			
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	0-1	2-3	4-11	12-21
<b>Persistent Organic Chemicals</b>								
Blood (whole)	1				1	1	1	1
Blood (serum)	1				1	1	1	1
Blood (plasma)	1				1	1	1	1
Urine	3				3	3	3	3
Saliva	3				NA	3	3	3
Hair	3				3	3	3	3
Nails	3				3	3	3	3
Adipose Tissue	1				NA	NA	NA	1
Feces	3				3	3	3	3
Semen	3				NA	NA	NA	3
Breath	3				NA	3	3	3
Teeth	3				NA	NA	3	NA
Cord blood	1	1	1	1	3	3	3	3
Meconium	3	2	2	2	3	3	3	3
Milk (maternal) *	1	1	1	1	1	3	3	3
Blood (maternal)	1	1	1	1	1	3	3	3
Urine (maternal)	3	3	3	3	3	3	3	3
Hair (maternal)	3	3	3	3	3	3	3	3
Adipose Tissue (maternal)	1	1	1	1	1	3	3	3
<b>Non-persistent Organic Chemicals (includes semi- and non volatiles)</b>								
Blood (whole)	1				1	1	1	1
Blood (serum)	1				1	1	1	1
Blood (plasma)	1				1	1	1	1
Urine	1				1	1	1	1
Saliva	2				NA	2	2	2
Hair	3				3	3	3	3
Nails	3				3	3	3	3
Adipose Tissue	3				NA	NA	NA	3
Feces	3				3	3	3	3
Semen	3				NA	NA	NA	3
Breath	3				NA	3	3	3
Teeth	3				NA	NA	3	NA
Cord blood	3	3	3	1	3	3	3	3
Meconium	3	3	2	2	3	3	3	3
Milk (maternal) *	3	3	3	3	2	3	3	3
Blood (maternal)	3	1	1	1	3	3	3	3
Urine (maternal)	3	1	1	1	3	3	3	3
Hair (maternal)	3	3	3	3	3	3	3	3
Adipose Tissue (maternal)	3	3	3	3	3	3	3	3
<b>Volatile Organic Chemicals</b>								
Blood (whole)	1				1	1	1	1
Blood (serum)	3				3	3	3	3
Blood (plasma)	3				3	3	3	3
Urine	2				2	2	2	2
Saliva	3				NA	3	3	3
Hair	3				3	3	3	3
Nails	3				3	3	3	3
Adipose Tissue	2				NA	NA	NA	2
Feces	3				3	3	3	3
Semen	3				NA	NA	NA	3
Breath	1				NA	1	1	1
Teeth	3				NA	NA	3	NA
Cord blood	3	3	3	1	3	3	3	3
Meconium	3	3	3	3	3	3	3	3
Milk (maternal) *	3	3	3	3	2	3	3	3
Blood (maternal)	3	1	1	1	3	3	3	3
Urine (maternal)	3	3	3	3	3	3	3	3
Hair (maternal)	3	3	3	3	3	3	3	3
Adipose Tissue (maternal)	3	3	3	3	3	3	3	3

**Table 4-1. Importance of Various Biological Matrices for Measuring Exposure During the Different Life Stages (continued)**

Matrices	Adult Pre-conception	Fetal			Years			
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	0-1	2-3	4-11	12-21
<b>Bioaccumulative Inorganic Chemicals</b>								
Blood (whole)	1				1	1	1	1
Blood (serum)	3				3	3	3	3
Blood (plasma)	3				3	3	3	3
Urine	2				2	2	2	2
Saliva	3				NA	3	3	3
Hair	2				2	2	2	2
Nails	2				2	2	2	2
Adipose Tissue	3				NA	NA	NA	3
Feces	3				3	3	3	3
Semen	3				NA	NA	NA	3
Breath	3				NA	3	3	3
Teeth	3				NA	NA	2	NA
Cord blood	2	2	2	1	3	3	3	3
Meconium	3	2	2	2	3	3	3	3
Milk (maternal) *	3	3	3	3	3	3	3	3
Blood (maternal)	1	1	1	1	3	3	3	3
Urine (maternal)	3	2	2	2	3	3	3	3
Hair (maternal)	2	2	2	2	3	3	3	3
Adipose Tissue (maternal)	3	3	3	3	3	3	3	3
<b>Non-bioaccumulative Inorganic Chemicals</b>								
Blood (whole)	3				3	3	3	3
Blood (serum)	3				3	3	3	3
Blood (plasma)	3				3	3	3	3
Urine	1				1	1	1	1
Saliva	3				NA	3	3	3
Hair	2				2	2	2	2
Nails	2				2	2	2	2
Adipose Tissue	3				NA	NA	NA	3
Feces	3				3	3	3	3
Semen	3				NA	NA	NA	3
Breath	3				NA	3	3	3
Teeth	3				NA	NA	3	NA
Cord blood	3	3	3	3	3	3	3	3
Meconium	3	3	3	3	3	3	3	3
Milk (maternal) *	3	3	3	3	3	3	3	3
Blood (maternal)	3	3	3	3	3	3	3	3
Urine (maternal)	3	1	1	1	3	3	3	3
Hair (maternal)	2	2	2	2	3	3	3	3
Adipose Tissue (maternal)	3	3	3	3	3	3	3	3
<b>Criteria Pollutants (CO only)</b>								
Blood (whole)	1				1	1	1	1
Blood (serum)	3				3	3	3	3
Blood (plasma)	3				3	3	3	3
Urine	3				3	3	3	3
Saliva	3				NA	3	3	3
Hair	3				3	3	3	3
Nails	3				3	3	3	3
Adipose Tissue	3				NA	NA	NA	3
Feces	3				3	3	3	3
Semen	3				NA	NA	NA	3
Breath	1				NA	1	1	1
Teeth	3				NA	NA	3	NA
Meconium	3	3	3	3	3	3	3	3
Milk (maternal) *	3	3	3	3	3	3	3	3
Blood (maternal)	3	1	1	1	3	3	3	3
Urine (maternal)	3	3	3	3	3	3	3	3
Hair (maternal)	3	3	3	3	3	3	3	3
Adipose Tissue (maternal)	3	3	3	3	3	3	3	3
<b>Bioallergens (IgE and endotoxins)</b>								
Blood (whole)	1				1	1	1	1
Blood (serum)	1				1	1	1	1

**Table 4-1. Importance of Various Biological Matrices for Measuring Exposure During the Different Life Stages (continued)**

Matrices	Adult Pre-con- ception	Fetal			Years			
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	0-1	2-3	4-11	12-21
Blood (plasma)	1				1	1	1	1
Urine	2				2	2	2	2
Saliva	3				NA	3	3	3
Hair	3				3	3	3	3
Nails	3				3	3	3	3
Adipose Tissue	3				NA	NA	NA	3
Feces	3				3	3	3	3
Semen	3				NA	NA	NA	3
Breath	3				NA	3	3	3
Teeth	3				NA	NA	3	NA
Cord Blood	3	1	1	1	3	3	3	3
Meconium	3	3	3	3	3	3	3	3
Milk (maternal) *	3	3	3	3	3	3	3	3
Blood (maternal)	3	1	1	1	3	3	3	3
Urine (maternal)	3	2	2	2	3	3	3	3
Hair (maternal)	3	3	3	3	3	3	3	3
Adipose Tissue (maternal)	3	3	3	3	3	3	3	3
Sample Volume Obtainable During Life Stage (mL unless otherwise specified)								
Blood (whole) **	100	0	0	0	9	22	38	90
Blood (serum) **	40	0	0	0	3.6	8.8	15.2	36
Blood (plasma) **	40	0	0	0	3.6	8.8	15.2	36
Urine	100 +	0	0	0	1-10	10-20	30-50	100 +
Saliva	2 mL per collection	0	0	0	0	1-2	1-2	2
Hair	0.5-4 g?	0	0	0	<0.5 g	0.5-2 g	0.5-4 g	0.5-4 g
Nails	??	0	0	0	??	??	??	??
Adipose Tissue	10 g ?	0	0	0	0	0	0	10 g?
Feces	10 g	0	0	0	3 g	5 g	10 g	10 g
Semen	2 mL per collection	0	0	0	0	0	0	2 mL
Breath	?	0	0	0	?	?	?	?
Teeth	0	0	0	0	0	0	6-10 teeth	2-4 teeth
Cord blood	30-60	30-60	30-60	30-60	NA	NA	NA	NA
Meconium	2 g	2 g	2 g	2 g	NA	NA	NA	NA
Milk (maternal)	100 +	100 +	100 +	100 +	100 +	NA	NA	NA
Blood (maternal)	100	100	100	100	100	100	100	100
Urine (maternal)	100 +	100 +	100 +	100 +	100 +	100 +	100 +	100 +
Hair (maternal)	??	??	??	??	??	??	??	??
Adipose Tissue (maternal)	10 g?	10 g?	10 g?	10 g?	0	0	0	10 g

\* Milk sample taken post-partum. All other maternal samples taken during respective trimesters.

\*\* Based upon blood collection from NHANES

\*\*\* Dioxins will drive the sample requirement to the maximum volume end; other chemicals can be measured near the lower end volume requirement

As = arsenic; Hg = mercury

**Rating**

**scale:**

- 1 Important matrix for most chemicals in category
- 2 Important matrix for one or two chemicals in category
- 3 Not an important matrix for assessing exposure for chemicals in the category
- NA Matrix not viable for life stage because it cannot be collected, the chemical can not typically be measured in the matrix, or doesn't represent exposures in a given life stage.

*Biological Matrices for Exposure Assessment*

The two primary matrices used to assess human exposure to chemicals are urine and blood (serum, plasma, blood cells, etc) (Pirkle et al., 1995a; Angerer and Gundel, 1996; Needham and Sexton, 2000). The volume of blood in a person's body is relatively constant and consistent from person-to-person. Adults typically have about 5 liters of blood, and children have much less—about 80 cubic centimeters per 10 kilograms of body weight. Urine volume is not constant.

### *Blood*

Researchers can measure many persistent (such as POPs) and non-persistent chemicals in blood (Angerer and Horsch, 1992; Patterson, Jr. et al., 1987; Blount et al., 2000; Barr et al., 2001; Angerer, 1988; Ashley et al., 1992; Weiss et al., 1999; Barr et al., 2002b; Anwar, 1997; Campillo et al., 2000; Cocker et al., 2002; Drevenkar et al., 1993; Leng et al., 1997). Although the amount of blood is nearly the same in all adults, the chemical composition of blood, such as lipid content, varies between individuals and within an individual, especially post-prandial (Phillips et al., 1989a). Blood concentrations of lipophilic chemicals, such as polychlorinated dibenzo-*p*-dioxins, polychlorinated biphenyls, and organochlorine insecticides, are routinely normalized using blood lipid concentrations to allow a direct comparison of their concentrations within and among individuals, irrespective of the time of day the blood was collected. However, other chemicals measurable in blood may not vary based upon the blood lipid content. For example, fluorinated chemicals in blood do not depend upon the lipid content; instead, they bind to blood albumin (Jones et al., 2003). Therefore, these measurements should not be adjusted based upon the blood lipid content; however, other adjustments, such as for albumin content, may be required, if deemed appropriate. Other examples of chemicals whose measurements should not be adjusted for the lipid content of blood include volatile organic compounds (Ashley et al., 1994), cotinine (Bernert, Jr. et al., 1997; Pirkle et al., 1996), contemporary-use pesticides (Barr et al., 2002a), and phthalates (Kato et al., 2003).

There is an inherent advantage for measuring a chemical in blood. Because researchers know how much blood is in the body, they can calculate the body burden (i.e., the amount of chemical relative to the amount of blood and the body) more accurately than if they measure the chemical or its metabolite in urine.

Although blood is a common matrix for biomonitoring, there are several limitations to its use. Blood collection is invasive which may severely limit the ability to collect it from infants and small children. In addition, non-persistent chemicals are usually found in very low concentrations in blood, typically about three orders of magnitude lower than urinary metabolite levels. Also, if testing is not performed soon after sample collection, which will likely be the case in the NCS, long-term storage of blood may be problematic, depending upon what form of blood is stored. Serum stores well at -70 degrees Celsius because it is low in protein and stays homogeneous. Plasma contains more proteins, which precipitate and makes plasma less homogeneous than serum. Whole blood does not store well because the cells tend to hemolyze.

### *Urine*

Urine's ease of collection (except in cases of 24-hour urine voids) makes it an excellent medium for biomonitoring. Generally, spot urine samples, whether first-morning voids or "convenience" samplings, are most commonly used for biomonitoring purposes. The major disadvantages of spot urine samples include the variability of the volume of urine and the concentrations of endogenous and exogenous chemicals from void to void (Barr et al., 1999). The issue on how best to adjust the urinary concentrations of environmental chemicals in a manner analogous to the adjustment of the concentrations of lipophilic chemicals in blood is a subject of continued research. Urinary creatinine concentrations, specific gravity, and osmolality are the most routinely used methods for adjustment and for determining if a spot urine sample is valid. The most widely used method is creatinine adjustment which involves dividing the analyte concentration by the creatinine concentration (in g creatinine/L urine). Analyte results are then reported as weight of analyte per gram of creatinine (e.g.,  $\mu\text{g}$  of analyte/g creatinine). This may work well when comparing analyte levels in a single individual because the intra-individual variation in creatinine excretion is relatively low (<10%). However, the inter-individual variation in creatinine excretion is quite high and is dependent upon lean muscle mass; therefore, a small individual such as an infant or child will

excrete far less creatinine than an older child, adolescent or adult. Thus, by creatinine-adjusting, you may be artificially elevating the analyte levels in a small child. However, a creatinine-adjusted concentration may be a good indicator of chemical dose per body weight if creatinine can be reliably used as a surrogate for body weight. Information on variables potentially affecting creatinine excretion (e.g., height, weight, sex, age, BMI, skin fold measurements) would assist in interpretation of creatinine-adjusted data.

Variations in urinary analyte concentrations due to changing water content in urine have been eliminated using urinary excretion rate (UER) calculations (Rigas et al., 2001). To calculate the UER, the metabolite concentration in urine is multiplied by the volume of the void then divided by the duration of time that the void was accumulating in the bladder. This model assumes that the entire bladder is emptied with each void and that the entire sampling void volume is known. Because this is based upon the mass in the sample, variability in urine concentrations due to urine dilution are removed, particularly for analytes where the rate of excretion varies with the urine flow (Boeniger et al., 1993). However, because the void volume and times of previous and current voids are required, this approach may not be practical for young children or for large, complex studies. For occupational monitoring, industrial hygienists typically use creatinine concentrations for excluding samples for testing instead of for adjustments. In adult males, creatinine levels normally fall between 30 mg/dL at the dilute end to a more concentrated 300 mg/dL (American Conference of Governmental Industrial Hygienists, 2001). If a creatinine level is within the range, the sample is considered suitable for testing. If the creatinine concentration is outside the range, the sample is discarded and another sample must be collected. This same practice has also been adopted for many non-occupational monitoring studies; however, there are several disadvantages to this protocol. Normal urinary creatinine concentration ranges for populations with a wide age and racial diversity may differ from the established range. In these instances, perfectly suitable samples may be discarded for no reason. In addition, a second sample is usually not collected because the analysis occurs some time after the collection; therefore, all measurement information from that particular individual would be lost.

### *Breast Milk and Adipose Tissue*

Many of the same chemicals measured in blood have been found in breast milk (Committee on Environmental Health, 1994; Korrick and Altshul, 1998; LaKind et al., 2001; Landrigan et al., 2002; Monheit and Luke, 1990) and adipose tissue (Patterson et al., 1987). For example, many persistent organic pollutants and non-persistent chemicals such as phytoestrogens have been detected in breast milk samples. Metabolites of some chemicals, such as phthalates, have also been found. Breast milk measurements are unique in that they provide data not only on ingestion exposures for the infant but also are indicators of maternal exposures. Breast milk and adipose tissue are more lipid-rich matrices than blood, so similar lipid adjustments are required for reporting concentrations of lipophilic analytes such as PCBs and dioxins. In general, these lipophilic analytes partition among the lipid stores in blood, breast milk, and adipose tissue on nearly a 1:1:1 basis (Patterson et al., 1987). More laboratory work needs to be done on the partitioning of less bioaccumulative analytes in these matrices.

### *Alternative Matrices*

Chemicals have been successfully measured in alternative matrices such as saliva (Bernert, Jr. et al., 2000; Lu et al., 1998), meconium (Bearer et al., 1999; Ostrea, Jr. et al., 1994; Whyatt and Barr, 2001), amniotic fluid (Bradman et al., 2003; Foster et al., 2002), and breath (Pellizzari et al., 1992). Because many of these matrices are not commonly analyzed, the resulting chemical concentration data are more difficult to interpret than urine, blood or breast milk measurements. However, because many of these matrices are available and could provide potentially useful information, they should not be discounted. Instead, preliminary studies evaluating the partitioning of chemicals in the various matrices should be conducted that will allow for comparison of data among matrices.

### *Specificity and Sensitivity Requirements*

When deciding how best to assess exposure, researchers need to consider both the chemical and the matrix. The first consideration should be specificity (i.e., how specific an analysis method is for a particular exposure). The second consideration should be the sensitivity of an analysis method (researchers' ability to measure the chemical at the desired level). The half-life of a chemical is a factor for sensitivity; however, because persistent chemicals have long half-lives, it is not nearly as important as it is for non-persistent chemicals, which metabolize rapidly. For instance, in adult men, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin has a half-life of about 7.6 years (Phillips et al., 1989b). Therefore, to assess exposure over a period of time, for example, nine months, the sample could be collected at any time period within the nine months or even afterwards and the biological measurement information would still be useful for accurate exposure classification (e.g., exposure quartiles—people whose exposure is high, medium, low, or none). When measuring exposure to persistent chemicals by analyzing adipose tissue, it does not make much difference which portion of the body the sample is taken from; however, because blood is easy to collect and readily available, blood is an ideal medium in which to measure persistent chemicals. In lactating women, milk is also frequently used.

Non-persistent chemicals have half-lives of hours or minutes (Griffin et al., 1999; Dirven et al., 1993; Lau et al., 1984; Nolan et al., 1984); therefore, the post-exposure fate of a non-persistent chemical is dramatically different (Needham and Sexton, 2000). After each exposure, the concentration of the chemical in blood declines rapidly. The window of opportunity for measuring non-persistent chemicals in blood is narrow and requires the use of a very sensitive technique. By measuring these chemicals in blood as the intact, or parent, chemical, researchers gain information on the exact chemical to which one was exposed. For example, if someone was exposed to chlorpyrifos, researchers can measure chlorpyrifos in the blood rather than its metabolite, which is formed from more than one parent chemical and is also the same chemical as environmentally degraded chlorpyrifos. In addition to blood, certain non-persistent chemicals, such as cotinine, have been measured in saliva because cotinine is in equilibrium in blood and saliva.

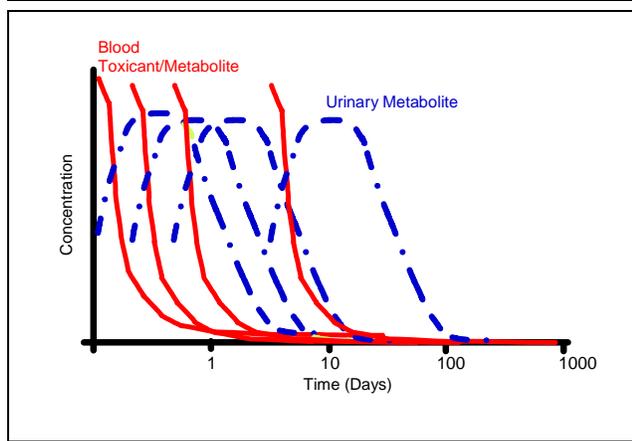
In urine, researchers generally measure metabolites of the chemical which may lack the desired specificity for analysis; however, measurements in urine allow a much wider window of opportunity in which to take the sample. The window may be only a few hours or two days, but there is more time in which to measure the chemical in urine than in blood. Generally, researchers assess exposure to non-persistent chemicals by measuring their metabolites in urine, even though this method does not have the specificity of the blood measurement.

When chronic exposure to a non-persistent chemical occurs, the exposure is continually replenishing the chemical in the blood and urinary elimination may reach a steady state; that is, the chemical or metabolite present in the urine stays at a relatively constant level (see **Figure 4-3**). Therefore, urine becomes a better matrix for measurement because researchers integrate exposure over a longer period. As a result, researchers gain an advantage by using urine as a matrix when exposures are chronic.

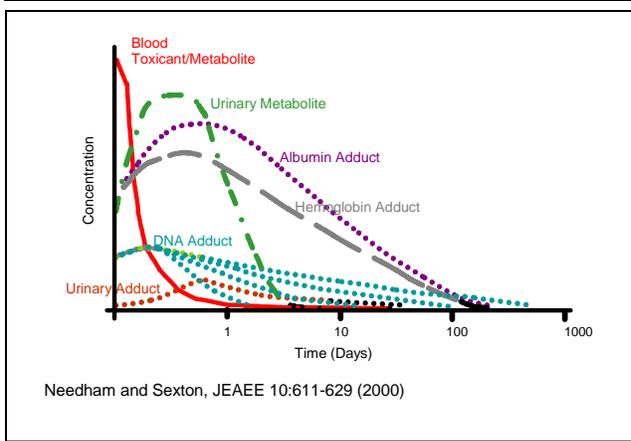
### *Biomolecular Adducts*

Persistent and non-persistent chemicals can also react with biomolecules such as DNA or hemoglobin to form biomolecular adducts (Angerer et al., 1998; Bergmark et al., 1991; Schettgen et al., 2002; Cadet et al., 1999; Dubois et al., 1995; Johannesson et al., 2001; Kristiansson et al., 2002; Lin et al., 2002; Oakley et al., 1996) (see **Figure 4-4**). By measuring these adducts, researchers are able to increase the amount of time after exposure that researchers can measure a non-persistent chemical because the

**Figure 4-3. Post-Exposure Fate from Chronic Exposure to a Non-persistent Toxicant in Blood and Urine**



**Figure 4-4. Post-Exposure Fate from Acute Exposure to a Non-persistent Toxicant in Blood and Urine**



amount of time the adduct remains in the body is largely dependent upon the lifetime of the biomolecule itself (Needham and Sexton, 2000). For example, the average lifespan of a red blood cell is about 120 days. If a chemical formed an adduct with hemoglobin on the day a red blood cell was created, the adduct should remain in the body about 4 months, allowing a much longer time after exposure to collect the sample. Other adducts are formed with DNA, albumin, and other prominent proteins. Because adducts are not formed from every chemical molecule to which one is exposed, adduct measurements must be very sensitive and usually a large amount of matrix is required. In addition, the measurements are usually cumbersome and time-consuming so the analytical throughput is very low and the cost is very high.

When measuring persistent chemicals, researchers do not gain much advantage by measuring them as adducts. Blood is still the matrix of choice because the concentration is higher in blood than in any adduct, and it provides researchers a wide window of opportunity (Barr and Needham, 2002). To form an adduct, the chemical must have an electrophilic site for the nucleophile on the biomolecule (usually sulfur or nitrogen) to attack which forms a covalent bond, and hence, the adduct.

### *Sampling Timeframe*

For persistent organic chemicals, the timeframe for sampling is reasonably straightforward. In general, researchers can take a blood sample at any time—up to several years—after exposure has occurred and the exposure can still be accurately identified; however, this will not provide any information about when the exposure occurred. For example, if a PCB concentration of 1000 ng/g lipid was measured in a blood sample, it is unknown if a recent exposure to this amount of PCB occurred or whether a larger exposure occurred many years ago and, though a portion of the PCB has been eliminated from the body over time, this amount is still circulating in the bloodstream. Coupling questionnaire data with these biological measurements, may clarify the timing of the exposure (e.g., breastfeeding, subsistence food consumption).

The sampling timeframe for non-persistent chemicals is not straightforward. Because these chemicals have short biological half-lives, researchers must collect the samples—whether blood or urine—soon after the exposure to appropriately quantify it. If the primary exposure medium is the air and the exposure is continuous, a first morning void urine sample is probably the best biological sample for measuring the exposure. However, if the exposure is from a source related to personal grooming (e.g.,

VOCs from showers or phthalates from personal care products), a first morning void urine sample or an early morning blood sample (prior to showering) would likely miss the exposure from the following day. Rather, a late morning or early afternoon sample would more accurately characterize the daily exposure to these chemicals. Similarly, samples designed to evaluate dietary exposures, such as pesticides, should be collected several hours after mealtimes so that researchers can identify these exposures.

In general, sample collection for non-persistent chemical measurements should reflect the residence time of the chemical in each individual matrix. The half-lives of non-persistent chemicals in blood are typically much less than in urine samples, thus blood samples may need to be collected within minutes or hours after the exposure whereas urine samples may be collected several hours, or in some instances days, after the exposure. Saliva samples will typically mimic blood, whereas meconium samples may provide a longer window for capturing the exposure. Measurements of biomolecular adducts need to consider the lifetime of the biomolecule, rather than the lifetime of the chemical, in the particular matrix; however, more adduct will likely be present immediately after exposure than several weeks after exposure.

### *Collecting Samples from Infants and Children*

Difficulty is often encountered when collecting urine samples from infants and small children which are not toilet trained. The traditional approach was similar to that in a clinical setting, using an infant urine collection bag. This technique is rather straightforward; however, it is usually bothersome to the child and often requires that the child be given liquids to encourage urination within a given timeframe. Encouraging urination with drinks will usually dilute the urine and make the analytical measurement more difficult. Other approaches for urine collection have also been investigated. Hu et al. (2000) described an approach where a cotton insert was placed into the diaper and the child was allowed to urinate as usual. When the diaper change occurred, the cotton insert was collected and the urine was expressed via a syringe into a vial for storage and analysis. Most of the target analytes were recovered well from the cotton insert. Calafat et al. (2004) used a similar approach for collecting urine samples from neonates for phthalate analysis except centrifugation was used to express the urine. They were able to recover about 1 mL urine from each neonate diaper. A variation on this method is the use of cotton diapers on the child then expressing the urine directly from the diaper. Another approach of ongoing interest is the collection of the target analytes directly from disposable diapers. The use of extraction techniques for solid materials such as accelerated solvent extraction and supercritical fluid extraction offers promise for extracting target chemicals from the coagulated gel matrix of the disposable diaper. This research is currently underway. If proven viable for isolating a broad array of target analytes, this method of collection would be most attractive as it is the least burdensome on the participant and the most logistically practical.

### *Temporal Variability in Urine and Blood Samples*

The variability of non-persistent target analyte levels in samples collected from an individual over time is of concern, whether the sample is biological or environmental. Temporal variability can include the variation of a given chemical in multiple samples collected on a single day or can include variation among days, months or seasons. How accurately can a single sample represent a day's exposure to a given chemical or how accurately can a single sample represent an individual's exposure over a longer period of time? Researchers can answer these questions more easily for chronic exposures to non-persistent chemicals because the exposure is repeated, thus the amount in a given sample would be representative of that average exposure. However, for episodic exposures, the questions become more difficult to answer and likely vary from chemical to chemical. For urine matrix, a 24-hour urine sample is preferred, rather than a single spot sample on a given day; however, this is very burdensome on the participant and often logistically difficult. If a 24-hour sample cannot be obtained, a first-morning void is often preferred because the urine is more concentrated and the collection represents a longer window of

accumulation (usually >8 hours). However, as mentioned *vide supra*, a first morning collection may not be ideal for certain exposures because the timing for capturing the exposure is off. To evaluate daily, monthly and/or seasonal variations of analyte in urine, sequential samples are often taken days and weeks apart to evaluate how the intra-individual variation over time compares to the inter-individual variation and whether an accurate classification of exposure is possible. These studies are important in interpreting the biomonitoring data and should be considered, at some level, in the NCS. These data will help to determine whether multiple samples should be taken and at what intervals. In most instances, sampling for non-persistent chemicals, whether environmental or biological, will require multiple samples taken over the course of the study at regular intervals (e.g., weekly, monthly, semi-annually, etc.).

### *Methodology*

**Organic Chemicals.** Most methods for measuring organic chemicals in biological matrices use a sample preparation step to isolate the target chemical(s) from the matrix, an analytical technique with a detection system, data processing, and quality assurance processes. For sample preparation, liquid-liquid extraction, solid phase extraction, or some permutation of the two is the most commonly employed, although some alternative extraction procedures, such as purge and trap isolation for VOCs, are also available. Sample collection and storage conditions are critical if data generated from the sample at a later time are to be useful. **Table 4-2** shows some sample storage requirements. **Table 4-3** shows characteristics of common analytical methods.

The sample preparation steps are usually the most common source of analytic error, whether systematic or random, because the sample is frequently handled by humans. Automated sample preparation techniques are usually more precise. If the chemical is inherently incompatible with the analytic system that follows, a chemical derivatization or reduction procedure may also be required. The addition of steps into the sample preparation procedure usually increases the overall imprecision of the method.

Common analytical techniques for separation of individual chemicals include gas chromatography, high-performance liquid chromatography, or capillary electrophoresis, which are coupled in-line to a detection system. Common detection systems include low resolution mass spectrometry, high resolution mass spectrometry, tandem mass spectrometry, electron capture (for halogenated chemicals only), flame photometric, nitrogen phosphorus, fluorescence and ultraviolet (UV) absorbance detection. Of the detection systems, mass spectrometers, especially high resolution and tandem systems, provide the most specificity whereas UV absorbance detection usually provides the least specificity. Most mass spectrometry-based methods have limit of detections (LODs) in the pg to ng/g matrix range, typically adequate enough to detect levels in the general population when 1 to 10 g of matrix is used. The analytical imprecision usually ranges from 10 to 20 percent. A typical throughput is about 40 samples per day.

**Table 4-2. Storage Requirements and Characteristics for Biological Matrices and Chemical Classes**

Chemical class	Chemicals	Storage Temperature (C)	Matrix	Duration of Matrix Stability (appx)	Duration of Chemical Stability (appx)	Container Requirements	Preservative Requirement for Chemical Stability
Persistent organic compounds	All	Preferably at or below -70	milk	several years at -70°C	several years at -70°C	polypropylene. NO glass or teflon	NA
	All	Preferably at or below -70	serum/plasma	several years at -70°C	several years at -70°C	polypropylene. NO glass or teflon	NA
	All	Preferably at or below -70	adipose tissue	several years at -70°C	several years at -70°C	polypropylene. NO glass or teflon	NA
Non-persistent organic compounds	All	Preferably at or below -70	urine	several years at -70°C	several years at -70°C	polypropylene or glass	NA
	Phthalates	Preferably at or below -70	serum/plasma	several years at -70°C	several years at -70°C	polypropylene or glass	125 micromoles phosphoric acid/mL matrix. To be added ASAP after collection
	Pesticides	Preferably at or below -70	serum/plasma	~ 5years	up to 1 year, less for many of the reactive pesticides such as OPs, carbamates, pyrethroids. Herbicides, repellants, fungicides are generally stable for longer periods	polypropylene or glass	none; possibly different anticoagulants can enhance stability of OPs and pyrethroids
	Others	Preferably at or below -70	serum/plasma	several years at -70°C	several years at -70°C	polypropylene or glass	NA
Volatile organic chemicals		4°C	whole blood	10 weeks	>10 weeks	heat and vacuum-purged glass grey top vacutainer; reassembled after treatment; vacuum-reestablished and gamma-irradiated to restore sterility	vacutainer contains sodium fluoride and potassium oxalate
Bioaccumulative metals		4°C	whole blood	indefinitely	indefinitely	Purple top liquid EDTA vacutainer; pre-screened collection materials	second or third draw if multiple draws

**Table 4-2. Storage Requirements and Characteristics for Biological Matrices and Chemical Classes (continued)**

Chemical class	Chemicals	Storage Temperature (C)	Matrix	Duration of Matrix Stability (appx)	Duration of Chemical Stability (appx)	Container Requirements	Preservative Requirement for Chemical Stability
Non-bioaccumulative metals		-20	urine	indefinitely	indefinitely	Prescreened collection materials	for mercury, treatment with TritonX 100 and sulfamic acid upon collection is necessary
		room temperature	hair	indefinitely	indefinitely	zipper bag	NA

**Table 4-3. Characteristics of Analytical Methods for Measuring Chemical Classes in Biological Matrices**

Chemical Class	Most Typical Matrices	Methodology Used	Detection Limits	Relative Standard Deviations	Throughput Per Day	Volume Needed for Analysis ***	Cost for Multi-Chemical Analyses
Persistent organic chemicals	Blood (serum or plasma)	GC-HRMS	fg/g to pg/g range	15-25%	20	2-30 mL	H
	Milk	GC-HRMS	fg/g to pg/g range	15-25%	20		H
	Adipose tissue	GC-HRMS	fg/g to pg/g range	15-25%	10		H
Non-persistent Organic Chemicals (includes semi- and non volatiles)	Blood (serum or plasma)	GC-HRMS or HPLC-MS/MS	low pg/g to low ng/g	10-20%	30	2-10 mL	H
	Urine	GC-MS/MS;HPLC-MS/MS; immunoassay	low pg/g to low ng/g	10-15%	50	1-4 mL	H
	Saliva	GC-HRMS; GC-MS/MS or HPLC-MS/MS	low pg/g to low ng/g	10-15%	30		H
	Milk	GC-HRMS; GC-MS/MS or HPLC-MS/MS	low pg/g to low ng/g	10-15%	40		H
Volatile Organic Chemicals	Blood (whole)	GC-MSD or GC-HRMS	low pg/g range	10-20%	10-20	5-10 mL	M
	Breath	GC-MSD	low ng/g range	10-20%	20	10-20 mL	M
Bioaccumulative metals	Blood (whole)	ICP-MS	low ng/g range	10-15%	40	1-2 mL	M
	Hair	ICP-MS	low ng/g range	10-15%	40		M
Non-bioaccumulative metals	Blood (whole)	ICP-MS	low ng/g range	10-15%	40	1-2 mL	M
	Urine	ICP-MS	low ng/g range	10-15%	40		M
	Hair	ICP-MS	low ng/g range	10-15%	40		M
Cost categories	Low (L) Medium (M) High (H)	\$0-100 \$100-500 >\$500					

Another analytical technique that is often employed with organic chemicals is immunoassay (IA) or bioassays (BA). For these techniques, a sample preparation step to isolate the chemical from the matrix may or may not be used. Many IA and BA are commercially available for selected chemicals. However, the development of an IA or BA for a new chemical is a lengthy process which typically requires the generation and isolation of antibodies, then the development of the assay itself. Usually UV, fluorescence, or radioactivity detection is used for the assays. IA and BA may be very specific for a given chemical or they may have a great deal of cross-reactivity that may limit their utility. The LODs for IA and BA can vary widely; however, many have adequate sensitivity for measuring levels in the general population. The imprecision usually ranges from 10 to 15% and the throughput is usually quite high (>100 samples per day).

Because organic chemicals are measured using expensive instrumentation and require highly trained analysts, these measurements are usually costly. The most selective and sensitive methods are usually the most complex and can range in cost from \$100 to \$1500 per sample analyzed. However, many of the analyses are multi-analyte panels so the cost per analyte per sample is much more reasonable. In general, IAs are less specific and less complex; therefore, their cost is usually less than \$50 per test. However, researchers can usually measure only one chemical per test, and new chemicals cannot be easily incorporated into the method. One IA test that could potentially be useful for the NCS is cotinine, a metabolite of nicotine. This test is adequate to differentiate passive smoking from active smoking; however, it likely lacks the sensitivity to differentiate between degrees of passive smoking (e.g., 2 smokers in home vs. 1 smoker in home). Other IAs may have potential also for screening samples to determine the necessity of further testing.

### *Inorganic Chemicals*

The sample preparation process for inorganic chemicals is typically much simpler than for organic chemicals. In some instances, the sample matrix just needs to be diluted with water prior to analysis. However, special precautions must be taken to avoid contamination, both pre-analytically and in the analytic system. For example, pre-screened collection materials should be used for sample collection, all analytic supplies should be appropriately free of the target chemicals and special clean rooms may be required for analysis.

Inorganic chemicals are usually measured using atomic absorption spectrometry (AAS) or inductively coupled plasma-mass spectrometry (ICP-MS). In some instances, a dynamic collision cell may also be used to eliminate potentially interfering salts from the system. When various forms of inorganic chemicals are speciated, such as for arsenic or mercury, the AAS or ICP-MS will be preceded in-line by some chromatographic unit. For lead screening, researchers can use an efficient portable lead analyzer for in-field measurements.

Similar to organic chemicals, because expensive instrumentation is used, the analyses are usually costly, ranging from \$50 for single chemicals to \$250 for multi-chemical panels. The LODs are comparable to those of organic chemicals and are suitable for general population studies. Because the handling of the sample is usually minimal, the precision is usually better, within 5 to 10%. In addition, the throughput is usually around 50 to 100 samples per day.

### *Quality Assurance and Control*

A vital component of all biomonitoring methodology is a sound quality assurance/control program (QA/QC). QA/QC programs are typically comprised of multiple testing procedures that easily allow the detection of systematic failures in the methodology. These testing procedures can include proficiency testing to ensure accuracy as measured against a known reference material, repeat measurements of known biological materials to confirm the validity of an analytical run and to measure analytical

precision, Around robin@ studies to confirm reproducible measurement values among laboratories analyzing for pesticides and/or metabolites, regular verification of instrument calibration, daily assurance of minimal laboratory contamination by analyzing Ablank@ samples, and cross validations to ensure that multiple analysts and instruments obtain similar analytical values. Many laboratories have adopted comprehensive QA/QC programs to ensure valid measurement results (Needham et al., 1983; Schaller et al., 1995). For instance, some public health laboratories in the US have been certified by the Health Care Finance Administration (HFCA) to comply with all QA/QC parameters outlined in the Clinical Laboratories Improvement Amendment of 1988 (CLIA >88) and many other laboratories have received ISO 9000 quality registrations. The Federal Republic of Germany has chosen to implement a rigorous internal and external quality assurance program for environmental and toxicological analyses (Lehnert et al., 1999; Schaller et al., 1995; Schaller et al., 1991). Many parameters for implementing or improving a quality assurance program have been published (Schaller et al., 1991; Taylor, 1987; Westgard, 2002).

### Conclusions

As a part of the NCS, many researchers will be competing for the matrices available for biological measurements, whether it is to assess nutritional status, genotyping or phenotyping, or to make chemical exposure measurements. Researchers should refine existing methodology to include as many chemicals as possible using as little blood or urine as possible. In addition, researchers should investigate ways to use more readily available, less-invasive matrices, such as urine, and alternative matrices such as saliva and feces. Researchers must consider all matrices and analytes that integrate exposure over longer periods to maximize the exposure information gained on an individual using the matrices available during a particular life stage.

Another consideration is the quality and cost of analyses. Researchers should evaluate low-cost techniques such as immunoassays for some applications. In addition to requiring smaller volumes of samples, these analyses are often less expensive and require less training to effectively perform the analyses. Before using these less-costly techniques, researchers should confirm that they can obtain quality exposure assessment information—as rated by method sensitivity, accuracy, specificity and precision—and that the resulting data will be comparable to data existing in the literature.

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## 5. Survey Collection Instruments for Use in the NCS

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### 5.1 Introduction

An important aspect of public health protection is the measurement or estimation of exposures to hazardous chemical contaminants that contribute, either directly or indirectly, to increased rates of pre-mature death, diseases, discomfort or disability. Children are exposed to a variety of potentially harmful chemicals in the air they breathe, the liquids they drink, the foods they eat, the surfaces they touch, and the products they use. There are two general ways of quantitatively estimating exposure: directly measuring the amount, duration, frequency of the agent, or estimating the exposure through an indirect assessment. Direct assessment includes point-of-contact or point-of-use measurements of air, water and food, and/or biological indicators of exposure such as chemicals or their metabolites measured in urine and blood. Indirect assessment may include some environmental monitoring of microenvironments combined with modeling of air, water supply, and food supply with information provided through other means to determine potential exposures, such as use of existing data bases. In addition to collecting direct or indirect exposure measurements, it is important to also collect questionnaire and time-activity diary data in NCS. Researchers can use this information not only to augment any measurement data collected, but also to estimate exposures in the absence of direct monitoring data.

Questionnaires and other survey instruments, including diaries and checklists, will be used extensively throughout the NCS to gather participant information for a variety of purposes, not just to estimate exposures. Therefore the survey instruments should be developed across the study to meet the needs of multiple hypotheses. Innovative planning about how to do so efficiently should precede the actual development to minimize the overall burden on the participants. The quantity of information that will be requested from each participant over the length of the study will be very burdensome. Survey instruments will likely be used to characterize the frequency and duration of exposures over the length of the study. Incentives used for participation, as well as additional incentives for the quality of the response (perhaps measured as completeness, or other internal measures of participation), will need consideration.

The form of the data collection instrument and mode of administration of the questionnaires, time and activity logs, diaries, source inventories, and check lists of sources or conditions vary from the very basic paper-pencil questionnaire to the web-based questionnaire. Automation and instrumentation development have made it possible to remotely monitor the status of source/product use, and voice or image record locations, sources, activities, and other exposure related factors. The extent to which these developments are affordable and ready for national implementation is not part of this chapter. However, once the final study design features and study hypotheses have been formally approved, options for technological enhancements, and how to incorporate these over the projected length of the NCS, should be weighed before field implementation. *Table 5-1* compares the advantages and disadvantages of administering a pencil and paper interview (PAPI). Self-administered questionnaires appear to be more favorable in many instances, but concerns over participants following instructions properly and returning questionnaires promptly are deterrents. Self-administered questionnaires usually require additional follow up to either return the questionnaires or complete missing, or questionable information.

**Table 5-1. Self vs. Interviewer Administered Pencil and Paper Questionnaires**

Category	Self-Administered	Interviewer Administered	Why Is There An Advantage?
Cost	Advantage	Disadvantage	Self-administered questionnaires do not require a visit from an interviewer
Participant convenience/burden	Advantage	Disadvantage	Study participants can complete the questionnaires at their own convenience
Compliance with instructions/protocol	Disadvantage	Advantage	The presence of an interviewer ensures compliance with protocol (ex. skip patterns)
Timeliness of completion	Disadvantage	Advantage	An interviewer can schedule the home visit and completion of questionnaire
Sensitive/personal questions	Advantage	Disadvantage	A respondent needs privacy for sensitive/personal questions
Follow-up	Disadvantage	Advantage	No follow-up and extra costs are likely required if administered by interviewer, self-administered usually requires follow-up to get respondent to return questionnaire

The type of survey instrument and mode of administration used will depend on the age of the child and/or the status of the parent. For example, the pre-pregnancy cohort may be asked sensitive and personal questions. Experience has shown that survey collection instruments such as audio computer-assisted self-interviewing (ACASI) and telephone audio computer-assisted self-interviewing (T-ACASI) are methods to obtain this type of information while respecting the participants’ sensitivities (Gfroerer, et al., 2002).

Each questionnaire’s content will be considered carefully before any decisions are made on how the questionnaire will be administered. Also, the framing and wording of a questionnaire will have a great effect on the responses to it. In general, the more standardized the question, the better it is for supporting inferences across studies (NRC, 1991), which is important because (1) it serves as an independent check on how representative and valid the study population might be; (2) the other data collection efforts can be used in calculating non-response bias; and, (3) the wording of the questions has been field tested, and reworked if necessary, to overcome many of the usual misunderstandings that often arise, especially across communities and cultures. As a result, each question should be examined for its appropriateness for the study. Each instrument should be pilot tested prior to full implementation to test the reliability (precision) and validity (accuracy) of respondents’ answers, especially for estimating frequency or duration of exposure to various pollutants.

The following sections discuss four general types of data collection that are implemented at various stages of the child’s development: (1) questionnaires; (2) time and activity logs; (3) diaries of specific activities such as foods eaten; and, (4) visual assessments, database inventories and check lists. Other forms of data collection instruments (e.g., chain of custody records, quality control meta-data, etc.) are related to actual measurements and will be discussed as part of the monitoring protocols. The sections also include a brief discussion of existing data sources together with many of the databases that can be used for this study. *Appendixes B through F* provide a number of survey instruments used in major past or planned environmental exposure studies. These survey instruments serve as sources of questions to be included in the various modules as discussed below. However, the final set of questions should be selected based on a review of their ability to provide the information specific to the needs of the NCS.

## 5.2 Questionnaires

Questionnaires will be used extensively throughout the study to determine the potential exposure for each participant, characterize the total population relative to the study population, and identify non-response bias. For this paper, questions related to the participants’ health status will not be included because this paper deals with exposures and not outcomes; however, it is extremely important to note that

many questions are valuable for both purposes. Consequently, in the final questionnaire development, questions should be reviewed to ensure completeness, but to avoid duplication whenever possible. In the design of the questionnaire, basic questions must be answered related to the questions being asked:

- Are the correct questions being asked?
- Are the participants interpreting the questions in the intended manner?
- Can the participant provide the information needed for an accurate estimate of the exposure?
- What level of detail can participants be expected to remember?

Many questions need only be asked once because the response would not vary with time, e.g., gender. Other questions may need to be answered only when specific conditions warrant, e.g., when a specific source is present, during a specific developmental period of the fetus or child (see **Table 5-2**), or apply to a specific health hypotheses, e.g., asthma. And others may be repeated when the conditions have changed, e.g., when the participants move to another residence. To the extent possible, these considerations have been grouped into modules that are described below. (Note: a **questionnaire** is defined by timing, purpose and/or intended use; **module** is defined as specific topic areas such that the questions are all related to that topic area; and **items** are the specific question based on need for information.)

**Table 5-2. Developmental Stage for Exposure Data Collection**

Time Period	Developmental Stage	Exposure Sources	Social Interactions <sup>1</sup>
Pre-pregnancy	Conception	Via mother and father	Family
Pre-natal	Fetal	Via mother	
Birth to 1 week	Infant	Food (formula, breast milk, water, beverages)	
1 week to 1 month	Infant	Food	
1 month to 3 months	Infant	Food (Infant foods, etc.)	
3 months to 6 months	Infant	Food, toys	
6 months to 1 year	Crawlers	Food, toys, surfaces	
1 year to 3 years	Walking	Food, surfaces	
3 years to 5 years	Pre-school	Food, air, surfaces	
5 years to 12 years	Pre-adolescent	Food, air, surfaces	
12 to 18 years	Adolescent (at home)	Consumer products, activities, food	Peers
18 years to 21 years	Post-adolescent (away)	Consumer products, activities, food	Peers
21 years and older	Adult	Consumer products, activities, food	Peers

<sup>1</sup>To be completed and coordinated with the Social Interaction WG.

For purposes of this discussion, questionnaires have been organized into six modules with topic areas delineated where appropriate:

- I. Baseline Questionnaires – Including Roster Of Family Unit
  - A. Demographics
  - B. Housing characteristics (physical); conditions and activities (window status, etc.); residential history (past, present); and renovation/remodeling activities
  - C. Parental Occupation (maternal and paternal)
  
- II. Personal Lifestyle Exposure Questionnaires—Parent/Child
  - A. Lifestyle or Risk Behaviors
  - B. Activities
  - C. Diet

- III. Source Questionnaires and Inventories of Sources
  - A. Consumer products
  - B. Use of Pesticides and Presence of Pets
  - C. Others
  
- IV. Microenvironmental Characterization Questionnaires
  - A. Neighborhood and Residential Outdoor Environment
  - B. Child's Bedroom Environment
  - C. School Environment
    - 1. Daycare
    - 2. Elementary
    - 3. Middle/Junior
    - 4. High School
    - 5. College/Technical School/Other
    - 6. Work Place Environment (all applicable household members)
    - 7. Playgrounds and Recreational Environment
  
- V. Hypothesis Specific Questionnaires (Refer Back To *Chapter 2*)
  - A. Microbiological and Particles
  - B. Specific to each chemical class
  - C. Specific to each hypothesis
  
- VI. Other Types of Questionnaires
  - A. Biological (when measurements are collected, e.g., when urine sample collected, pre-void interval, and void- volume)
  - B. Questionnaire of participant/family unit during monitoring period (say 24-hrs)
  - C. Questionnaires related to specific activities, e.g., breastfeeding
  - D. Questionnaires related to specific life stage exposure events (*Table 5-2*)
  - E. Data collections logs and forms

### 5.2.1 Baseline Questionnaires

#### *Household Roster, Demographics and SES*

The first item of importance is to obtain a complete roster of each individual living in the household unit. For the NCS, the roster may be administered in the clinic by a health care professional, or at home by a trained interviewer. In the examples provided, questions specific to the study objective were included so that exposure related information about all household members could be ascertained. In a complex study with many hypotheses, obtaining even this limited amount of detail for each member would be difficult, but it may be worth the effort.

The Demographics and SES module is designed to be administered one time to the family unit (father, mother, and if appropriate, other siblings and family members). However, selected items may be repeated during the course of the study should information change, e.g., marital status, household income, etc. Researchers can use this questionnaire for all chemical classes and for all hypotheses (all health end points). The following shows the types of information needed (items have been selectively chosen primarily from the CHAMACOS [Eskenazi et al., 2003]).

- date and location of birth
- gender
- marital status
- country of origin
- total number of years in U.S.
- country of mother's birth
- country of father's birth
- ethnic group and race
- last grade completed in school
- household income/month
- number of people supported by this income
- height in feet and inches
- weight in pounds
- language spoken at home

### *Housing Characteristics, Conditions, Activities and Residential History*

Many excellent questionnaires have been used in past studies that collected housing characteristics, conditions, activities, and residential history information. In particular, HUD has an active program in surveying homes, both those with lead (and lead remediation projects), and with their healthy homes program. (See, for example, <http://www.icfhosting.net/HUD/HH/HealthyH.nsf/>). Data required for probabilistic exposure assessment models include basic demographic information on each participant home and location relative to sources and geography. This information often serves as a preliminary indirect exposure estimate for potential risks for chemicals with major indoor (at home) sources. Researchers' should attempt to obtain in-depth information about the residential environment to compare with data from other surveys (e.g., national census information and previous health and exposure studies). From a perspective of time spent, the residential environment is of greatest importance relative to exposures to the child and the parents, which accounts for the large amount of information requested of the participants. Types of information needed are shown below. These data are very useful in a probability-based survey to adjust the statistical analysis weights to reduce nonresponse bias (Whitmore et al., 1999). In addition to using the questionnaire information, researchers can capture location by question or with a GPS unit.

Questionnaires that have been used successfully for those purposes in other exposure assessment studies include the Baseline Questionnaire and Descriptive Questionnaires used in EPA's NHEXAS field studies (see [http://oaspub.epa.gov/heds/study\\_list\\_frame](http://oaspub.epa.gov/heds/study_list_frame)) and, also, the "Standard Basic Environmental Inventory Questionnaire" (Lebowitz et al., 1989). The references at the end of this chapter provide other sources for additional information (e.g., Alameda County Community Development Agency, 2001; Burge, 1999; Daisey and Angell, 1998; Flannigan and Morey, 1996; McNeel and Kreutzer, 1996; NYCDH, 2002; Odom and DuBose, 1996; EPA, 2000; ISIAQ, 2004).

Researchers may need to survey multiple housing units to account for time spent with parents not living together, grand parents, and other relatives or child care members, as well as homes used by the participants such as vacation homes. The school environment is excluded from this residence questionnaire because it is addressed in a separate microenvironment questionnaire. This questionnaire seeks the following types of information:

### *Housing Characteristics*

- Housing Type
- When was this building built
- How long have you lived at this address
- Number of rooms in Dwelling
- Size of dwelling
- Source of tap water
- Telephone service available in Dwelling

- Does your kitchen stove have top burners that use natural or LP gas? Does it have a constantly burning pilot light?
- Primary heating fuel used in Dwelling
- Type of fuel used for cooking
- Presence of a wood burning fireplace or stove
- Air conditioning and type
- Carpeting: age and where (% of total living area)
- Type of flooring material (other)
- Does your kitchen have a hood/exhaust fan? If yes, where is it vented? How often is it used?
- Garage? Attached? Is there a door leading from living quarters to the attached garage? Number of vehicles? Other gasoline engines? Gasoline and solvents stored?
- Gas appliances? Which ones have pilot lights?
- Porches and condition
- Foundation
- Number of bathrooms
- Presence of a washer/dryer and dishwasher inside the main living area

### *Housing Conditions*

- Ventilation: type, hours, by room, day of week, season
- Window and door status: hours, by room, day of week, season
- During the past 3 years, has it been remodeled? Redecorated with new carpeting, new upholstering or drapes? Repainted? If so, did someone sand, scrape or burn off any of the old paint?
- Are there signs of paint peeling, flaking or chipping?
- If an evaporative cooler is used for cooling (Arizona, Texas and other parts of the Southwest), how often are the pads changed on the coolers? How often is the water drained and the cooler cleaned? How often is a water treatment added to the cooler?
- Signs of water leakage on walls or ceiling
- Rodents and other pests
- Other potential sources (humidifiers, cooking practices)
- Number of personal motor vehicles kept at dwelling by household members
- Monthly electrical cost (previous month)
- During the past year, have you had damp or wet spots on surfaces inside your living quarters other than in the basement or bathroom?
- During the past year, have you had molds (including mildew or fungus) growing on any surface inside your living quarters
- Have there been leaks, flooding, or water damage in your living quarters or basement?
- How frequently are the beddings where your child sleeps washed?
- Have you changed mattresses or added/removed mattress covers in the past 6 months?
- Have you or your child(ren) acquired any stuffed animals in the past 6 months?

### *Residential History*

- For the mother, father, and participating child, starting with current residency, list address of each location lived in during the past 5 years. Include additional residences such as vacation homes where more than 4 weeks were spent during the year. Indicate how long lived in each location.
- A subset of questions related to housing characteristics and conditions from above would be asked for each location other than the current place of residence.

### *Household Activities*

In general, questionnaires are used to collect data to assess the activities that are occurring during a specified period of time, usually when there are monitoring instruments in operation. An example of a questionnaire of this type found in the appendices is the planned Longitudinal Study of Young Children's Exposures in their Homes to Selected Pesticides, Phthalates, Brominated Flame Retardants, and Perfluorinated Chemicals – A Children's Environmental Exposure Research Study (CHEERS): Monitoring Period Questionnaire.

- Combustion appliance use: hours, weekday/weekend, season (this includes wood stoves/fireplaces, gas range, ovens, and gas water heaters).
- House cleaning activities
- Use of specified products, e.g., pesticides (indoors)
- Cooking
- Hobbies
- Construction or renovation

### *Occupational History*

The objective of this questionnaire is to obtain in depth information about the occupational environment for two different reasons related to this study. The first is to determine the potential pre-conception exposures of the father and the mother that may influence the conception and fetal development period. The second reason is to estimate potential take-home exposures that might influence the residential environment. Also, older children often work part- or full-time jobs, thus receiving direct occupational exposures to work place chemicals if they come into contact with them. Researchers can compare responses to many of these questions with other occupation survey information and occupational exposure literature. An occupational history questionnaire seeks the following types of information:

- place of work, the job duties, hours per week. Specify hours worked outside, and hours work around expected sources
- potential sources (additional topics may be included for specific hypothesis or pollutant class, e.g., gases, PM, pesticides, metals)
- use of protective clothing
- potential for bringing occupational sources into home (e.g., removing clothing and shoes before leaving worksite or coming into the home)
- laundering (washing work clothing separate from child's clothing)
- job history
- exposures to specific chemicals or products. This list would be made specific to each hypothesis, to relate to the existing scientific knowledge of potential source-effects studies.

#### 5.2.2 Personal Lifestyle Exposures Questionnaires

These questionnaires relate to lifestyle behaviors, including use of certain medications and drugs, and exercise. The resulting information can be closely related to medical information collected during pregnancy and also establishes a link to specific health outcomes of interest to the NCS. Also included in this section are questionnaires related to hobbies, travel time, and physical activities. These questionnaires are specific to the parent or others who might be the source of exposure to the child. Once the child is older, the behaviors of the child become the primary source of the exposure.

Obtaining accurate information related to lifestyle behaviors is very difficult and the responses often reflect perceptions of what is the right answer instead of accurately reflecting the actual behaviors of

interest. Questions about alcohol, drug and tobacco use are sensitive in nature and should be addressed in a self-administered instrument.

### *Lifestyle Behaviors*

- Smoking habits (pre- and post-pregnancy)
- Intake of alcohol, coffee, caffeinated sodas, water
- Illicit drugs (marijuana, cocaine)
- Vitamins, herbal supplements
- Medications
- Exercise routine
- Does anyone in the household participate in a routine physical exercise program at least three days a week, on a regular basis
- How many stair steps do you usually climb up each day
- How many city blocks or equivalent do you regularly walk each day
- For the child, number of friends who routinely participate in activities with the child outside of the school
- Hours of sleep and sleep patterns
- Nutritional questions otherwise not included in the dietary questionnaire

### *Hobbies, Physical Activity/Exercise, and Travel*

- List hobbies
- How many hours each week do you engage in hobbies
- List any sport recreation or other physical activity you engaged in over the past week. List average number of minutes/activity and number of days during the week. Is this (number of activities, duration and days per week) similar to other weeks during the past six months?
- How many hours during an average week do you spend in a motor vehicle
- How many hours during an average week do you spend vacuuming, dusting, sweeping or cleaning the home?
- Laundry (if washer is in/connected to main living area), dishwasher use
- Cleaning bathrooms (with spray, solvent based cleaners)

### 5.2.3 Source Questionnaires and Inventories of Sources

These questionnaires relate to use of various products, including a more detailed questionnaire about smoking activities. Many consumer products, such as products used while cleaning, are sources for Volatile Organic Compounds (VOCs). Smoking produces vast amounts of aerosols and vapor phase organic compounds, including PAHs. House cleaning activities stir up particles that have been tracked in or have deposited from the air. Pets are a source of pet allergens and often are a primary vehicle for tracking in chemicals deposited on the soil or other outside services, including pesticides. If not captured else, use of specific combustion appliances and sources (e.g., wood stoves) would be captured here. Depending on the specific hypothesis of interest (e.g., asthma), more detailed questions would be asked about how and when these appliances are used.

### *Consumer Products*

The purpose of this type of questionnaire is to collect information on the purchase and use of specified consumer products that might influence exposure to the participant(s) in the study. Note, for example, the Monthly Cleaning Products Purchase, Inventory, and Use Log. This questionnaire is to be used in the planned EPA CHEERS project. Types of information of importance include:

- Use of various consumer products: paints, solvents, candles, air fresheners, cosmetics, fragrances,
- Use of cleaning products
- Use of automotive products (car wax, tire polish, etc.)
- Use of charcoal starter
- Use of any product producing smoke, fumes, or strong odor
- Gasoline storage location
- Use and storage of devices with a gasoline engine
- In locations with odors: perfume or cologne, pesticide or insecticide, drying paint, new carpet, car exhaust, natural gas (gas stove or fireplace), disinfectants, chlorinated water, soft plastics (e.g., new shower curtain), room air fresheners or deodorizers, tobacco smoke, burning candles, personal deodorants, hair care products, finger nail products, etc.
- Upholstered furniture location, age, and availability for use by dogs/cats/animals
- Type and age of mattress
- Type of window covering

### *Pesticides*

Often specific questionnaires are developed for a specific consumer product, or in this case, a class of chemicals, pesticides. It may also include questions about how it is used, location of use (which rooms and surfaces treated), how much and how it is applied. There are several examples of such a questionnaire found in the appendices, related to pesticides. (See, for example, Monthly Pesticide, Purchase, Inventory, and Use Log taken from the planned CHEERS project.)

- Do you or someone in your household apply pesticides for control of insects or other pests? What is applied? How often?
- Do you have pets? If so, how many? Do they spend time indoors? Where do they sleep? Do you apply flea control on your pets? If yes, what type?
- Household use of pesticides. (what, how applied, when, where, how often)
- Garden and outside use of pesticides. (what, how applied, when, where, how often)
- Storage of unused pesticides. (location, near food, appliances [safety issues])

### *ETS Exposure*

ETS exposure has been shown to be a major source of many chemicals and there are many excellent questionnaires available as examples to use to characterize exposures to each of the participants in the study. Furthermore, several of these questionnaires have been validated with biomarker measurements, usually measurements of cotinine or nicotine in several matrices (Hammond et al., 1987; Repace et al., 1998; Tunstall-Pedoe et al., 1995; Jenkins and Counts, 1999). In a general way, these questionnaires can provide an indirect, but validated measure of exposures occurring to the participants. In addition, the ETS biomarker, cotinine, has been measured in the blood of NHANES III participants so that there is a national population distribution for reference. Several examples of ETS questions are provided in the appendices.

### *Dietary Questionnaires*

The diet is the major route of exposures to many of the chemicals of concern throughout the life stages of the child. As discussed in **Chapter 3**, there are both direct methods and indirect methods for measuring dietary exposures. The indirect method uses questionnaires or an appropriate database from which to obtain information about what foods are being consumed and the quantities of that food consumed. This information is then tied together with knowledge of what levels of the chemical is typically found in the food from databases such as the FDA Total Diet Study and Pesticide Residue

Database. The Continuing Survey of Food Intake for Individuals and the NHANES are national population-based surveys that provide detailed information on food intake and distributions of intake in the general population and for some sub-populations. These are often personally administered questionnaires that researchers can augment with models to improve accuracy for portion sizes, and prompts for the food form (e.g., frozen, canned, fresh) and preparation methods. Researchers use this information to code the food items in ways linkable with existing food contaminant databases to provide indirect estimates of dietary exposures. (See, for example, the [Food Diary](#), that will be used by the U.S. EPA in the planned CHEERS project.)

There are basically three types of dietary intake instruments used in exposure studies. They include [dietary recall](#) in which questions are asked about what one ate for the last 24-hours, or in some cases even longer such as month or six months. The shorter the time period from the eating event and the reporting of the data would normally increase the accuracy of the reported data and the association with the levels of the chemical within the body. The second type is the [food frequency questionnaire](#) in which questions are asked about how frequently over a period of time one ate specific types of foods. The resulting information is normally used as an indication of longer term exposures for chronic health measures. The third type is the [food diary](#) wherein one records the foods and amounts actually consumed during each eating event. In general this form of data collection is usually considered to be the most accurate and most directly related to a specific exposure event and/or internal biomarker of exposure. Additional information about this methodology is provided in section 5.4.1.

In addition to types of questions that ask about what is being consumed and how much is being consumed, there is also a need to know how the food is prepared and also how the food is eaten. For young children, especially those unable to eat with utensils, it is often important to ask questions about what a child is eating, but also something about the child's eating behaviors, such as:

- On a typical day, how many times does your child engage in the following:
  - uses fingers to eat;
  - washes hands before eating;
  - washes hands after eating;
  - uses napkin;
  - licks fingers while eating;
  - uses fingers to eat snacks;
  - washes hands before eating snacks;
  - eats while walking around;
  - wipes fingers on clothes or on furniture;

Some types of food contain substances that are known to be potential toxins. Often questions about these foods are asked. Because many of these chemicals bioaccumulate in the body, it is important to know how much of the food is eaten and how often the food is eaten. For example, questions related to the following products are often included in the dietary portion of questionnaires:

- intake of fish and type
- intake of locally grown fruit
- intake of locally grown vegetables
- intake of wild meats (deer, birds, etc.)
- intake of local milk (not pasteurized)

#### 5.2.4 Microenvironmental Characterization Questionnaires

These questionnaires are designed to characterize the potential sources of exposures in important locations (microenvironments) for the participants of a study. As mentioned above in section 5.2.1, the household environment is the single most important location because that is the location where most of the participants will spend much of their time. Furthermore, there are a number of potential sources and factors that can contribute to the actual exposure levels experienced by the participants. To a lesser extent, other microenvironments can potentially account for additional exposures to various chemicals and microbiologicals, and the primary ones are identified below.

*Neighborhood and Residential Outdoor Environment*

- describe neighborhood (or better, provide a digital image of outside of house, and surrounding area)
- location of roads, sidewalks, parks and play areas in relation to the household
- volume of traffic and type
- location of nearby commercial and industrial sources
- description of noise levels during busiest time of work day (on a scale of quiet, intermittent low level noise, constant low to medium noise, loud, very loud)
- description of odors, if applicable

*Child's Bedroom Characterization*

- amount of carpeted area or area covered by rugs
- number of windows
- type of window treatment (e.g., blinds, curtains, etc.)
- type of mattress, pillow, blanket/comforter
- bed frame made of what material.
- Number of children who use the room
- Is food allowed in the room
- Television in room? Other entertainment devices?
- Average number of hours spent in room per day
- How often is the room cleaned, vacuumed, or mopped?

*School or Day Care Environment*

- If the child goes to school (or will go in the coming year), what school does (will) he/she attend during the coming year?
- Provide location of school, grade levels of school, other physical information about the school, include approximate age
- How many hours a day does this child usually go to school?
- How far does this child travel to get to and from school each day from home? Mode of transportation?

The following questions would be addressed to someone who can answer on behalf of the school. Most parents would not have the information readily available. (Note that this could potentially be obtained by a third party on behalf of the NCS.)

- Number of children in school; Number of children in child's classroom; number of staff
- Percent of floor area in classroom that is carpeted
- Is there a routine schedule for pesticide application?
- Are windows open during the day, weather permitting?
- How frequently are the floors swept? Mopped? Vacuumed?
- Is there a water fountain in the classroom? Outdoors?
- Is the classroom cleaned daily?
- Note: if detailed information is required about the school environment, a separate questionnaire and/or checklist should be used with information provided by a knowledgeable school employee.

### *Child's Workplace Environment*

- Does this child work part or full time? If yes, job title or position. Number of hours per week during the summer and during the rest of the year.
- Is the work inside or outside?
- How far does this child travel to get to and from work each day? What is the mode of transportation?
- Does it involve use of cleaning products, pesticides, dust, tobacco smoke, food preparation

### *Playgrounds and Recreational Environment*

- List type of recreational area
- If the area includes recreational use of water, provide type (pond, lake, running water, indoor or outdoor swimming pool)
- List activities, average amount of time, and levels of exercise

#### 5.2.5 Hypothesis Specific Questionnaires

These questionnaires are specific to the hypotheses discussed in *Chapter 2*, but, hopefully, researchers can use them across all hypotheses for aggregate exposure estimation. The following are examples of the types of questionnaires sorted by type of information requested.

- Microbiological and Particles
- Questionnaires specific to each chemical class
- Questionnaires designed specifically to address a study hypothesis
- Specific to each stage of Child Development (refer to *Table 5-1*)

#### 5.2.6 Other Types Of Questionnaires

These questionnaires relate to information collection associated with other aspects of the study. They are listed here primarily to ensure that they are included in the total package of information requested about the participants as NCS designers proceed with the study development.

- Biomarker Questionnaire(s) e.g., urine volume, duration since previous void, recent exposures for VOCs
- Breastfeeding Questionnaire
- Questionnaires related to sample collection, storage, chain of custody

### 5.3 Time and Activity

Participant location and activities are critical to exposure research. Pollutant concentrations and sources vary over space and time, and also, people move about during the course of any time period. Studying activity patterns is relatively new to the field of environmental assessment. However geographers, social scientists, and urban planners have a long tradition of studying the nature of people's movement and location patterns. Time activity information is usually collected in diaries, which are discussed in the following section. Research in exposure assessment has demonstrated that information about an individual's location and activity over time is critical to accurately assessing his or her exposures. Because of this importance, time and activity measurements are discussed as a separate section of this paper, apart from the other types of diary information described elsewhere.

To capture activity levels and related parameters, automated methods have been designed to provide individual measurements of location, time, energy level, and activity (video imaging). Although plausible, detailed measurements of this type can be extremely intrusive on the participant and can compromise confidentiality as well as generate ethical issues. Less intrusive automated methods include the use of handheld computers, motion sensors, and GPS units. However the most common, but most burdensome, technique for capturing this information is with an activity log completed by hand such as the following example format. (See detailed examples provided in the appendices, such as the Activity Time Line, taken from the planned CHEERS project.) Robinson (1983) has reported good reliability results from adults using the recall method, even though other researchers question how reliably one might remember specific exposure related events.

**Format 1:** On an event basis (e.g., every time the activity or location changes) and/or time basis (e.g., every ½ hour) for a defined period (e.g., 24 hours) record:

Start time	Location	Activity	Energy level	Stop Time
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**Format 2 RECALL:** Over the past 24-hours, record the number of hours (minutes) in the following locations, or engaged in the following activities:

	Indoors		Outdoors		Commuting (Specify Type)
Home	_____	Home	_____	Home	_____
School	_____	School	_____	School	_____
Work	_____	Work	_____	Work	_____
Other	_____	Other	_____	Other	_____

Often recall questions are asked in a very general way, such as:

- On most weekdays, how many hours does the child spend in the following places: indoors, outdoors, in a car or other motor vehicle?
- Does this child engage in other activities that take him/her out of the home for several hours each day?

There have been a number of time usage surveys as evident in the literature. The State of California has conducted a statewide exposure related time survey of California residents (Wiley et al., 1991). California has also conducted a statewide survey specific to children (Wiley, 1991). The U.S. Environmental Protection Agency (USEPA) has conducted a national exposure related time survey (U.S.EPA, 1996), and have consolidated existing activity information into one database, Consolidated Human Activity Database (CHAD). This database contains human activity data collected in surveys conducted throughout the U.S. and Canada.

Newer technologies are being tested to obtain time and activity information. For example, a passive photo diary has been used for collecting detailed activity and microenvironmental location information (Johnson et al., 2001). Researchers can configure the camera for self-activation on a periodic basis, with color pictures taken and stored electronically with a time stamp. Motion sensors have been used to collect activity levels (Johnson et al., 2001).

Capturing time and activity information is generally quite burdensome and considered by many participants to border on potential invasion of privacy, especially when photo technologies are used. However, the resulting data are extremely useful for understanding how exposures occur and the duration

of the exposures. The importance of this information is also becoming more evident in unexpected ways. For example, Roy et al. (2003) reported that time activity data is a better predictor of blood concentrations of benzene and lead than the measurements in environmental and dietary media.

## 5.4 Diaries

Diaries have been used in exposure and epidemiological studies for many years. The diary provides information in a structured manner about an individual for specific purposes. In addition to the time – activity information discussed above, diaries have been used for many other purposes, including the three below.

### 5.4.1 Diet Diaries

For many environmental pollutants, such as pesticides, ingestion exposure is the largest component of one's exposure. In the case of diet, pesticides are applied to many of the food items humans consume. With the diet diary that contains information about what was eaten, how much of the item was eaten; one can use information contained in pesticide residue databases to estimate dietary exposures. FDA also provides information about nutritional information, as well as other chemical information. Dietary exposure research has provided results that can improve the dietary exposure estimation estimates. Factors that have been found important for exposure, in addition to what is eaten and how much, include how the food was packaged and prepared, how it was eaten (fingers versus utensils) and if it might have been in contact with a contaminated surface. It is also important to know if the food is home-grown in gardens or greenhouses, and location of origin of the seafood (if possible).

The USDA Food Surveys Research Group (USDA/FSRG) has developed an automated multiple-pass method that improves recall for foods respondents have eaten over the previous day (Moshfegh et al., 1999). The method has automatic cues that prompt the user to recall details of each food and how it was prepared. Data accuracy is enhanced by the use of a new Food Model Booklet that contains life-size photographs, two-dimensional drawings, and interactive geometric shapes that help respondents recall portion sizes of the foods they have consumed. The Automated Multi-Pass Method and Food Model Booklet are being used in the current NHANES data collection efforts conducted throughout the nation. In this context, a computer-aided dietary interview (CADI) is administered by an NHANES staff member during the initial interview with each participant. Four to ten days later, the CADI is administered over the telephone to obtain a second day of food consumption data for each participant in the study. The multiple pass format prompts the participant to report the time and place each food was eaten.

### 5.4.2 Source Use Diaries

Diaries are often used to record participant's use of various sources, including tobacco, alcohol, and other consumer products. In conjunction with on-going activities within a residence (see section 5.2.1.2.4), diaries are used to record when windows and doors are open or closed, the air conditioner or heater are on or off, when the stove is being used, and the like. This information is then used to estimate indoor air quality measurement results.

Evaluation of potential aggregate and cumulative exposures associated with consumer products used in and around residences presents unique challenges as a result of the complex and dynamic nature of this environment. Potential exposures can occur as the result of the spatial and temporal interaction of product application and human activities in affected microenvironments.

New technology can be a useful replacement for source use diaries. For example, new RFID technology used by grocery stores and warehouses, can detect product use remotely. In the case of the NCS, these systems could trigger a telephone call to the home to request related or follow-up information.

The signal could also be integrated into on-site monitoring equipment to allow for emission concentration measurement.

#### 5.4.3 Other Use of Diaries

The existing literature provides other uses of diaries. Many health studies provide a symptom diary for the participant to record his or her symptoms. Also, research subjects can use diaries to record specific activities when eating, playing, or for about any purpose requiring a detailed accounting of the activity. Unfortunately such use can be quite burdensome on the participants, and response accuracy and detail often fall off over time.

#### 5.4.4 Discussion

Diary methods relying on recall are not highly reliable and have a relatively high respondent burden, which negatively impacts participant compliance. Post-study processing of diary entries is labor intensive unless simplified reporting protocols are employed and automatic processing systems are developed. Voice diaries have been researched with subjects dictating requested information into a portable recorder (RTI, 2001). Automatic word recognition and activity/location coding reduced the human coding by as much as 60 percent.

### 5.5 Existing Databases, Visual Assessments, and Checklists

#### 5.5.1 Databases

As the science of exposure assessment has grown, so have the databases that store the results of numerous studies related to many of the key factors/variables which influence one's exposures. It is possible to obtain a first order approximation of the exposure levels of some chemicals based on knowledge of one's location relative to a source, activities that are occurring at or near the location, nearby spatial and temporal measurement data of the chemicals of interest, wind direction and speed, and time of day. Availability of all this data may be limited, so the estimate may be less accurate than one with additional information. **Table 5-3** provides some of the databases that are frequently used for exposure assessment. These databases are used in conjunction with models that actually forecast the exposure distribution for the time period of concern.

Most human exposure models have not been validated and could be grossly inaccurate, especially for use as a predictor of a single individual's exposure. However, use of available data and models should not be overlooked in this study because of the expenses required for implementation of alternative methods. Combining the various methods available including questionnaires, diaries, models, databases, and measurements would be a worthy compromise for most exposure study designs.

#### 5.5.2 Visual Assessments

Visual assessments prove to be an invaluable aid for the field team conducting the study. They serve as a reminder of what is observed, as well as what the field personnel should be looking for and reporting. A camera snap shot or video often proves to be an invaluable asset for identifying potential sources for unexpected results obtained through other means, such as monitoring. They are also helpful for many other reasons such as medical history reporting, discussions with participants about history of the residence, immediate vicinity, and unusual events. Numerous references provide forms for visual assessments in the survey of housing conditions (HUD Guidelines), Indoor Air Surveys (EPA Indoor Air Program), and there are also ASTM standard guidelines for use in building visual assessment surveys.

**Table 5-3. Databases Available for Guiding Study Design and Estimating Exposures**

Medium/Other	Database	Comment
GIS	City level County level State Level	Usually includes land parcels, street centerlines, sources, streets, roads, other major features
Census Datasets	Extensive information, SES, Demographic, housing and population, etc.	Tiger/Line files critical for identifying boundaries for all counties and current addresses. Note: past datasets can be used for historical exposure modeling
USGS Digital Line Graphs (USGS topographic maps)	Roads, boundaries, hydrology	Includes land use and vegetative cover
USGS	Land Use and Land Cover LANDSAT	Satellite images at 30-m resolution (1980 to present)
Urban maps	Planning maps	Identifies community areas, sources, past and future development
Traffic Counts	City, County and State	
Water	1. community wells 2. public water supply 3. Pipelines 4. WATSTORE 5. STORET 6. PCS 7. State Water Use	Data provide monitoring results by county. Usually maintained by county, but provided to State Environmental Health as required by law. Data available for when systems installed and upgraded in community, including repairs and materials. USGS estimation of private well contamination. USEPA national data on water quality USEPA Permit Compliance System (sources permitted to discharge waste water into rivers) USGS – water withdrawal levels and purposes, water consumption, and water return to source from 1950 to present
Air	1. AIRS 2. County and state air quality data	USEPA national data on air quality and emissions. Data available in addition to data submitted to AIRS
Air – source	NESHAPS NEDS TRI	National New Source Emissions Data for permit requirement (states and local communities permit commercial and industrial sources) USEPA Toxic Release Inventory (releases of 650 toxic chemicals emitted to air, water, and land)
Superfund Sites	CERCLIS	USEPA – national
Hazardous Waste Sites	RCRIS BRS Chemical Bulk Storage Major Oil storage Facilities	USEPA – national. Generators, transporters, treaters, storers, and disposers of hazardous waste. Biennial Reporting System– USEPA. Dataset contains information about the generation, management, and minimization of HW by large quantity generators. States maintain inventories of location and quantities of chemicals in storage. Same as for chemicals.
Toxic Spills database	Most states store information about toxic spills	
Nuclear Power Reactors	NRC	Nuclear power facilities – location(?), size, etc.
Meteorological and Climatological Data	NOAA – Asheville, NC	Also local data available from nearest airports
Agricultural	USDA	Census statistics at the county level
Soils	Natural Resources Conservation Service	Soil erosion and conservation, land use and crop history at the county level
Pesticides	USEPA/OPP USDA Pesticide Residue Data base	National survey of pesticide use
Activity	USEPA (NHAPS) USEPA (CHAD)	National survey of time activity Database of time activity information from literature and surveys (Comprehensive Human Activity Database)
Food consumption Food contaminants and nutrients	USDA – CSFII FDA FDA – TDS USDA PDP	National survey National database updated periodically Total diet survey Pesticide Residue Program
Biomarker	NHANES	Chemical constituents in blood and urine
Houses and Daycare Centers	HUD	

General items as examples of what information an inspector may be looking for in a visual assessment are provided below. These items are taken from a Visual Assessment Checklist, developed by

Partnerships for Children’s Respiratory Health, a demonstration project of Integrated Health and Housing Interventions ([www.aclppp.org](http://www.aclppp.org)).

- type of roof
- type of siding
- overall building condition
- site, exterior and general interior
- evidence of roaches, debris, accumulation of food waste, trash, rodents, pets
- evidence of safety hazards, electrical hazards, Environmental Tobacco Smoke
- paint condition (interior and exterior)
- structural defects
- odors, moldy smells, standing water, water stain marks, visible mold
- flooring (carpet, hardwood, resilient)
- window coverings (cloth drapes, blinds)
- housekeeping, clutter
- GFI Protection near sinks
- smoke detector
- broken windows or doors
- interior walls have large cracks or hole

### 5.5.3 Checklists

Checklists are often used as a standard method for collecting data during a visual inspection. However, they are often used for a variety of purposes as reminders for the field inspectors. As such, checklists are an invaluable tool for quality control purposes.

A field inspection checklist follows as an example of the information requested in an exposure inspection visual assessment.

- unusual activities/events
- inspection of hot water heater
- inspection of furnace
- inspection of space heaters
- inspection of fireplace
- use of pesticides
- use of coolers ( a/c, swamp cooler, evaporative cooler, or other type)
- use of humidifiers
- use of vaporizers
- look for leaks, moisture, water problems
- look for dust control effort –cluttered, dust on window sills

## 5.6 Innovative Approaches

Technological advancements have primarily focused on ways to administer questionnaires to ease the burdens for the participant, facilitate administration by the field staff and handling the resulting questionnaire information. Advancements of this type include Computer Assisted Telephone Interview (CATI) and Computer Assisted Personal Interview (CAPI) whereby the field survey specialist asks questions from the questionnaire and enters the response information into the computer. Skip logic is programmed into the computer so that responses direct the sequence of information requested. For the NCS, it would be expected that similar methods would be employed at all stages of the information gathering process – programmed instruments would be used extensively to reduce burden and improve the quality of information. Approaches utilizing web-based technology for collecting questionnaire information have been pilot tested by EPA in anticipation of needing technology to reduce participant burden for the NCS study (RTI, 2002).

For those cases where self-reporting on paper may be the only option for information collection, standardized forms can now be optically scanned. Accuracy of response is improved if the responses are marked onto the form in closed format. However, open-ended questions are problematic. Paper-based diaries, electronic diaries, voice-recorded diaries, and observational techniques have all been used to

collect data about temporal activities, special locations, product use events, and dietary consumption events of research participants (Johnson et al., 2001).

If possible, NCS researchers should consider implementing such newer and emerging technologies as voice diaries, passive microenvironment identification, wireless interfaces, intelligent prompting, and automated daily review to collect data accurately with low participant burden. *Table 5-4* provides additional details for several of these innovations. Others can be found in a workshop report held on the topic of Remote Collection of Data for the NCS (EPA, 2003). For older children, a pre-programmed Pocket PC would allow the participant to answer a set of queries. Such a device could also be an incentive for continued participation as the child grows older.

**Table 5-4. Technologies for Acquiring Questionnaire-type Information**

Method/Technology	Advantages	Limitations and Issues to be Resolved
Paper diary instrument	Easy to use. Does not require equipment training	Not reliably used by respondents. Data entry and processing costs are high.
Electronic diary instrument on a hand-held computer	Easy to use menu driven system. Automated time-stamp if user confirms entry made at time of activity/location change.	Interface design (e.g., menu format, impact of screen size) may be complex. Screens can be hard to read for certain populations. Battery life.
Voice-recorded diary	Natural and easy to use. Applicable to activity/location, dietary, and product use data. Post-collection speech to text processing is possible.	Speech to text processing requires respondent "calibration" for optimal speech recognition.
GPS devices	Tracks location and velocity without reliance on respondent reports.	Provides location coordinates only, requiring post-acquisition location mapping. Ineffective indoors.
Passive photo diary	Easy to use and compact. Overcomes reliance on respondent to make diary entries.	Participant privacy concerns. Requires participant censoring and post-acquisition coding.
Passive location diary (RFID transmitters)	Easy to use and compact. Overcomes reliance on respondent to make diary entries.	Requires placing devices in user microenvironments. Requires carrying a recording device (PDA).
Bar Code Reader (for product coding)	Easy to use. Minimal training. Minimizes participant burden for post-inventory indication of each specific consumer product use event.	Requires development of bar-code based labeling system. Post-processing required. Expensive hardware.

To ensure that the data obtained are of the highest quality and interpretable for exposure assessment, input verification would be an added feature to the NCS study. Such a procedure would remind participants to make diary entries throughout the data collection period. An automated system would be designed to check for diary entries at regular intervals, provide reminders to participants, and could also prompt for diary entries whenever changes in physiological responses (heart rate or motion) of a given magnitude are detected, or products are used. Entries may also be prompted whenever a passive location change is detected and a diary entry is not made. An automated, multi-pass review of daily activities would ensure data quality and minimize missing information. If data are available in real time, an automated review could be conducted daily. Such review would be fully Pocket PC-assisted, allowing the subject to interact with a set of queries once each day to review and validate collected information.

## 5.7 Limitations and Other Considerations

Survey instruments will be used in one form or other throughout the implementation of the NCS. The type of survey instrument and mode of administration used will depend on the age of the child and/or the status of the parent. Most researchers are aware that these instruments will have varying degrees of

success, depending on what the instruments are seeking to obtain. For example, obtaining a roster of household participants and related information such as birth dates, together with basic information about the residence is relatively easy to obtain, and should be considered to be highly reliable. Most participants will have had previous experiences that ask for similar information, so the overall impact/burden on the participants will be minimal.

On the other hand, exposure specific questionnaires are more problematic for several reasons. First, the participants are less familiar with this venue. They may be asked about uses of specific products or activities that on the surface appear to be quite invasive and personal. For example, when exploring the potential exposures of toxic compounds, the questionnaire may ask for uses of deodorants, cosmetics, or the age of their carpet (assuming there is one). Most likely there will be questions asked about their use of alcohol, tobacco, medications, and nutritional supplements. These questions are often too personal for the individual to give an accurate account, and under-reporting of their usage is the norm. Another problem is that parents may choose to give an “acceptable answer” about their child, when specific questions are asked. For example, questions about how often the child washes his/her hands before eating food is grossly exaggerated. Or asking the parent if the child ever eats food that has dropped on the floor will generally result in a “never” answer. Researchers can overcome many of these problems with field staff-administered questionnaires, but these increase cost and participant burden. For example, explaining to the participant “why” the question is being asked provides the rationale for more representative responses. Another problem is with terminology uncommon to the participant. For example, to many of us, the term “pesticides” means “something used to kill insects.” Weed killers applied to lawns are not pesticides; neither are fungicides used on roses. And, there are the problems with recall. The good news is that these problems are known and successful ways to overcome (or limit) them are available in the literature.

Survey instruments will in some cases be the only method available to get at possible exposures that have occurred. This is because it will not be possible to monitor every participant for every possible exposure for the duration of the study. Furthermore, methods may not be available to measure the chemical/biological agent in the environment or in the body. As the body of knowledge grows about the relationship between measurements and questionnaire response using appropriate questions, so will confidence in relying on the survey instrument to provide an estimate of exposure. Recent successes in this regard include a study by Kieszak et al. (2002) where they reported that people who indicated that they had recently used specific pesticides of concern had higher average urine pesticide (or pesticide metabolite) levels than did people who reported not recently using these chemicals. In another study by Al-Delaimy et al. (2000), the authors reported a consistent relationship between questionnaire responses of exposure to ETS and dose measured by nicotine levels in the hair. In another study of ETS by Kaufman et al. (2002), the authors observed that the number of household smokers was a statistically significant indicator of the variation measured in serum cotinine, but it was not sufficient to provide an adequate estimation of ETS exposure levels as measured by serum cotinine.

In spite of the usefulness of survey instruments for obtaining vital information for use by the researchers in the NCS, three general limitations are worthy of further discussion. The first limitation to survey information is a recognition that most questionnaires that have been used and reported in the literature have not been validated in the broadest sense. The instrument may provide worthwhile information for a select community or state, but it may not work well across the country for a national survey. Furthermore, there are no available, appropriate exposure questionnaires that have been used for a very long period of time, so the instruments’ ability to produce valid results may be limited. There is a need to develop methodology as to how best to measure the reliability (precision) and validity (accuracy) of respondents’ answers, especially over time. In the past, questionnaires regarding the presence of, or contact with, potential sources of exposures to indoor and outdoor environmental chemicals have been used in various community health studies. However, the reliability of these survey instruments in predicting exposures to chemicals of concern in the absence of exposure measurement, especially for

individuals in the population, is uncertain and should be evaluated carefully. As discussed further in the next chapter, to-date very little effort has been devoted to making comparisons of exposure estimates obtained from questions with measurements (especially personal measurements), with biomarker measurements, or with modeled estimates. Hopefully this will change in the future. Thus, the use of questionnaires in the NCS will require proper validation against direct exposure-related measurements, prior to their broad use in the field.

The second limitation is the participant burden. As the preceding sections show, expectations for information could exceed the time available for participant response. NCS designers will need to plan for this by developing a schedule for administering the questionnaires (either directly or indirectly) that would lessen the burden AND obtaining information via other alternative means, including using some of the innovative techniques discussed in **Section 5.6**. Given the large sample size of the planned NCS cohort, and the high costs and burden associated with environmental sampling, it will be difficult to collect detailed exposure information across the entire cohort and at all time periods to support multiple exposure and health hypotheses. Thus, it is likely that low cost, low burden methods, such as the acquisition of questionnaire information, perhaps in conjunction with low burden biomonitoring or passive environmental or community based environmental sampling, may be employed across the full cohort. As discussed further in the next chapter, a smaller subset of the study population could then be recruited to participate in a more extensive (aggregate) exposure assessment study, relying upon more accurate environmental and personal exposure measurements. For certain select chemicals of interest, questionnaires and other indirect techniques may not provide the accuracy needed for the exposure assessment. In these instances, researchers must use higher-cost personal and biological sampling methods to quantify these unique exposures and their sources.

Another limitation is that survey information is often poorly used in subsequent analyses. Almost all environmental health studies have depended on some kind of questionnaire to identify the background of participants as well as demographic information, general life styles, and residential information. Beyond that, the questionnaire analysis is often limited to categorizing the participants into exposure categories, without further attempts to identify sources, explore factors associated with the categories, and the like. In many cases, the wealth of information obtained from these surveys has gone unreported, or under-reported, perhaps because the information content is difficult to extract. Recently there has been greater emphasis on developing or applying statistical methodologies to extract information already collected. It is likely that these methods could be used in the NCS to the fullest.

There are also other limitations, of which many have been reported in the literature together with methods to overcome them, at least to a partial extent. For example the literature has many examples of misclassification error associated with questionnaire response. But often the single most difficult problem for the exposure scientist is to be able to know which sources and combination of factors might lead to high exposures. In nearly every reported exposure study, the perception of the problem is different from what is actually causing the problem. This suggests that the survey instruments used in the NCS may need to be modified as knowledge is gained during the course of the study.

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## 6. Exposure Assessment Implications for the Design and Implementation of the NCS

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### 6.1 Role of Exposure Assessment in the NCS

Examining the influence of environmental exposures on various health indices is a critical component of the NCS, one that will require determining (1) the likely chemical and biological agents of interest, (2) the most cost-effective approaches to measure these chemicals in environmental and biological matrices, (3) how to best design and administer questionnaires, and (4) cost-effective statistical sampling strategies for gathering the necessary environmental and personal exposure-related information with minimum burden to the participants. However, designing a scientifically sound, yet cost-effective exposure measurement program for the NCS can be quite challenging. Obtaining high quality exposure information is vital to the investigation of complex linkages between exposures to different environmental agents and various health indices that will be measured in the NCS. In the past, some of the community studies that have been investigating the impacts of environmental pollution on human health have suffered from the limitations of available exposure data. Thus, a key objective of the planned NCS is to assure that the final study design has sufficient statistical power for testing various hypotheses regarding different types of associations between exposures to selected chemical and biological agents and measured health outcomes in children. In addition to designing an optimum study that achieves the targeted goals of the study, it is important that low-cost, low-burden monitoring techniques, and new and emerging technologies be included in the study, as they become available in the future. In this Chapter, we discuss optimum study design considerations and alternative approaches for properly assessing exposures of the study subjects by different life-stage.

In general, children and adults are exposed to a wide variety of persistent and non-persistent chemicals in the environment, some of which are either known or suspected to cause health effects and/or exacerbate health conditions. The NCS hypotheses attempt to link certain types of exposures with specific health effects. For example, one NCS hypothesis holds that exposure to several indoor and outdoor air pollutants (including PM, ozone, and certain VOCs) and bioaerosols (including allergens, endotoxin, and mold) is associated with increased risk of incidence of asthma in children. Much of the epidemiological asthma research to date has focused on the acute effects of air pollution and aeroallergen exposures and on housing and personal factors that may trigger asthma attacks. For example, researchers have shown that acute air pollution (PM<sub>2.5</sub> and sulfur dioxide) exacerbates asthma and also may increase its incidence (Dockery and Pope, 1994; Tolbert et al., 2000; Schwartz et al., 1993). Additionally, children who live near a busy road and are exposed to motor vehicle emissions have been shown to be at increased risk of wheezing, a symptom of asthma (Venn et al., 2001). Researchers have also shown associations between wheezing or asthma incidence and exposure to indoor allergens, such as dust mites or cockroach-related allergens (Platts-Mills et al., 2001; Finn et al., 2000). Chronic asthma studies have shown increased prevalence of respiratory symptoms for areas with higher air pollutant levels (Sunyer, 2001). Because the long-term effect of these air pollutant and allergen exposures on asthma incidence and severity are not well understood, the NCS is planning to study the effects of indoor and outdoor air pollution and allergen exposures on asthma incidence after adjusting for potential confounders.

Recent studies have also shown associations with pre-natal exposures to ambient particulates and gases (e.g., CO, SO<sub>2</sub>, and NO<sub>x</sub>) with adverse birth outcomes, such as pre-term birth or fetal mortality (Bobak et al., 2000; Pereira et al., 1998; Ritz et al., 2002; Rogers et al., 2000; Xu et al., 1995). In addition, pre-natal exposures to residential-use pesticides, such as chlopyrifos and diazinon, have been associated with undesirable birth outcomes, such as low birth weight or size (Whyatt et al., 2004; Berkowitz et al.,

2004). Moreover, diagnoses of autism and attention deficit disorder (ADD) have been on the rise in recent years, prompting concern over potential relationships between such neuro-behavioral outcomes and exposures to chemicals in the environment. Associations between exposures to lead and IQ deficits in children have already been documented (Needleman, 1995; Bellinger et al., 1992; Koller et al., 2004). Similarly, one NCS hypothesis holds that repeated low-level exposure to non-persistent pesticides *in utero* or post-natally increases risk of poor performance on neurobehavioral and cognitive examinations during infancy and later in childhood, especially for those with genetically decreased paraoxonase activity. Many of the organophosphate and carbamate pesticides used in agricultural and residential settings are neurotoxic and are suspected to cause neurobehavioral deficits in children. For example, members of the pyrethroid and organophosphate classes of synthetic insecticides have been identified as toxic to developing nervous systems (Olson et al., 1998; Roy et al., 1998; Weiss, 2000). The ages during which children are most vulnerable to disruption of their neural development due to exposure vary by substance, dose of the substance, and mechanism of action (Adams et al., 2000). In addition, some animal toxicology studies have shown that *in utero* and subsequent exposures to environmental agents—such as bisphenol A, atrazine and lead—can affect the endocrine system, which has led to a hypothesis that children’s exposure to these chemicals could lead to an altered age of puberty.

A number of other important NCS hypotheses have also recognized the contribution of personal activities and exposures—such as dietary practices—as either confounders or effect modifiers in the hypothesized environmental factors resulting in various adverse health conditions in children. Recording dietary intake and consumption amounts is an integral part of assessing nutrition and exposures to persistent and non-persistent chemicals from the dietary pathway.

As the preceding examples show, determining what to measure and when to measure it is a very complex issue for consideration in the design of the NCS. Environmental exposures can be quantified by three methods: direct environmental or personal measurements, collection and analysis of biological samples (e.g., blood, urine, hair, saliva, etc.), and indirect measurement—including questionnaires, time-activity diaries, or GIS techniques—often combined with environmental data using existing exposure models. Choosing an appropriate method can be daunting. Choices that might eliminate measurements of certain chemicals might also mask the synergistic effects of the chemicals on the fetus or developing body and lead to erroneous conclusions about the outcomes of concern. Additionally, participant burden and the costs of sample collection and analysis can have a major influence on method choice in a study of the size of the planned NCS.

Before choosing the various measurement methods to be used in exploring the NCS hypotheses and formulating an exposure monitoring program for NCS, study designers must first identify the chemicals or chemical classes of interest for each hypothesis and then the key media, routes, and pathways of exposure for each chemical type or class. However, the primary sources and routes of exposures to chemicals vary by age of the study subject, and the specific media and routes of exposure that are of concern to children start to dramatically change during the course of early infancy and into the toddler stage. Young infants and children exhibit considerable hand-to-mouth or object-to-mouth behavior. Crawling on carpets and hard surfaces increases the potential for dermal and non-dietary ingestion of pesticides, other household chemicals, and chemicals in soil or dirt tracked in from outdoors. Exposures in day care and school settings can become a concern for children under 1 through 6 years of age. The NCS measurement program should thus consider monitoring non-home environments as well as residential environments to fully assess the role of early childhood exposures in the development of asthma and both neurobehavioral and other developmental disorders. As children get older they become more active and mobile, and their activities and behaviors become more variable. Consequently, identifying and monitoring the different microenvironments in which young children spend most of their daily waking hours becomes more difficult. These children often engage in outdoor sports and episodic eating behaviors at home, in school, or in local restaurants. Inhalation and dietary ingestion exposure

routes become more significant for school-age children. During teenage and young adult years, times spent in friends' homes, school, malls, movie theaters, other public places, and in commuting increase the diversity of locations and sources that contribute to exposures of children older than 12 years of age.

Identifying key media and routes of exposure will help focus the design of the study's exposure component and will also allow for the dedication of valuable study resources to the study of major sources and factors of childhood exposures. For example, the exposure pathway for many chemicals of concern for the nursing infant is mother's milk. Accordingly, mother's milk would be collected and analyzed during the nursing stage of the infant. Characterization of most significant contributors to children's exposures to pollutants will enable researchers to employ more extensive methods for measuring these important exposures while administering less-detailed measurements (e.g., integrated samples or measures with lower precision, accuracy, and sensitivity) to quantify secondary routes or pathways of exposures.

## 6.2 Exposure Measurement Considerations

As aforementioned, the important locations, media, and routes of exposures to environmental agents may vary by chemical type and by the age of the child. Exposures to some of these chemicals—such as outdoor concentrations of fine particulates or pollen—are more widespread, but concentrations of many other pollutants—such as combustion related pollutants (e.g., NO<sub>x</sub>, air toxics from motor vehicles)—are higher near roadways or in cars or buses. Exposures to pesticides are highly variable depending on the proximity to agricultural fields or during times of indoor or outdoor residential application. Consequently, concentrations of most of the chemicals may vary considerably over time and by season. As a result, quantifying exposures to short-term or intermittent acute exposures requires a measurement system that incorporates periodic monitoring (perhaps triggered by reported chemical use, such as a residential-use pesticide or consumer products) as well as more routine surveillance-type monitoring. However, measurements collected as a result of a particular event constitute a type of adaptive sampling, and those data are likely to result in biased estimates of the distribution of exposures if great care is not used to analyze them properly (i.e., researchers need to consider the frequency of use events over time as well as the magnitude of exposure per event).

Environmental sampling methods vary by analytical sophistication and level of precision. Unfortunately, increased sensitivity, accuracy, precision, and temporal resolution often come at the cost of more expense (including both instrumental and operating costs) and larger instrument size. Personal monitoring is not always possible for all of the environmental agents due to sample volume constraints dictated by analytical requirements. Moreover, active personal samplers are often heavy and bulky and are not suited for use by children younger than 7 years old. Passive samplers such as the 3M or Ogawa badges are lightweight and may be used by small children. However, all types of personal samplers require parental supervision and collection of accurate activity and instrument use information. Active or passive devices can be used for fixed-site indoor or outdoor environmental monitoring applications. Use of these sampling devices, especially active samplers, requires technician visits to homes, schools, and other selected microenvironments for the study subjects. Less detailed measurements may be more feasible to collect from many homes. Passive or active devices could be shipped by mail or installed in homes by a field technician. The parents of the study subjects can return these devices on a pre-specified schedule. Results from the analysis of these monitors can then be used to determine if additional more-accurate or shorter-term sampling is recommended for a given household. Many biological samples will most likely be collected during technician home visits or during check-ups at doctors' offices. However, biological samples collected in a non-invasive manner (e.g., hair, nail, saliva, lost teeth, and perhaps urine samples) could be collected directly by the parents without a technician visit. Where and how these samples are collected depends on the biological sample and the age of the participant.

In addition to collecting environmental and biological measurements, collecting questionnaire and time-activity diary data is also important. This information will not only be used to augment any measurement data collected but can be used to estimate exposures in the absence of direct monitoring data due to sub-sampling of participants or time periods to be measured. In essence, such indirect data may provide surrogate or indirect estimates of exposures to environmental agents. Furthermore, questionnaires will be used to obtain background information from the study population cohort—so that inferences are strengthened when sub-sampling is required—and to adjust for item non-response. Questionnaire information will also be cross-compared with other survey information, where appropriate, to relate item response and generate a measure of representativeness of the cohort (e.g., to compare participant and household characteristics with census data).

Given the size and long-term duration of the NCS, questionnaires are expected to be a key component of any planned exposure study design for the NCS. They will be used to enroll the participants and gain understanding about the family, family structure and relationships, education, occupational and residential history, type and nature of potential exposures, activity and behavioral profiles, and medical and health-related information. The content of the questionnaires and the frequency and mode of administering them will vary depending on the nature of the chemical or chemical class, the hypothesis of concern, and the age of the child (or fetus). For example, once a questionnaire has been completed relating to occupational and residential characteristics, it does not need to be updated unless there is some noteworthy change (which would be described in the instructions). Also, questionnaires may provide some information on past exposures to the fetus, especially during the first trimester, when knowledge of conception is unknown to the parent. Nevertheless, recruiting a sample of women before they are pregnant and obtaining early pregnancy (e.g., first 20-30 days of gestation) exposure measures may be possible, as well as recruiting women reporting for pre-natal care during the first trimester. Hamilton et al. (2003) suggest that 84% of all births in the United States receive pre-natal care during the first trimester, although this percentage varies greatly by race (the percentage is about 75% for blacks). (For additional discussion of pre-pregnancy enrollment as part of the NCS, see the report from the NCS Sampling Design Workshop at <http://nationalchildrensstudy.gov/events/workshops/samplingdesign032004.cfm>.) Collecting both questionnaire information and early pregnancy measures for a sample of women could provide a way to check for potential recall bias.

Questionnaires regarding the presence of, or contact with, potential sources of exposures to chemicals in homes, schools, and other key locations where a child spends his or her time each day have been used in various community health studies. However, the reliability of these survey instruments in predicting exposures to chemicals of concern in the absence of actual exposure measurements is uncertain and should be used cautiously. All survey instruments in the NCS should be pilot-tested and used in conjunction with direct exposure-related measurements for a sample of participants to obtain some measure of validity.

Technological advancements may reduce the time burden of obtaining questionnaire information. For example, wireless-coupled infrared technologies may provide updated consumer source information, which would be more accurate and useful for exposure modeling, without participant burden. Greater use of web-based technology may improve data collection and data processing, generating savings for the participants and the researchers. Accuracy and completeness of the item response can be improved with automation of responses via PDAs or similar devices because the data checking could be done very quickly. Questionable responses could be verified in a timely manner via human or machine interaction.

The discussion presented so far has addressed the important strengths and weaknesses of alternative exposure measurement methods. However, an important operational question for NCS is how to determine an optimum strategy for a measurement program (i.e., one that uses environmental monitoring, personal monitoring, biomonitoring, questionnaires, or other indirect methods in a most cost-effective,

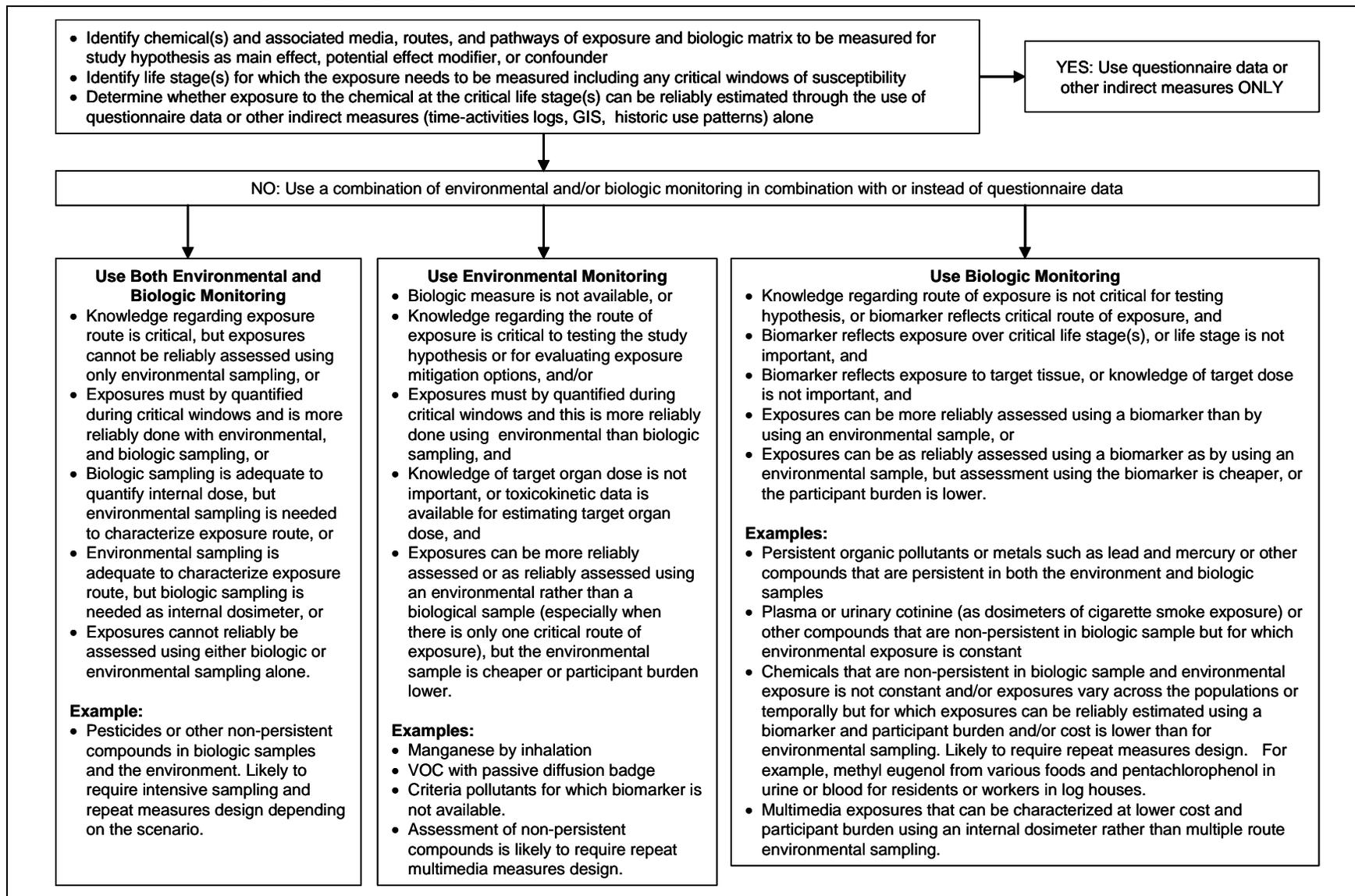
reliable, and minimally burdensome manner) for the selected health hypotheses. We have examined this complex issue and developed a recommended approach for selecting an appropriate exposure measurement method (or methods) for different classes of chemicals and exposure situations.

*Figure 6-1* provides an overview of the steps in selecting the appropriate exposure measure(s). Initially, the researcher must identify the chemical(s) and associated pathways of exposures that need to be quantified (either as main effects or as potential confounders or effect modifiers) to test the study hypothesis. The life stage(s) at which the exposure(s) need(s) to be measured should also be determined. The initial step in selecting the exposure measures will include an evaluation of whether or not the exposure at the critical life stage can be reliably estimated using only questionnaire data or another indirect low-cost, low-burden measure of exposure (e.g., ambient monitoring data, emissions inventories, time-activity logs, consumer product use information, etc.) alone. When such indirect methods exist and offer an acceptable measurement error for testing a given hypothesis, they can then be used with the aid of GIS tools because of lower potential cost and participant burden. Prior epidemiologic studies indicate that when relative risks are high and exposure misclassification is not too high, questionnaire data can be used as a surrogate for direct measures. Examples include questionnaire-derived estimates of cigarette smoking in relation to lung cancer and alcohol consumption in relation to fetal alcohol syndrome. However, we again recommend that prior to using questionnaire-derived or other indirect measures of the exposure, the NCS validate the measure against more direct measures (e.g., biologic or environmental monitoring). In many instances, questionnaire data alone will not provide a reliable dosimeter for the environmental chemicals of concern for the NCS and may need to be supplemented with other direct measures, such as passive environmental sampling or biomonitoring. Nevertheless, the questionnaire data might still be useful in estimating the contact time (frequency and duration) that an individual may have with the environmental media that contains the chemical of interest, or in identifying changes in environmental/residential conditions and sources over time. Therefore, questionnaire data will provide a valuable addition to the direct measures.

In selecting the direct measures, the researcher must decide whether to collect a biological or an environmental measure, or some combination of both. In addition to technical factors, participant burden and cost are among the key issues to consider in selecting one or both of these sample types. Furthermore, regardless of which measure is selected, timing of sample collection and the averaging period represented need to be tailored to coincide with critical life stages of vulnerability. Biologic measures have the advantage over environmental measures of providing an integrated dosimeter, reflecting exposures from all sources and pathways. They also indicate intake/uptake and absorption into the body across all routes. However, biomarkers alone cannot normally be used if knowledge of route of exposure is necessary for testing the study hypothesis. Moreover, when associations are detected between chemical exposure and health outcome, researchers must determine how to mitigate or prevent the exposure, which typically requires knowing the source and pathways of the exposure related to the effect. Environmental measures, conversely, can provide pathway/source-specific exposure estimates but cannot provide information on the target organ dose unless toxicokinetic data are also available. Often biologic and environmental samples provide a snapshot of exposures and may require repeat measurements when exposure conditions are not stable over time. Combining biologic and environmental measures allows for the comparison of the relative contribution of different routes and media to internal dose, facilitates the identification of missing exposure measurements (e.g., locations that were not sampled), and provides a link to identify locations and sources of exposure, all of which help researchers to determine how to reduce exposures and risks.

In general, a biologic measure (e.g., serum levels of PCBs and DDT) could be a dosimeter of choice for many of the persistent organic pollutants listed in *Table 1-1*. Prior studies have also shown that biologic measures can provide excellent dosimeters for some of the metals, such as lead and mercury measured in blood. In addition, biologic measures can provide reliable dosimeters for some of the

Figure 6-1. Selecting an Appropriate Exposure Measure



non-persistent compounds listed in *Table 1-1*, particularly when exposures are constant, intra-individual variability is low, and pathway-specific information is not needed or exposure occurs principally from one pathway, such as in the measurement of plasma or urinary cotinine as a dosimeter of cigarette smoke exposure. In some instances, however, collecting one or more types of biologic measures from a very young child may not always be easy (e.g., from newborn or young infants). In some of these situations, questionnaires and low-cost direct environmental measurements may be used instead, when feasibility and other factors limit the use of biomonitoring. For example, questionnaire information has been shown to be a good indicator of exposure for ETS. There are low-cost methods for measuring cotinine on a filter that has been shown to have very high association with biomarker levels. Also, depending on what other information is needed about the environment or the exposure, researchers may choose an alternative type of an environmental sampling approach. For example a dust sample might be a good indicator for a number of potential or historical exposures to the infant/fetus, especially when concurrent biologic or environmental measures are not available.

An environmental measure will be necessary when no biologic measure is available, as is the case for most of the criteria air pollutants and bioallergens. In addition, an environmental sample may be the measurement of choice for exposures that occur predominantly by one route. For example, inhalation exposures to many of the volatile organic compounds listed in *Table 1-1* may be measured with the lowest cost and participant burden by using passive diffusion badges. The internal dose is then estimated based on models. In general, whenever possible, collection of both biological and environmental measurements are encouraged, because together they provide a much more complete picture of media, routes, pathways, and physiologic factors that influence exposures of a child.

Quantifying exposures to the non-persistent compounds listed in *Table 1-1* will be difficult, particularly in instances of multimedia sources and sporadic exposures—such as the non-persistent pesticides when exposures are variable, can occur simultaneously from multiple routes (dietary and non-intentional ingestion, inhalation, and dermal absorption), can vary dramatically within a particular group or across populations depending on use patterns, and are difficult to quantify by questionnaires only. These situations will likely require intensive sampling and a repeat measures design, and they may require a combination of both environmental and biologic monitoring supported by questionnaire information. Questionnaires or check-lists have been used in past exposure studies to estimate/classify individuals by frequency of exposures to household products.

Additional technical and feasibility issues that should be considered in selecting a particular exposure measurement method include evaluation of:

- The selected method scientifically meeting data quality objectives (DQO's)
  - limit of detection
  - accuracy
  - precision
  - specificity
  - sensitivity
  - robustness/ruggedness
- Adaptability of samples to long-term storage
- Required laboratory capabilities and throughput adequacy for sample analysis
- Single sample measurement of multiple analytes (i.e., analysis produces multiple results, such as more than one metal)
- Multimedia adaptability (i.e., analysis is suited for more than one medium)
- Reliability across laboratories in analytic criteria
- Total costs, including

- Collection, on-site processing, and shipping requirements
- Sample preparation, extraction and analysis
- Storage of sample or extract
- Burden and nuisance factors
  - participant burden
  - sample collection (amount needed; invasiveness of collection procedures), processing, and shipping
  - sample processing, preparation, extraction and analysis
  - sample storage, including specialized storage conditions and sample stability
- Adaptability to low burden modifications.

### 6.3 Epidemiological Study Design Considerations

In addition to determining what measurement methods to use, NCS researchers must also determine optimum sample sizes for obtaining measurement data. Sample size determinations should be made on an epidemiological basis. More common health outcomes or relative risks that are greater than 1.3 can be readily tested on a large portion of, or on the full, NCS cohort. However, rarer outcomes (e.g., autism, certain birth defects, or reproductive health outcomes) or exposures that are unique to certain subgroups may be more efficiently tested using a case-control or a nested case-control study design involving fewer subjects. For example, in studying the cases of autism, researchers might use a nested case-control design in which a large screening sample is used to identify the cases and a sub sample of the non-case sample members is selected for the control sample. However, some environmental or exposure samples must still be collected for the entire cohort because case status will be unknown until later in the study. Properly analyzing the exposure and outcome data from this type of design will require considerable care.

The large sample size and longitudinal nature of NCS raise unique statistical issues, such as obtaining enough samples to provide adequate statistical power to detect health effects attributable to environmental and personal exposures with a minimum amount of burden, while still being cost-effective and staying within the study's overall budget. Rather than measuring the full cohort for every hypothesis, researchers could draw a sample randomly using a stratified or matrix sampling approach to minimize overlap and burden. The sample could then be assigned to sub samples that are targeted to answering specific hypotheses or having common measurement requirements (e.g., hypotheses requiring similar exposure measures, collected at similar time-points, might be grouped together). Unrestricted randomization may not be practical for this purpose, but academic medical centers, primary sampling units, or other geographical sample areas can be randomly assigned to test specific hypotheses or collect more detailed exposure measures so that no sample household is overburdened with excessive numbers of environmental measurements, biological samples, or questionnaire items.

In developing an exposure assessment strategy for NCS, researchers should carefully analyze each hypothesis to determine the various appropriate measures of exposure, including both basic (or core) and more detailed direct measures, as well as indirect measures (e.g., ambient monitoring data and time-activity diaries). The resulting measurement design and statistical analysis plan should consider cost, burden, and level of detail (i.e., accuracy, precision, sensitivity, specificity, and temporal resolution). Given the measurement design, statistical analysis plan, and the basic features of the sampling design for recruiting participants (e.g., multistage probability-based sample), researchers should determine the required sample size for the full cohort, and possibly for a sub-sample in which more detailed measures are collected. If the full NCS cohort, likely to be drawn by using a probabilistic sampling frame, is not required to answer the questions of interest, researchers should develop a plan for random assignment of NCS cohort members to a sub-sample to support the specific hypothesis. With this main objective in mind, EPA, Battelle, and Harvard University researchers have undertaken a project to develop cost-effective statistical sampling strategies and optimal design considerations for the NCS. In this study they

have identified potential sources of bias and/or uncertainty in the environmental/personal estimates of exposure (in particular, non-responses, subject over-burden, drop-out rate, and measurement error) and developed strategies to address these issues, including sample weighting techniques and replicate sampling to assess measurement error variance. The following paragraphs discuss the design strategy for collecting exposure-related information that is mostly derived from the recent Battelle/Harvard report (Strauss et al., 2003).

Given the large sample size and long duration of the planned NCS and the potentially high costs and burden associated with environmental sampling, collecting detailed longitudinal exposure information across the cohort and at all time periods to support multiple hypotheses relating environmental exposure to potential adverse health outcomes will be difficult. Well-designed sub-studies, however, can be carried out within the NCS cohort—using only a small fraction of the sample size—to estimate and adjust for exposure measurement errors, with sufficient power to characterize the relationship between exposure and health outcome for most hypotheses. This methodology allows the exposure-response relationship to be tested on the whole cohort, while the detailed validation sub-samples provide the relationship between different exposure measures. Potential methods for selecting a sub sample include stratified sampling (e.g., specific sampling of high-exposure and/or high-risk individuals), multistage sampling, two-stage case-control studies, and other outcome dependent designs, case cohort designs, and nested case-control studies.

As aforementioned, low-cost, low-burden methods, such as the use of questionnaires, may be employed across the entire NCS cohort (e.g., selected using a representative probabilistic sampling survey design), with smaller subsets of respondents undergoing more extensive environmental exposure assessment using more expensive and detailed environmental and exposure measurements. For example, low-cost and low-burden methods could be used to estimate exposure (e.g., environmental tobacco smoke, cooking, attached garages, fireplace usage, etc.) and physical activity. Available data on ambient air pollution (e.g., particulate matter, NO<sub>x</sub>, SO<sub>2</sub>, CO, Pb) and contaminants could then be used to complement these exposure estimates. The low-cost, low-burden methods could easily be applied to a large cross section of the NCS. However, these methods are not likely to be sufficient for completely characterizing the participants' actual exposures, and even questionnaires do not have a low burden unless they are very short, which is not likely to be the case for the NCS. The lower level of detail and quality (i.e., accuracy, precision, specificity, and temporal resolution) associated with these methods can be problematic in generating data across the entire cohort. However, biological samples or low-burden environmental samples that can be collected in a non-invasive manner (e.g., urine or passive air samples) may be appropriate in some instances for the entire cohort. Participants are more likely to understand the value of these measures, and, for certain chemicals, these samples are likely to be more informative than are the survey data alone. In general, questionnaire data should be restricted to items directly related to exposures of interest, or they should cover time periods that are not included in monitoring (e.g., retrospective or changes over time between monitoring visits). Moreover, to reduce respondent burden, the questionnaire sections can be randomly assigned so that not everyone answers all the same items. Surveys would include some core items and other items that may be used only for sub-samples addressing specific hypotheses. In addition, if numerous questionnaire items are relevant to certain hypotheses, a short version for the primary sample and a long version for the sub-sample participating in more detailed monitoring may be appropriate. For quantifying exposures to some chemicals, more-detailed but higher-cost methods may be required, such as personal air monitoring and microenvironmental sampling. However, assignment of these sampling methods would require identification and recruitment of the study subjects prior to the development of conditions such as asthma, which may be more difficult.

Although recruiting study subjects may be difficult, keeping them in the study throughout the full period of 21 years may be even more difficult. Due to the study's length, both non-response over the course of a monitoring period (i.e., wave non-response) and attrition or dropout are concerns. Non-

response refers to a study subject missing data for one or more planned sampling events but remaining in the study. Wave non-response occurs for several reasons, including respondent unavailability, difficulty in obtaining a sample, and difficulty in analyzing the sample. Attrition refers to a respondent ending his or her participation in the study, for such reasons as the burden of the study, the respondent moving away from the study area, and potential human subjects issues. Strauss et al. (2003) evaluated the influence of both these factors on the estimated study power, as did reports developed for the NCS Sampling Workshop (<http://nationalchildrensstudy.gov/events/workshops/samplingdesign032004.cfm>). Assuming reasonable levels of attrition and wave non-response (ranging from 10 percent to 30 percent), Strauss et al. (2003) found that these factors seem to have minimal effect on the resulting power and efficiency of the sub-study samples.

Strauss et al. (2003) formulated a tentative design approach for the environmental component of the NCS, which centers on hierarchical methods of sampling from the NCS cohort. This strategy, however, will likely support the hypotheses formulated for exposure-related research in the NCS. In principal, the recommended design for the exposure component of the NCS assumes that the NCS will collect information related to major health outcomes of interest and basic (or core) exposure information on all study subjects over time. However, many of the NCS hypotheses will require much more detailed exposure assessment, which should be addressed using sub-sampling or other focused surveys. For each set hypothesis relating health outcome to similar types of exposures, the recommended approach to this sub sampling would entail combining information from two different samples drawn from the NCS cohort (i.e., the core and the detailed analyses samples).

The primary or the core sample should be designed to characterize the relationship between the health outcomes of interest and a less-detailed measure of exposure. These exposure measures should include, but are not limited to, cost-effective environmental, exposure, or biomarker measurements, as well as such measures of exposure-related behavior as activity patterns, diet, or consumer product use. The selected measure of exposure may be assessed either prospectively or retrospectively (i.e., after certain outcomes or cases have been identified), depending on the design. The optimal statistical design for this first core sample would be unique to the hypotheses of interest, based on several factors, including prevalence of the health outcome, the availability of appropriate measures of exposure at the appropriate time period(s) of vulnerability for the child (which is typically unknown), and the expected relationship between the health outcome and the measure of exposure.

A representative sub-sample, drawn from the total NCS cohort, can then be used for conducting more focused and detailed environmental and exposure measurements and for characterizing the relationship between (1) the basic (or core) measures of exposure likely to be explored in the full cohort (e.g., low-cost, low-burden measurements) and (2) the more detailed exposure measurements collected in sub-samples. Establishing the relationship between these different levels of measurement will be an important consideration in both the planning of the study design and the statistical analysis of the data to be collected. Detailed exposure measures would require the collection of environmental pollution and aeroallergen samples from all relevant media of exposure (i.e., air, soil, dust, water, food) as well as time-activity, consumer product use, and food consumption information. The specific design of this sub-sample would include cross-sectional components to capture person-to-person variability across the NCS cohort, as well as longitudinal components to provide estimates of within-person variability in exposure over time. Additionally, this sample should be designed to provide statistically valid estimates of the relationship between biomarker concentrations and pathways of exposure for all life stages during the study, from pre-conception through early adulthood. In most cases, according to Strauss et al. (2003), these carefully designed sub-samples provide adequate power and precision for characterizing the relationship between health outcomes and measures of exposure using sample sizes in some cases as low as a few thousand respondents, with exceptions typically occurring when the prevalence of the health

outcome is very low (e.g., autism) and the relationship between the core and detailed measures of exposure is very weak.

This environmental and exposure sampling strategy allows for the use of fewer measures of exposure, supplemented by more comprehensive sub-studies. To ensure the viability of this strategy, certain information must be collected within the study to characterize the measurement error or uncertainty in the core measure(s) of exposure. Collecting repeated samples from a small validation sample of participants from the sub-study to estimate the appropriate within-person variability, considering both lag-times and possible seasonal effects, would work well. These validation sub-studies will be designed to provide information regarding the uncertainty and variability in exposure information resulting from routine or screening-level exposure data collection. The studies would be conducted on very small yet representative sub-sets of the NCS cohort, and may include additional repeated sampling for biological specimens to capture temporal variability in biomarker chemical concentrations; concurrent analysis of a subset of biological and environmental samples to measure VOCs, SVOCs, and biological pathogens to characterize measurement error in questionnaire and other methods used to act as surrogates for these types of exposures; and higher-technology methods to capture exposure-related behavior (e.g., GPS, accelerometer, or heart-rate monitor to capture physical activity) with a higher degree of precision.

Because some of the efficient design options for linking health outcomes to exposure metrics are outcome dependent, collecting basic (or core) exposure measures from all study subjects in a consistent manner, with a sampling plan that provides coverage across life stages, will be critical. Having exposure-related information available for all study subjects at different stages of development for the subject child will also be critical to support health outcome-oriented research in which the biological cause of disease is not well understood and the disease is rare. The collection and archiving of biological samples (e.g., blood, hair, or urine) could serve as a foundation for some, but not all, exposure-related research. For example, although archived biological samples can be used for retrospective investigation of heavy metals, persistent and some non-persistent pesticides, other persistent pollutant chemicals, and potential genetic links to disease, they cannot be used directly for most volatile organic compounds, semi-volatile organics, allergens, most criteria air pollutants, tobacco smoke (with the exception of cotinine biomarker), or biological pathogens. To provide coverage across exposures that cannot be assessed retrospectively using archived environmental or biological specimens, the NCS will likely need to employ the prospective collection of less-detailed exposure-related information, including the use of questionnaires to capture exposure-related behavior information on activity, diet, and consumer product use; collection of housedust samples; abstraction of medical records and/or diaries during pregnancy to capture fever and exposure to biological pathogens; and reliance on independent data sources such as ambient air monitoring data obtained from EPA's Aerometric Information Retrieval System (AIRS).

The hypothesis on neurobehavioral or neurocognitive health effects from exposures to environmental pesticides highlights how combined biomarker, environmental, and questionnaire information can be used in the NCS. Some health effects might be related to long-term average pesticide exposure, in which case an environmental measure (e.g., a housedust or passive air sample) might be an appropriate measure of exposure for use in these studies. Alternatively, if an adverse health effect is related to an acute pesticide exposure event, questionnaire information regarding consumer product use and other exposure-related behavior combined with periodic biological monitoring (e.g., for urinary pesticide metabolites triggered by the occurrence of periodic events) might be better suited to estimate the impact from these episodic events. Generally the urinary metabolite measurements represent roughly only 24-72 hour exposure time-frame, whereas dust or semi-permeable membrane diffusion (SPMD) would cover weeks or months.

One possible sampling strategy proposed in Strauss et al. (2003) for the detailed exposure study is randomly selecting and recruiting a sub-sample of participants—roughly 1,000 to 5,000 participants among women planning pregnancy or in early stages of pregnancy. However, the actual sample size

necessary to provide detailed exposure assessment information to the NCS and to serve as a basis for adjusting relationships for measurement error in basic measures of exposure may, in fact, be different than the 1,000 to 5,000 subjects chosen here as an example at each stage of life. The sample size and timing of detailed measurements will be an important topic of research, especially if the recommended approach is adopted as part of the overall strategy for exposure assessment. Of these 1,000 to 5,000 women who participate in the aggregate exposure study during this first stage (e.g., the first year of study) 40% (or 400 to 2,000) women could be selected at random to participate in the aggregate exposure study during the first two stages of vulnerability, and 16% (or 160 to 800 women) would be encouraged to participate in the aggregate exposure study for the first three stages of vulnerability. At each subsequent stage of vulnerability covered by the NCS, the aggregate exposure study would be replenished to achieve a total sample size of 1,000 to 5,000 study subjects by enrolling 600 to 3,000 study subjects for the aggregate exposure study from a pool of available NCS study participants who previously had not participated in the aggregate exposure study. Of the 600 to 3,000 study subjects who are chosen for participation in each subsequent stage or year, 240 to 1,200 would participate in two consecutive phases, of which 160 to 800 would participate in three consecutive phases. This hierarchical sampling or recruitment strategy offers the advantage of both samples (i.e., the core and the detailed samples) being selected from the same finite study population. As a result, there will be a small number of study participants occurring in both samples that can provide data to assess the assumption of transferability of findings. **Table 6.1** summarizes the required number of subjects that would need to be recruited under this rolling enrollment strategy for a hypothetical total sample size of 1000 subjects. Alternative but similar recruitment and sampling strategies could also be considered.

**Table 6-1. A Conceptual Example on Rolling Enrollment**

Sampling Event	Cohort								Total Participants	
	1	2	3	4	5	6	7	8		
1	1000									1000
2	400	600								1000
3	160	240	600							1000
4		160	240	600						1000
5			160	240	600					1000
6				160	240	600				1000
7					160	240	600			1000
						160	240	600		1000

Note:

1. Cohort 1: 1000 recruited for 1st sampling event; 400 of 1000 (40%) retained for 2nd sampling event; 160 of 400 (40%) retained for 3rd sampling event.
2. Cohort n: 600 recruited for nth sampling event; 240 of 600 (40%) retained for (n+1)th sampling event; 160 of 240 (66%) retained for (n+2)th sampling event (assume n>1).

Additional design considerations for the aggregate exposure study could be considered, such as stratified sampling approaches to over-sample important subpopulations. The hierarchy of the proposed study design facilitates collecting data across various age groups and core and detailed study cohorts. The result of combining the data across the two study samples would then be the identification of substances or behaviors that are significant risk factors for causing adverse health effects (from the first core group), and the identification of important environmental exposures and/or exposure pathways (from the second sub-study). Finally, the results from these partially overlapping studies can then be used for conducting more specific epidemiological analyses or for identifying optimum exposure mitigation strategies, the ultimate aim of the planned NCS.

## 6.4 References for Chapter 6

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