C. Blood and Urine Collection

Venipuncture

Public Health Objectives:
Venipuncture is performed to obtain laboratory results that provide prevalence estimates of disease, risk factors for exam components, and baseline information on health and nutritional status of the population.

Staff:
Certified Phlebotomist

Protocol:
Methods
Blood is drawn from the examinee's arm. In the laboratory the blood is processed, stored and shipped to various laboratories for analysis. The complete blood count (CBC) results are reported in the MEC and all other results are reported from NCHS to the participant.

The volume of blood drawn by age follows:

- 1–2 years, 9 ml (0.3 ounces), 0.6 tablespoons
- 3–5 years, 20 ml (0.7 ounces), 1.3 tablespoons
- 6–11 years, 28 ml (1.1 ounces), 2.3 tablespoons
- 12+ 115 ml (3.5 ounces), 7.0 tablespoons

Time Allotment:
Depending on age of participant. Range 5–10 minutes.

Health Measures:
Laboratory test results.

Eligibility:
Sample persons aged one year and older who do not meet any of the exclusion criteria.

Exclusion Criteria:

- Hemophiliacs;
- Participants who received chemotherapy within last four weeks; and/or
- The presence of the following on both arms: rashes, gauze dressings, casts, edema, paralysis, tubes, open sores or wounds, withered arms or limbs missing, damaged, sclerosed or occluded veins, allergies to cleansing reagents, burned or scarred tissue, shunt or IV.

Steps to Minimize Risk:
After the blood draw, the phlebotomist has the participant place two fingers on the gauze to hold it in place, and asks the participant to raise the arm straight up, elevating the arm above the level of the heart, without bending the elbow. The participant should remain in this position for 2 to 3 minutes to help prevent hematomas.

The phlebotomist then checks the venipuncture site for clotting. An adhesive bandage or new strip is applied over the gauze pad. The participant is instructed to remove it in no less than 45 minutes if the bleeding has stopped. Also, the phlebotomist suggests that the participant sit quietly for a few minutes. If bleeding continues, keep direct pressure on the site for 5 minutes or more.

Any adverse reaction to the venipuncture is reported to the physician immediately. For those participants younger than 12 years of age, a snack and juice is offered.

Justification for Using Vulnerable Populations:
Minors are included in this component because they are an important target population group. Laboratory data are linked to other household interview and health component data and are used to track changes that occur in health over time. There is no reason to exclude mentally impaired or handicapped individuals because there is no contraindication.

Risks:
The following are known risks associated with venipuncture:

- Hematoma;
- Swelling, tenderness and inflammation at the site;
- Persistent bleeding; and
- Vasovagal response—dizziness, sweating, coldness of skin, numbness and tingling of hands and feet, nausea, vomiting, possible visual disturbance, syncope and injury fall from fainting.

Rare Adverse Effects:
Thrombosis of the vein due to trauma.
Infection which results in thrombophlebitis.

Special Precautions:
Sterile equipment issued with all sample persons.
Physician on call in case an adverse effect occurs.

Report of Findings:

MEC:	Complete Blood Count (CBC)
NCHS:	Other laboratory results

Urine Collection

Public Health Objectives:
Urine is collected to obtain laboratory results that provide prevalence estimates of disease, risk factors for exam components, and baseline information on health and nutritional status of the population.

Staff: MEC Coordinator

Protocol:
Methods
Urine is collected from individuals ages six years and above.

Time Allotment:
2 minutes

Health Measures:
Laboratory test results.

Eligibility:
Sample persons aged six years and above.

Exclusion Criteria:
None

Justification for Using Vulnerable Populations:
Minors are included in this component because they are an important target population group. Laboratory data are linked to other household interview and health component data and are used to track changes that occur in health over time. There is no reason to exclude mentally impaired or handicapped individuals because there is no contraindication.

Risks:
None

Special Precautions:
None

Report of Findings:
MEC: Pregnancy Test **(Attachment 48 – Pregnancy Testing)**
NCHS: Other laboratory results

Bone Mineral Status Markers

Laboratory Measures:
Vitamin D

Public Health Objectives:
Evaluation of bone mineral status will utilize an evaluation of vitamin D status based on two analytes: serum 25-hydroxyvitamin D. Vitamin D is essential for active intestinal calcium absorption and plays a central role in maintaining calcium homeostasis and skeletal integrity. In addition, vitamin D has recently been linked to other non-skeletal conditions of public health significance, such as hypertension, and cancer. Vitamin D is derived mainly from cutaneous synthesis in the presence of ultraviolet sunlight while dietary intake constitutes a minor fraction. Serum 25(OH)D is the best indicator of vitamin D status. It is converted in the kidney,

stimulated by parathyroid hormone (PTH), to the hormonally active metabolite 1,25-dihydroxyvitamin D (1,25 $(OH)_2D$).

Inclusion of serum 25(OH)D in NHANES will allow us to continue to assess vitamin D status in the population. Interest in vitamin D status in the US has increased significantly in recent year. For example, questions have been raised recently about the extent of vitamin D deficiency and insufficiency in the U.S. population. Furthermore, the adequacy of the 1997 Dietary Reference Intake recommendations for vitamin D in the U.S. are now being questioned, especially since new data suggests that optimal serum 25(OH)D levels may be noticeably higher than previously thought. Finally, recent studies have clarified that rickets still occurs in the U.S. Thus, it is important to include these two measures of vitamin D status in the NHANES survey. In addition, these measures can be linked with other measures included in the survey, such as blood pressure and bone mineral density, in order to evaluate its role in both skeletal and nonskeletal conditions.

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
Vitamin D	1+	500–700 uL			

Vitamin D deficiency leads to a decrease in calcium absorption in the gastrointestinal tract and overproduction of parathyroid hormone.

Diabetes Profile

Laboratory Measures:

- Fasting Glucose
- Insulin
- Glycohemoglobin
- Oral Glucose Tolerance Test (OGTT)

Public Health Objectives:

Diabetes mellitus will be assessed by fasting measures of plasma glucose, insulin, and glycohemoglobin and an oral glucose tolerance test in examinees ages 12 years and over who are examined in the morning sessions.

Diabetes is a large, growing, and costly public health problem in the United States and disproportionately affects racial and ethnic minorities. About 17 million Americans have diabetes and over 1 million new cases of diabetes are diagnosed each year. Diabetes is the leading cause of kidney failure, non-traumatic lower extremity amputation, and blindness in working-age adults, and an estimated $135 billion were spent on direct and indirect medical costs for diabetes in 2002. Alarmingly, type 2 diabetes (formerly considered an adult disease) is now being diagnosed in children and adolescents and there has been a large increase in diagnosed diabetes among adults < 40 years of age.

Information on the prevalence of diabetes disease, especially in its early stages, and associated risk factors will be used to help develop early intervention and prevention programs for the disabling consequences of this condition. Specifically, the diabetes disease examination will provide population data to:

- Determine a national estimate of diabetes disease prevalence (diagnosed and undiagnosed), including those at high risk for the late complications of the disease;
- Identify the risk factors of diabetes disease;
- Permit a national cohort to be established for follow-up studies of this condition; and
- Provide critical information to clinicians and public health officials for the development of preventive care and community-based interventions.

In NHANES 2005, an oral glucose tolerance test was added in to the survey to reassess the prevalence of diabetes and impaired glucose tolerance (IGT) in the US population. Because of the increasing occurrence of diabetes in younger ages, our collaborators, the National Institute of Diabetes, Digestive and Kidney Diseases, NIH and the Division of Diabetes Translation at CDC have proposed that NHANES conduct the OGTT in participants aged 12 and older who are examined in the morning sessions.

Persons with (IGT)—15.6% of the U.S. population—are at high risk for developing diabetes. Also, IGT is an important risk factor for a number of other adverse health conditions and mortality. IGT is defined on the basis of an abnormal oral glucose tolerance test (OGTT). Persons without diabetes but with an OGTT 2-hour value of 140–199 mg/dl are considered to have IGT. Recent national and international randomized controlled trials have shown that diabetes can be delayed or prevented among persons with IGT. Furthermore, NHANES III data indicate a tremendous opportunity for the prevention of diabetes—over 12 million persons aged 4–74 years have pre-diabetes (defined as overweight persons with either IGT or impaired fasting glucose metabolism). These data also indicated that over 50% of persons with pre-diabetes are only detected by IGT findings. As risk factors for diabetes, IGT, and pre-diabetes increase (e.g., physical inactivity, obesity, and aging), consequently the prevalence of these conditions is also likely to increase.

The inclusion of OGTTs on NHANES will allow estimation of the prevalence of IGT and, thus, pre-diabetes in the U.S. population, surveillance of trends in the prevalence and awareness of these conditions, study of the risk factors for IGT and pre-diabetes, and examination of IGT as a risk factor for health conditions and mortality. Timely data on IGT and pre-diabetes are particularly important as the nation initiates efforts to prevent diabetes among persons with pre-diabetes. These data on IGT and pre-diabetes are critical to targeting, designing, and evaluating prevention efforts, such as DHHS's STEPS program and efforts by the National Diabetes Education Program.

A fasting glucose blood test is performed on all participants 12 years and older who are examined in the morning session after a nine-hour fast. After the venipuncture, participants are asked to drink 75 milligrams of Trutol® and to have a second venipuncture two hours (plus or minus 15 minutes) after the first venipuncture.

Exclusion Criteria for Blood Draw:

- Hemophilia; and
- Receiving cancer chemotherapy.

Additional exclusion for the OGTT:

- Taking oral medications for diabetes;
- Taking insulin;
- Pregnant; and
- Fasting for nine hours has not occured.

Risks/Benefits:

There are minimal risks associated with this procedure. The package label for Trutol® lists the following rare but known adverse reactions: nausea, vomiting, abdominal bloating and headache. In addition, there is a rare incidence of hypoglycemia. The risks associated with venipuncture include excessive bleeding, fainting/feeling lightheaded, hematoma, infection, and multiple punctures to identify veins. Participants eligible for OGTT will have to endure the discomfort of a second venipuncture; however, they will benefit by the report of findings that will inform them if they have impaired glucose tolerance (IGT).

Management of Adverse Reactions:

Board certified physicians are members of the NHANES exam team. If an adverse reaction occurs, a staff member will ask the physician to evaluate the participant and provide basic medical support. The physicians are prepared to refer participants to their own physician, community clinics, or the emergency room. MEC staff are certified in American Heart Association Basic Life Support. Emergency procedure drills are conducted twice a year.

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
Glucose	12+	500uL		Y	Y
Insulin	12+	1mL			
Glycohemoglobin	12+	400uL		Y	Y
Oral Glucose Tolerance	12+	500uL		Y	Y

Thyroid Profile

Laboratory Measures:

- Total and Free Thyroxine (T4)
- Total and Free Triiodothyronine (T3)
- Thyroblogulin
- Thyroblobulin Antibodies
- Thyroid Peroxidase Antibodies

- Thyroid Stimulating Hormone

Public Health Objectives:
Thyroid function is crucial for maintaining normal metabolic function in adults and for proper neurological development of the fetus. Serum levels will be used to assess thyroid function and will provide population-based reference information on these hormone levels.

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
Total T3 and T4	12 +	1 mL			
Free T3 and T4	12 +	1 mL			Y
Thyroglobulin	12 +	1 mL			
Thyroglobulin Antibodies	12 +	1 mL			
Thyroid Peroxidase Antibodies	12 +	1 mL			
Thyroid Stimulating Hormone	12 +	1 mL		Y	Y

Infectious Disease Profile

Laboratory Measures:

- Hepatitis viruses
- CMV Antibodies (IgG and IgM)
- Tuberculin skin test
- Quantiferon-TB test (QFT)

Public Health Objectives:
Hepatitis Viruses:
Viruses that primarily infect the liver constitute a major public health problem because of the morbidity and mortality associated with the acute and chronic consequences of these infections. New immunization strategies have been developed to eliminate transmission of hepatitis B and hepatitis A viruses in the United States. Because of the high rate of asymptomatic infection with both viruses, NHANES will provide the best means for determining the age-specific effectiveness of immunization strategies to prevent these infections. In addition, NHANES provides the means to better define the epidemiology of hepatitis viruses that were recently characterized, such as hepatitis C and G virus along with D and possibly F. In NHANES testing for markers of infection with the hepatitis viruses will be used to determine secular trends in infection rates across most age and racial/ethnic groups, and will provide a national picture of the epidemiologic determinants of these infections.

Among children age 2–5 years anti-HBs (a maker of immunity) testing will be performed to assist in the evaluation of the hepatitis B immunization program. If sufficient sera is available, other hepatitis markers will be measured.

Antibodies to Cytomegalovirus (CMV: IgG and IgM antibodies) (ages 1–5 years):
CMV does not usually cause significant disease in healthy individuals, but pregnant women can transmit CMV to their unborn babies, who are then at risk. Congenital CMV infection is a significant source of morbidity among children, causing a wide range of clinical outcomes including hearing loss, mental retardation, and even death.

Young children with CMV infection shed the virus in high titers and are a major source of transmission to other susceptible children and adults, which is of special concern for pregnant women. For this reason, characterizing infection among children less than six years old is essential to expanding our understanding of important transmission exposures, mainly breastfeeding and close contact during childcare, and patterns of primary and recurrent infections. Such information would inform the development of prevention strategies to protect vulnerable populations including pregnant women and their fetuses.

Additionally, young children have been identified as a potential target population for CMV vaccine development, recently ranked of highest priority by the Institute of Medicine. Describing the serological profile of children under the age of six would improve our understanding of immunity during early childhood and facilitate vaccine development.

CMV-specific IgM and IgG antibodies are a sign of active infection. They develop within a few days following primary infection and IgM remains detectable for four to nine months. During the first three months of a primary infection, medium to high levels of CMV IgM can be detected, after which the levels decline. The detection of IgM antibody is not sufficient to prove a primary infection, since IgM can sometimes be found during reactivation. Therefore, the case definition for primary infection for prevalence estimates will be the presence of both positive IgM antibody and IgG avidity antibody.

Tuberculosis (Tuberculin skin test and Quantiferon Blood Test):
Tuberculin skin testing (TST) has been used for years as an aid in diagnosing latent tuberculosis infection (LTBI) and includes measurement of the delayed type hypersensitivity response 48–72 hours after intradermal injection of PPD.

The QuantiFERON®-TB test (QFT) was approved by the Food and Drug Administration (FDA) as an aid for detecting latent *Mycobacterium tuberculosis* infection. This test is an in vitro diagnostic aid that measures a component of cell-mediated immune reactivity to *M. tuberculosis*. The test is based on the quantification of interferon-gamma released from sensitized lymphocytes in whole blood incubated overnight with purified protein derivative (PPD) from *M. tuberculosis* and control antigens.
This report should assist public health officials, health-care providers, and laboratorians who are responsible for TB control activities in the United States in their efforts to incorporate QFT testing for detecting and treating LTBI.

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
Hepatitis Viruses	2–5 (anti-HBs) 6+ for all other Hep Viruses	400 uL 1.5mL		Y	
Antibodies to CMV	1–5 years	350uL		Y	
Tuberculin Skin Test	6+	Intradermal injection, no blood required		Y	Y
QuantiFERON® Blood Test	6+	3.0mL		Y	Y

Miscellaneous Laboratory Assays

Laboratory Measures:

The Standard Biochemical Profile includes:

- Alanine Aminotransferase (ALT)
- Albumin
- Alkaline Phosphatase (ALP)
- Aspartate Aminotransferase (AST)
- Bicarbonate (HCO_3)
- Blood Urea Nitrogen (BUN)
- Calcium
- Cholesterol
- Creatinine
- Creatine Phosphokinase (CPK)
- Gamma Glutamyltransaminase (γ-GT)
- Glucose
- Iron
- Lactate Dehydrogenase (LDH)
- Phosphorus
- Sodium
- Potassium
- Chloride
- Total Bilirubin,
- Total Protein
- Triglycerides
- Uric Acid

Public Health Objectives:

This battery of measurements are used in the diagnosis and treatment of certain liver, heart, and kidney diseases, acid-base imbalance in the respiratory and metabolic systems, other diseases involving lipid metabolism and various endocrine disorders as well as other metabolic or nutritional disorders.

a. Alanine Aminotransferase (ALT)

Alanine aminotransferase measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart diseases. Elevated levels of the transaminases can indicate myocardial infarction, hepatic disease, muscular dystrophy, or organ damage. Serum elevations of ALT activity are rarely observed except in parenchymal liver disease, since ALT is a more liver-specific enzyme than asparate aminotransferase (AST).

b. Albumin

Albumin measurements are used in the diagnosis and treatment of numerous diseases primarily involving the liver or kidneys.

c. Alkaline Phosphatase (ALP)

Increased ALP activity is associated with two groups of diseases: those affecting liver function and those involving osteoblastic activity in the bones. In hepatic disease, an increase in ALP activity is generally accepted as an indication of biliary obstruction. An increase in serum phosphatase activity is associated with primary hyperparathyroidism, secondary hyperparathyroidism owing to chronic renal disease, rickets, and osteitis deformans juvenilia due to vitamin D deficiency and malabsorption or renal tubular dystrophies. Increased levels of ALP are also associated with Von Recklinghausen's disease with bone involvement and malignant infiltrations of bone. Low levels are associated with hyperthyroidism, and with the rare condition of idiopathic hypophosphatasia associated with rickets and the excretion of excess phosphatidyl ethanolamine in the urine.

d. Aspartate Aminotransferase (AST)

AST measurements are used in the diagnosis and treatment of certain types of liver and heart disease. Elevated levels of the transaminases can signal myocardial infarction, hepatic disease, muscular dystrophy, or organ damage.

e. Bicarbonate (HCO_3)

Together with pH determination, bicarbonate measurements are used in the diagnosis and treatment of numerous potentially serious disorders associated with acid-base imbalance in the respiratory and metabolic systems.

f. Blood Urea Nitrogen (BUN)

BUN measurements are used in the diagnosis of certain renal and metabolic diseases. The determination of serum urea nitrogen is the most widely used test for the evaluation of kidney function. The test is frequently requested in conjunction with the serum creatinine test for the differential diagnosis of prerenal, renal, and postrenal uremia. High BUN levels are associated with impaired renal function, increased protein catabolism, nephritis, intestinal obstruction, urinary obstruction, metallic poisoning, cardiac failure, peritonitis, dehydration, malignancy, pneumonia, surgical shock, Addison's disease, and uremia. Low BUN levels are associated with amyloidosis, acute liver disease, pregnancy, and nephrosis. Normal variations are observed according to a person's age and sex, the time of day, and diet, particularly protein intake.

g. Calcium

Elevated total serum calcium levels are associated with idiopathic hypercalcemia, vitamin D intoxication, hyperparathyroidism, sarcoidosis, pneumocystic carinii pneumonia and blue diaper syndrome. Low calcium levels are associated with hypoparathyroidism, pseudohypoparathyroidism, chronic renal failure, rickets, infantile tetany, and steroid therapy.

h. Cholesterol

An elevated cholesterol level is associated with diabetes, nephrosis, hypothyroidism, biliary obstruction, and those rare cases of idiopathic hypercholesterolemia and hyperlipidemia; low levels are associated with hyperthyroidism, hepatitis, and sometimes severe anemia or infection.

i. Creatinine

Creatinine measurement serves as a test for normal glomerular filtration. Elevated levels are associated with acute and chronic renal insufficiency and urinary tract obstruction. Levels below 0.6 mg/dL are of no significance.

j. Creatine phosphokinase (CPK)

Measurements of creatine phosphokinase are used in the diagnosis and treatment of myocardial infarction, skeletal muscle diseases, and diseases of the central nervous system.

k. Gamma Glutamyltransaminase (γ-GT)

γ-GT measurement is principally used to diagnose and monitor hepatobiliary disease. It is currently the most sensitive enzymatic indicator of liver disease, with normal values rarely found in the presence of hepatic disease. It is also used as a sensitive screening test for occult alcoholism. Elevated levels are found in patients who chronically take drugs such as phenobarbital and phenytoin.

l. Glucose

Glucose measurements are used in the diagnosis and treatment of pancreatic islet cell carcinoma and of carbohydrate metabolism disorders, including diabetes mellitus, neonatal hypoglycemia, and idiopathic hypoglycemia.

m. Iron

Iron (non-heme) measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia, chronic renal disease, and hemochromatosis (a disease associated with widespread deposit in the tissues of two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin).

n. Lactate Dehydrogenase (LDH)

LDH measurements are used in the diagnosis and treatment of liver diseases such as acute viral hepatitis, cirrhosis, and metastatic carcinoma of the liver; cardiac diseases such as myocardial infarction; and tumors of the lungs or kidneys.

o. Phosphorus

There is a reciprocal relationship between serum calcium and inorganic phosphorus. Any increase in the level of inorganic phosphorus causes a decrease in the calcium level by a mechanism not clearly understood. Hyperphosphatemia is associated with vitamin D hypervitaminosis, hypoparathyroidism, and renal failure. Hypophosphatemia is associated with rickets, hyperparathyroidism, and Fanconi syndrome. Measurements of inorganic phosphorus are used in the diagnosis and treatment of various disorders, including parathyroid gland and kidney diseases and vitamin D imbalance.

p. Sodium, Potassium, and Chloride

Hyponatremia (low serum sodium level) is associated with a variety of conditions, including severe polyuria, metabolic acidosis, Addison's disease, diarrhea, and renal tubular disease. Hypernatremia (increased serum sodium level) is associated with Cushing's syndrome, severe dehydration due to primary water loss, certain types of brain injury, diabetic coma after therapy with insulin, and excess treatment with sodium salts.

Hypokalemia (low serum potassium level) is associated with body potassium deficiency, excessive potassium loss caused by prolonged diarrhea or prolonged periods of vomiting and increased secretion of mineralocorticosteroids. Hyperkalemia (increased serum potassium level) is associated with oliguria, anuria, and urinary obstruction.

Low serum chloride values are associated with salt-losing nephritis, Addisonian crisis, prolonged vomiting, and metabolic acidosis caused by excessive production or diminished excretion of acids. High serum chloride values are associated with dehydration and conditions causing decreased renal blood flow, such as congestive heart failure.

q. Total Bilirubin

Elevated levels are associated with hemolytic jaundice, paroxysmal hemoglobinuria, pernicious anemia, polycythemia, icterus neonatorum, internal hemorrhage, acute hemolytic anemia, malaria, and septicemia. Low bilirubin levels are associated with aplastic anemia, and certain types of secondary anemia resulting from toxic therapy for carcinoma and chronic nephritis.

r. Total Protein

Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow, as well as other metabolic or nutritional disorders.

s. Triglycerides

Triglyceride measurements are used in the diagnosis of diabetes mellitus, nephrosis, liver obstruction, and other diseases involving lipid metabolism and various endocrine disorders and in the treatment of patients with these diseases.

t. Uric Acid

Uric acid measurements are used in the diagnosis and treatment of numerous renal and metabolic disorders, including renal failure, gout, leukemia, psoriasis, starvation or other wasting conditions and in the treatment of patients receiving cytotoxic drugs.

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
Biochemistry Profile	12+	800 uL			
Alanine aminotransferase ALT				Y	Y
Aspartate aminotransferase AST				Y	Y
Albumin				Y	Y
Alkaline Phosphatase					Y
Bicarbonate (HCO_3)				Y	Y
Blood urea nitrogen (BUN)				Y	Y
Calcium				Y	Y
Cholesterol				*	*
Creatinine				Y	Y
Creatine Phosphokinase (CPK)				Y	Y
Gamma-glutamyl transaminase (GGT)					Y
Glucose				*	*
Iron					Y
Uric Acid					Y
Phosphorus				Y	Y
Sodium				Y	Y
Potassium				Y	Y
Chloride				Y	Y
Total Bilirubin				Y	Y
Total Protein					Y
Triglycerides				*	*

* Value may be reported from different assay

Kidney Disease Profile

Laboratory Measures:

- Serum Creatinine
- Blood urea nitrogen (BUN)
- First and second collections of urine albumin and creatinine,
- Urine flow rate
- Urine albumin creatinine ratio (ACR)

Public Health Objectives:

Serum Creatinine, and Blood Urea Nitrogen (BUN):

The incidence of end stage kidney failure is increasing rapidly in the U.S. in adults of all age groups which implies that the prevalence of progressive renal impairment is also increasing. However, little information is known about the prevalence of chronic renal impairment on a national level. Urologic disease, including urinary incontinence affect a large proportion of the population. Little nationally representative data on the prevalence and risk factors associated with these conditions are available.

The purpose of the kidney and urologic diseases portion of the NHANES is to determine prevalence of specific nephrologic and urologic conditions in the population; to determine the association between health conditions such as diabetes and hypertension and the development of kidney and urologic diseases; to monitor trends in the prevalence of these diseases and their risk factors over time. These data will be used to assist in planning for initiatives and other programs for the prevention and treatment of nephrologic and urologic diseases.

Blood specimens will be used to obtain measures of serum creatinine and blood urea nitrogen. Urine specimens will be used to obtain first and second collection measures of urine volume and time between previous collection (for determination of urine flow rate), as well as albumin and creatinine. Self-reported information on chronic analgesic use and incontinence will be collected

First and Second Collection of Urine Albumin and Creatinine:

Chronic kidney disease (CKD) is a serious condition associated with premature mortality, decreased quality of life, and increased health-care expenditures. Untreated CKD can result in end-stage renal disease and necessitate dialysis or kidney transplantation. Risk factors for CKD include cardiovascular disease, diabetes, hypertension, and obesity. Persistent albuminuria is used to determine kidney damage for categorizing persons as having stage 1 and stage 2 CKD. Two urine samples are needed to assess persistent albuminuria and confirm the presence of kidney damage.

Albumin/Creatinine Ratio (ACR):

Various large cohort studies have shown that microalbuminuria is a strong risk predictor for cardiovascular morbidity and all-cause mortality. Because urinary albumin excretion follows a circadian rhythym, the preferred method to collect urine for 24 hours for albumin assessment. However, a 24-hour urine collection is inconvenient and impractical in NHANES for logistical reasons. Urinary albumin concentrations for the albumin:creatinine ratio (ACR) should be measured in a first morning void. Since the ACR depends not only on urinary albumin but also

on urinary creatinine excretion (which is related to muscle mass), the ACR will be affected by gender and age because muscle mass is lower in females than in males and decreases with age.

Urine Flow Rate:
Urine analyte concentrations from single determinations (spot urines) are used to determine the exposure to environmental chemicals; however, they can vary depending on the water content of the urine. The urine excretion rate of an analyte is a more accurate measure of the exposure to environmental chemicals. The urine excretion rate (mg/min) is the product of the urine flow rate (mL/min) and the urine analyte concentration (mg/mL). Participants ages 6 years and older will be asked to record their time of last void before coming to the Mobile Examination Center and then asked to void in the Mobile Examination Center where the time of collection and volume of the urine will be recorded and a urine flow rate will be calculated.

Urinary Osmolality:
Urine osmolality measures the amount of solute particles contained in urine and indicates if the urine is overly diluted or concentrated due to hydration status or impaired renal function. The concentration of urine analytes (such as environmental chemicals) can fluctuate in spot (single determination) urine specimens depending on whether the urine is too diluted or concentrated. To compensate, the urine analyte concentration is divided by the urine osmolality to "standardize" the spot urine analyte concentration. Urine osmolality will be measured in the Mobile Examination Center

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
First urine albumin and creatinine	6 +	3 mL			
Second urine albumin and creatinine	6 +	3 mL			
First albumin/creatinine ratio (ACR)	6 +	3 mL		Y	Y
Second albumin/creatinine ratio (ACR)	6 +	3 mL			Y
Urine Flow Rate	6 +	3 mL			
Urine Osmolality	6 +	3 mL			

Pregnancy Test

Laboratory Measures:

- Pregnancy test (Urine)

Public Health Objectives:

Information on current pregnancy status will be used to exclude participants from the DXA examination and the OGTT test and for interpretation of current nutritional status and body measures.

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
Pregnancy Test (Urine)	8 - 59 females	1 mL (Urine)			Y

Report of Findings:
Details and ethical considerations of reporting pregnancy test results are in **Attachment 48– Pregnancy Testing.**

Testosterone Test

Laboratory Measures:

- Testosterone test

Public Health Objectives:
Measurement of testosterone is highly valuable in assessing disease risk, diagnosing disease, and monitoring treatment, as reflected in many clinical guidelines, recommendations, and review articles. However, lack of generally accepted reference ranges for people of all ages, gender, and ethnicities profoundly limits progress in disease research and translating valuable research findings into information useful for patient care and disease management as stated in several editorials and research publications. The analyses of testosterone levels can be used to establish reference ranges for all ages, gender, and ethnicities.

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
Testosterone	6 +	500 uL			Y

Nutritional Biochemistries and Hematologies

Laboratory Measures:

- Complete blood count
- Serum folate
- RBC folate
- Vitamin B_{12}

- Methylmalonic acid (MMA)
- Caffeine (Urine)

Public Health Objectives:
The objectives of this component are to:

- Provide data for monitoring secular trends in measures of nutritional status in the U.S. population;
- Evaluate the effect of people's habits and behaviors such as physical activity and the use of alcohol, tobacco, and dietary supplements on people's nutritional status; and
- Evaluate the effect of changes in nutrition and public health policies including welfare reform legislation, food fortification policy, and child nutrition programs on the nutritional status of the U.S. population.

These data will be used to estimate deficiencies and toxicities of specific nutrients in the population and subgroups, to provide population reference data, and to estimate the contribution of diet, supplements, and other factors to serum levels of nutrients. Data will be used for research to further define nutrient requirements as well as optimal levels for disease prevention and health promotion.

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
Complete blood count	1 +	1.5mL		Y	Y
Serum and RBC folate	1 +	700 uL–1 mL		Y	Y
Vitamin B_{12}	1 +	100 uL		Y	Y
Methyl Malonic Acid	3 +	1 mL			
Caffeine (Urine)	6+	10 mLs (Urine)			

Sexually Transmitted Disease Profile

Laboratory Measures:

- Chlamydia Trachomatis Herpes simplex 1 and 2
- Human Immunodeficiency Virus (HIV)
- Human Papillomavirus Virus (HPV) (vaginal swabs, females age 14–59 years)
- HPV (serum and oral fluid, males and females age 14-59 years)

Public Health Objectives:
Chlamydia Trachomatis:
Sexually transmitted infections caused by *Chlamydia trachomatis* may lead to pelvic inflammatory disease, ectopic pregnancy, infertility, and chronic pelvic pain in women. They may also increase the risk of HIV transmission in women. Pregnant women may transmit infection to their newborn causing serious medical complications. Presently, the prevalence of

chlamydial infection in the general population of the United States is unknown. NHANES offers an opportunity to assess the prevalence of chlamydial infection in the general population and to monitor trends in prevalence as prevention programs are established and expanded.

Herpes Simplex 1 and 2 (Blood Test):
Sera from NHANES subjects ages 14–49 will be tested for antibody to Herpes simplex 1 and 2 (HSV-1/2) to continue to monitor the prevalence of HSV-1/2 infection in the U.S. HSV-1 is a common chronic infection that is associated with lower socioeconomic status. HSV-2 is an index of sexually transmitted infections. In addition, questions about those sexual behaviors that are risk factors for sexually transmitted infections and that are the focus of major national HIV and sexually transmitted diseases risk reduction efforts will be included. The joint availability of sexually transmitted infection and risk factor data in a national sample on a periodic basis is a unique and invaluable resource for evaluation of national HIV/STD risk reduction efforts and for risk-based modeling of the frequency and trends of sexually transmitted infections.

HSV-2 infections are rarely life threatening, but morbidity due to recurrent genital ulcerations is substantial. Just as important, HSV-2 infection is the best current marker of sexual behavior risk factors leading to sexually transmitted infections, generally, because: (a) HSV-2 infections are common and, thus, HSV-2 rates are a sensitive measure of sexually transmitted infection risk factors; (b) HSV-2 infection is almost always a result of sexual transmission and, thus, a specific measure of sexually transmitted infection; (c) HSV-2 infections are not curable and, thus, HSV-2 risk is not influenced by health care seeking factors; and (d) sensitive, specific, and relatively inexpensive tests for HSV-2 antibody are available. HSV-2 is a very important index of the success of large national efforts, motivated by the acquired immunodeficiency epidemic, to reduce risky sexual behaviors

HIV Antibody (Blood or Urine Test):
The estimated prevalence of human immunodeficiency virus (HIV) infection in the United States population is an important measure of the extent of the medical and financial burden the nation faces due to this virus. NHANES III data on HIV infection during 1988–1994 will serve as a baseline for monitoring the changes in the epidemic over time in the general population of the United States. In addition to HIV testing in NHANES, whole blood samples will be collected and stored for future CD4 testing once the HIV status of the sample is known. This will allow CDC to determine the distribution of CD4 cells in a random sample of HIV positive individuals. NHANES is now the only national survey collecting blood on a population based sample, therefore it will be a key element in future estimates. If the participant refuses phlebotomy but does not refuse the HIV test urine will be tested for HIV antibody.

Human Papillomavirus (HPV) (Vaginal swab—DNA test; Oral fluid—DNA test; Blood test for HPV antibody):
Genital human papillomavirus (HPV) infection is likely the most common sexually transmitted infection in the U.S., and cervical infection with certain types of HPV, especially HPV-16, is the single strongest risk factor for cervical cancer. No surveillance systems exist for HPV infections, the majority of which are subclinical. Serum from participants age 14–59 years will be tested for antibody to HPV-16, the antigenic type most linked with cervical cancer to estimate the percentage of individuals of both genders who have ever been infected with this virus.

Testing of HPV DNA from vaginal swabs from women 14–59 will provide an estimate of current infection. Vaginal swabs will be tested for HPV DNA by the FDA approved Hybrid Capture II method (Digene) and by consensus PCR with type specific analysis. The Hybrid Capture assay will detect overall high risk HPV types, but cannot identify specific types. The PCR will allow identification of specific HPV type. Participants will be notified of their Hybrid Capture results and specific messages will be developed to explain the implications of the findings based on their age group.

Additionally, a rinsed oral fluid specimen will be obtained to test for oral Human Papilloma virus (HPV) infection. Molecular evidence supports a role for HPV, particularly HPV-16, in the pathogenesis of a subgroup of squamous-cell carcinoma of the head and neck. Epidemiologic evidence of the role of HPV in squamous-cell carcinomas of the head and neck is less rigorous. Studies are underway to evaluate the natural history of oral HPV infection and its potential health consequences. NHANES will provide information on the prevalence of oral HPV infection in the general population.

If a guardian is present, the mentally impaired person is allowed to participate. The guardian will call NCHS on behalf of the mentally impaired participant for the STD results. The impaired participant will not be asked to do the self-administered vaginal swab if she is unable to follow directions and complete the task alone in the bathroom. Mentally impaired persons will be excluded from the STD component if a guardian is not present.

Deaf persons are excluded from the STD testing. Reasons for not testing include "communication problems". If a participant cannot hear instructions because of deafness or a language problem (non English, non Spanish speaker with no interpreter), the STD testing is not done.

Participants are asked to call NCHS for STD test results 4 weeks after their exam. If NCHS has not heard from the participant 38 days after his/her exam, a reminder letter is sent to the adult participant; a reminder phone call is made to the minor participant. Three letters or three calls are initiated at 10-day intervals if the participant does not contact NCHS for his/her results.

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
Chlamydia Trachomatis	14-39	10 mlLs urine			
Herpes 1 and 2 antibody	14-49	200 uL		*	Y
HPV DNA test	Females 14-59	Vaginal swab		*	Y
HPV 16 antibody	14-59	500uL			
HIV antibody	18-59	800 uL		*	Y
HPV oral rinse	14-59	10 mLs (oral rinse)			

* Persons with positive STD or HIV findings will be referred for counseling and treatment.

Justification for Using Vulnerable Populations:
Teenagers are included because they are at increasing risk for STD's. A pilot study in NHANES III demonstrated an increased prevalence in chlamydial infection starting at age 14 years (whites 4%, blacks 12% Mexican Americans 6%). Mentally impaired persons will be excluded from the STD profile due to NCHS' inability to provide adequate support and counseling to this group with the test result.

Report of Findings:
See section on STD/HIV reporting in Reporting Examination Findings.

Inflammatory Disease Tests

Laboratory Measures:

- Tissue transglutaminase antibodies
- Endomysial antibodies

Public Health Objectives:
Celiac Disease:
Detection of tissue transglutaminase and endomysial antibodies, in conjunction with IgA antibodies, is an aid in diagnosis of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis. This test is intended for providing added sensitivity when testing IgA deficient patients.
An Enzyme-Linked Immunosorbant Assay (ELISA) is used for the semi-quantitative detection of IgA antibodies to tissue transglutaminase (endomysium) in human serum.

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
Endomysial antibodies Tissue transglutaminase antibodies	6 +	500 uL		Y	Y

Blood Lipids Profile

Laboratory Measures:

- Total Cholesterol
- HDL- Cholesterol
- LDL-Cholesterol
- Triglycerides
- Apolipoprotein B

Public Health Objectives:
The goals of this component are to:

- Monitor the prevalence and trends in major cardiovascular conditions and risk factors in the U.S.; and
- Evaluate prevention and treatment programs targeting cardiovascular disease in the United States.

The main element of the cardiovascular disease laboratory component in NHANES is blood lipid levels. Cardiovascular disease is the leading cause of death in the United States. An estimated 4.8 million Americans have congestive heart failure. Increasing prevalence, hospitalizations, and deaths have made congestive heart failure a major chronic condition in the United States.

The data will be used to:

- Monitor the status of hypertension prevalence, awareness, treatment and control and the success of the National High Blood Pressure Education Program;
- Monitor the status of hyperlipidemia and the success of the National Cholesterol Education Program; and
- Estimate the prevalence of congestive heart failure and compare to the baseline data from the NHANES I.

Health Measures, Eligibility, Report of Findings:

Health Measure	Eligibility	Volume Required	Report of Findings Level		
			1	2	3
Total cholesterol	6 and older	+++		Y	Y
HDL-cholesterol LDL-Cholesterol	6 and older	+++			Y
LDL –cholesterol*	12 and older	calculated			Y
Triglycerides *	12 and older	+++		Y	Y
Apo B*	12 and older	+++			

+++ For all 4 assays and 2ml used for persons 6 years and older
*Only done on fasting morning sample. Individuals aged 12 and older are asked to fast.

Environmental Health Profile

Laboratory Measures:
Environmental Chemical Exposures

Public Health Objective:
The NHANES environmental health component was expanded in 1999 in collaboration with laboratories of the National Center for Environmental Health (NCEH). NHANES 1999–2004 protocol includes approximately 150 measures of environmental chemicals or metabolites in blood and urine specimens collected from survey participants. The protocol was amended numerous times to add additional analytes as the NCEH laboratories developed and refined analytic methods.

The NCEH laboratories continue to develop laboratory methods that will expand the list of environmental chemicals that can be measured through NHANES. These include new classes of chemicals as well as additional analytes in the classes of chemicals already included in the 1999–2004 protocol. The laboratory methods are not finalized in many cases. This list is comprehensive; however, methods for some chemicals may not be fully developed for 2011–2012. ERB approval of this comprehensive list will minimize the need for multiple additional amendments to the protocol during 2011–2012.

The uses of the NHANES environmental exposure information by the public health community include the following:

- To determine the types of chemicals and concentration levels to which Americans are exposed;
- For chemicals with a known toxicity level, determination of the prevalence of persons above that toxicity level (e.g., blood lead > 10 µg/dL);
- To establish reference ranges that may be used by state and local public health physicians and scientists to determine whether an individual or group has an unusually high exposure;
- To assess the effectiveness of efforts to reduce exposure to specific chemicals;
- To determine whether exposure levels are higher among minorities, children, women of childbearing age, and other vulnerable groups;
- To observe time trends in the levels of exposure within the population; and
- To set priorities for human health effects research.

These analytes fall under the following classes of chemicals:

- Cotinine/NNAL (Urine)
- Metals (Trace) (Urine)
- Metals (Heavy)
- Serum Selenium, Copper, and Zinc
- Blood Selenium, and Manganese
- PhthalatesPolycyclic aromatic hydrocarbons (PAHs)
- Organophosphate insecticides: dialky phosphate metabolites
- Organophosphate insecticides: specific metabolites
- Pyrethroid pesticides
- Organochlorine pesticides
- Other pesticides and fungicides
- Herbicides
- Halogenated phenolic compounds
- Perfluorinated compounds
- Polychlorinated and polybrominated dibenzo-p-dioxins and dibenzofurans
- Polychlorinated biphenyls (PCBs)
- Polybrominated diphenyl ethers
- Toxaphenes
- Volatile organic compounds (blood)
- Perchlorate

Additional information on the classes of environmental chemicals is described as follows:

Cotinine:
Cotinine, a metabolite of nicotine, is measured in the blood as a biochemical marker to substantiate self-report of smoking and to define exposure to environmental tobacco smoke (ETS). The harmful effects of cigarette smoking have long since been established, and evidence has accumulated linking exposure to ETS with lung cancer, respiratory and other chronic diseases. Measurements of cotinine have been included in the survey since NHANES III. At that time, findings from NHANES showed a preponderance of exposure to ETS. While major efforts have been made to limit tobacco smoking in public places and restaurants in order to minimize ETS exposure, the inclusion of this biochemical marker is useful to examine trends and track progress in this area.

NNAL (4-(methylnitrosamino) -1-(3-pyridyl)-1-butanol) (Urine):
Another tobacco biomarker of importance is 4-(methylnitrosamino) -1-(3-pyridyl)-1-butanol (NNAL)NNAL, a tobacco-specific nitrosamine (TSNA) which is a metabolite of NNK (4-methylnitrosamino 1-3-pyridyl-1-butanone) in the body, and which has been detected in the urine of smokers, and in many cases, in nonsmokers exposed to Second Hand Smoke (SHS). NNK is formed in tobacco and in cigarette smoke from nicotine, so it and its NNAL metabolite are as specific for tobacco and cigarette smoke exposure as cotinine or nicotine itself. Both NNK and NNAL are known to be potent pulmonary carcinogens in rodents, and are believed to be lung carcinogens in humans. Total NNAL in urine samples in humans will be measured to characterize the concentration levels of this tobacco biomarker in the US population of both smokers and nonsmokers, and to compare the findings with previous estimates based on a currently proposed retrospective assessment of residual samples from the prior NHANES 2007–2008 survey.

Tobacco Biomarkers:
As tobacco processing and cigarette manufacturing continue to change, and as newer tobacco delivery devices such as the “potentially reduced exposure products (PREPS)” are introduced, changes in carcinogen levels such as the TSNA may occur in people. Thus, by testing for these tobacco biomarkers, NHANES will be able to monitor exposure levels in both smokers and nonsmokers over time.

Trace Metals (Urine):
Trace metals have been associated with adverse health effects in occupational studies or laboratory studies, but these substances have not been monitored in general population. Urinary antimony (Sb), barium (Ba), cadmium (Cd), cesium (Cs), chromium (Cr), cobalt (Co), lead (Pb), molybdenum (Mo), manganese (Mn), tin (Sn), strontium (Sr), thallium (Tl), tungsten (W), and uranium (U) levels were measured in previous NHANES. Exposure information will be used to establish population-based reference ranges and to evaluate the need for regulations to reduce levels of exposure by other agencies and health authorities. Manganese, tin, and strontium are of interest to the Environmental Protection Agency.

Iodine is a trace element required by the thyroid gland for the production of the thyroid hormones thyroxine and triiodothyronine, which are necessary for multiple processes related to growth and development. Recent data indicate the need to carefully assess the iodine status of pregnant women in the US. Urine iodine measurements will be performed on females (15–44) to determine the criteria for iodine deficiency for pregnant women.

Heavy Metals:

Lead: Lead is a known environmental toxin that affects the nervous, hematopoietic, endocrine, renal and reproductive systems. In young children, lead exposure is a particular hazard because children more readily absorb lead than do adults, and children's developing nervous systems also make them more susceptible to the effects of lead. The primary sources of exposure for children are lead laden paint chips and dust as a result of deteriorating lead-based paint. The risk for lead exposure is disproportionately higher for children living in older housing containing lead-based paint, who are poor, non-Hispanic black, and living in large metropolitan areas. Among adults, the most common high exposure sources are occupational.

Blood lead levels measured in previous NHANES programs have been the cornerstone of lead exposure surveillance in the U.S. The data have been used to document the burden of and dramatic decline of elevated blood lead levels; to promote the reduction of lead use; and to help to redefine national lead poisoning prevention guidelines, standards and abatement activities.

Cadmium:

Cadmium is used in batteries, pigments, metal coatings, and plastics. Cadmium enters the environment from the weathering and mining of rocks and minerals that contain cadmium. Contaminated water sources, foods, and combustion sources may also result in human exposure. Cadmium exposure occurs from inhalation of cigarette smoke. Exposure to cadmium may occur in industries, such as mining or electroplating, which use or produce the chemical. Once absorbed into the body, cadmium may remain for decades. Low level chronic exposures over many years may result in accumulation of cadmium in the kidneys. Chronic ingestion also has produced painful osteomalacia, a bone disorder similar to rickets in children. Large, acute airborne exposures to dusts and fumes, as occurs for example from welding on cadmium-alloyed metals, may result in severe swelling of the lungs (edema) and subsequent scarring (fibrosis). Other cadmium toxicity, as seen in animal studies, includes reproductive and teratogenic effects. The International Agency for Research on Cancer has determined that cadmium is a known human carcinogen.

Mercury (methyl, ethyl, and inorganic):

Mercury is widespread in the environment and originates from natural and anthropogenic sources. The general population may be exposed to three forms of mercury: elemental, inorganic, or organic (primarily methylmercury). Elemental and inorganic mercury exposure can result from mercury spills, dental amalgams, and occupational exposures. Methylmercury is formed, through microbial action from inorganic mercury that deposits in aquatic environments and bioaccumulates in the food chain. Exposure occurs primarily through consumption of seafood and/or freshwater fish, particularly larger predatory fish. Methylmercury is a well-established human neurotoxin and the developing fetus is most sensitive to the adverse effects. The concentration of total mercury in blood is a reasonable biomeasure of methylmercury exposure. The concentration of total mercury in urine is a biomeasure of exposure to inorganic mercury. NHANES 1999–2002 provided the first estimates of exposure for US children and women of childbearing years based on measurements of total mercury in blood and total mercury in urine (women only). NHANES 2011–2012 will include measurements of mercury species (methyl, ethyl, and inorganic) in blood in order to define exposure to various sources of mercury more precisely. Mercury assessments will be conducted in persons one year of age and older; urinary mercury will be measured in persons 6 years of age and older.

Serum Selenium, Copper, and Zinc, Blood Selenium, and Manganese:
Selenium is part of several enzymes necessary for the body to properly function. Generally, selenium functions as an antioxidant that works in conjunction with Vitamin E. Copper is involved in the absorption, storage, and metabolism of iron and the formation of red blood cells. Zinc is important in a number of key activities, ranging from protein and carbohydrate metabolism to the immune system, wound healing, growth and vision. Manganese, is of interest to the Environmental Protection Agency.

Arsenic:
Arsenic is widely distributed in the earth's crust and is found most often in ground water rather than surface water. People encounter arsenic in many chemical forms that vary greatly in toxicity. The most toxic of the naturally-occurring arsenic compounds are inorganic forms of arsenic and their methylated metabolites. Less toxic are the organic arsenic compounds. Exposure to inorganic arsenic can result in a variety of adverse health effects, such as skin disorders, nerve impairment, accelerated atherosclerosis, cancer of the liver, bladder, kidneys, skin, and lungs, and even death from large doses. People may be exposed to inorganic arsenic through activities such as drinking water contaminated from geological sources or because of occupational exposure, especially breathing air contaminated with sawdust or smoke from wood treated with chromated copper arsenic preservatives. Organic arsenic compounds are generally less toxic and may be encountered by ingesting various types of fish, shellfish, poultry or seaweed. In January 2001 the Environmental Protection Agency (EPA), in compliance with the 1996 Safe Drinking Water Act (SDWA) proposed a lower Maximum Contaminant Level (MCL) for arsenic in drinking water. The previous MCL was 50 ppb, a standard that was set by the U.S. Public Health Service in 1947. The new level proposed by the EPA is 10 parts per billion (ppb), the same limit is used by the World Health Organization (WHO).

Pesticides and Other Chemicals:
Phthalates:

Phthalate acid esters (phthalates) are used extensively as plasticizers in a wide range of applications such as children's toys, food packaging, and medical supplies. Putatively, these chemicals are weakly estrogenic and have been associated with cancers and reproductive toxicity. Governments in Europe and Japan have become increasingly concerned about levels in food packaging materials and children's toys. Biomeasures of phthalates in humans is necessary to evaluate potential human health threats from exposure to these chemicals.

Polycyclic Aromatic Hydrocarbons (PAHs) (Urine):
PAHs constitute a group of chemicals which are formed during the incomplete combustion of coal, oil and gas, garbage, and other organic substances. These compounds require metabolic activation prior to their interactions with cellular macromolecules. PAHs are ubiquitous, thus exposure to them is widespread. In general, people are exposed to mixtures of PAHs, the sources of which include tobacco smoke, vehicle exhausts, asphalt roads, coal, coal tar, wild fires, agricultural burning, charbroiled foods, and hazardous waste sites. Although most of the data regarding the carcinogenicity of these compounds comes from rats and mice, epidemiologic studies have shown increased mortality due to lung and bladder cancer in humans exposed to coke-oven emissions, roofing-tar emissions, and cigarette smoke. PAHs enter the body quickly and easily by all routes of exposure and are metabolized to hydroxylated metabolites as well as glucuronide metabolites. These metabolites are excellent indicators of exposure to the parent PAHs. While background level ranges of PAHs in air and water are known, the equivalent metabolite background levels in humans are not known.

Non-persistent pesticides (organophosphate insecticides, pyrethroid pesticides, other pesticides and fungicides, and herbicides): In 1999, about five billion pounds of pesticide active ingredients were used in the US, most of it for agricultural applications. The most recent registration data provided by the US EPA showed over 800 pesticidal active ingredients available in about 21,000 different formulations. Widespread use of the contemporary pesticides for agriculture and residential applications makes it virtually impossible for the average person to completely avoid exposure. Pesticide residues and their metabolites in human tissues and fluids can be indicative of pesticide exposure and the total body burden of these pesticides. Exposure to several pesticides was assessed by measuring urinary pesticide metabolites during NHANES 1999–2002. However, determination of the specific pesticide linked to the exposure can be inaccurate because some metabolites are common to multiple pesticides. Beginning in NHANES 2003–2004, specific pesticides in blood were also measured.

Little information is available concerning residential or household exposures to pesticides among the general population. Sufficient data do exist, however, from surveys or other focused research efforts to suggest that household exposure to certain common pesticides can be extensive and might be of significant public health concern. Pesticides of interest are: chlorpyrifos, 2,4-D, diazinon, permethrin, ortho-phenyl phenol, methyl parathion, and organophosphate pesticides.

Persistent organochlorines (organochlorine pesticides, polychlorinated and polybrominated dibenzo-p-dioxins and dibenzofurans, polychlorinated biphenyls (PCBs)): Organochlorines are diverse, synthetic chemicals that are persistent in the environment and tend to bioaccumulate. Most of these chemicals are banned in the U.S. Assessment of exposure to persistent organochlorines in a representative sample of the U.S. population is needed to determine current prevalence and level of exposure and the potential for human health threat from exposure to these chemicals.

Perfluorinated compounds:
Organic fluorochemicals are used in multiple commercial applications including surfactants, lubricants, paints, polishes, food packaging and fire-retarding foams. Recent scientific findings suggest that several perfluorinated surfactants, a group of these fluorochemicals, are ubiquitous contaminants found both in humans and animals worldwide, and there is increased concern regarding the toxicity of these perfluorinated compounds, including perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS). PFOS has been used in a wide variety of industrial and consumer products including protective coatings for carpets and apparel, paper coatings, insecticide formulations, and surfactants. In May 2000, the 3M Company, the sole manufacturer of PFOS in the United States and the principal manufacturer worldwide, announced that it was discontinuing the production of fluorochemicals, including PFOS. PFOA and its salts are used in the production of fluoroelastomers and fluoropolymers, such as polytetrafluoroethylene (PTFE) and polyvinylidine fluoride (PVDF). PFOA is still being produced (e.g., by DuPont). PTFE has numerous uses in many industrial and consumer products, including coatings on textiles and carpet; uses in the automotive, mechanical, aerospace, chemical, electrical, medical, and building/construction industries; personal care products; and non-stick coatings on cookware. PVDF is used primarily in electrical/electronics, building/construction, and chemical processing industrial sectors.

Polybrominated Diphenyl Ethers:
Brominated flame retardants (BFRs) are heavily used as additive or reactive chemicals in polymers and textiles. Increasing levels of polybrominated diphenyl ethers (PBDE) have been observed in mothers' milk from Sweden, Germany and Norway. PBDE concentrations found in North Americans are considerably higher than those found in Europeans. There is an increasing usage of PBDEs worldwide and results of several studies indicating that concentrations in North American populations may be increasing. Such information suggests that more information is needed to evaluate the degree of human exposure in the US population.

Toxaphenes:
Toxaphene is a mixture of chemicals that was one of the most commonly used insecticides in the United States prior to 1982. It consists predominantly of polychlorinated camphenes that are lipophilic (dissolve well in lipids) and persist for years in the environment. EPA banned the use of toxaphene in the U.S. in 1990. In 1993, EPA banned the importation of food that contained toxaphene residues. Toxaphene is considered a probable human carcinogen by EPA and the National Toxicology Program.

Volatile Organic Compounds (blood):

Exposure to volatile organic compounds (VOCs) is ubiquitous. Chronic exposure to extremely high levels of VOCs can lead to cancer and neurocognitive dysfunction. VOC exposure assessment will be expanded to include additional analytes of toxicological significance to include chemicals that are on priority toxicant or critical contaminant lists, and thus of – toxicological concern. Hexane is a widely used solvent with neurotoxic properties. Acrylonitrile is a probable human carcinogen used widely in the polymer industry. Cis- and trans-1,3-dichloropropenes and 1,2-dibromoethane are widely used as soil fumigants resulting in unknown human exposure. Furan also became a VOC toxicant of interest on May 7, 2004 when FDA released extensive data showing levels of this potential human carcinogen in food products.

Perchlorate:
Perchlorate is an anion that can disrupt thyroid function by competitively inhibiting iodide uptake. Despite the potential health effects of perchlorate exposure, widespread use of perchlorate salts coupled with little regulation concerning its disposal has led to widespread environmental contamination. Perchlorate is primarily produced as ammonium perchlorate for use as an oxidant in solid fuel propellants for rockets and missiles. Lesser amounts of perchlorate are used in matches, fireworks, and automotive airbags. Industries using perchlorate in the past have legally dumped large amounts into unlined lagoons resulting in large plumes of contamination in many areas of the United States.

Eligibility:
For the perchlorate measurement in urine, eligibility will be from a 1/3 sample of person six years and older.

Additional analytes proposed for 2011–2012 within a class of chemicals already included in the NHANES protocol do not require any additional specimen volume or change in eligibility. For the new classes of chemicals proposed for 2011–2012, the eligibility for serum-based measures is a one-third subsample of respondents 12 years and older and for the urine-based measures, it is a one-third subsample of respondents six years and older. These new classes of chemicals will be measured utilizing vials and volumes already available in the current laboratory protocol for environmental analytes.

Report of Findings:
Abnormal levels of blood lead, cadmium, and mercury; serum copper, manganese, selenium, and zinc; abnormal urine levels of arsenic; and high total trihalomethane levels in water are reported to participants. **(Attachment 31 – Early Reporting Letters)**

Table 2: Complete List of Environmental Toxicants, NHANES 2011

Chemical / Metabolite Name	Matrix	Eligibility	ROF		
			1	2	3
Tobacco Smoke					
Cotinine[0]	serum	3+			
4-(Methylnitrosamino)-1-(3-pyridyl)-1-Butanol (NNAL)	urine	6+			

Chemical / Metabolite Name	Matrix	Eligibility	ROF		
			1	2	3
Metals					
Lead[1]	whole blood	1+		Y	Y
Lead	urine	Heavy metal 6+, 1/3 subsample			
Cadmium[1]	whole blood	1+		Y	Y
Cadmium	urine	Heavy metal 6+, 1/3 subsample			
Manganese[1]	whole blood	1+		Y	Y
Selenium[1]	whole blood	1+			
Mercury (total) [1]	whole blood	1+		Y	Y
Mercury (total)/Iodine	urine	6+, 1/3 subsample			
Inorganic Mercury[1]	whole blood	1+			
Ethyl Mercury[1]	whole blood	1+			
Methy Mercury[1]	whole blood	1+			
Arsenic (total)	urine	6+, 1/3 subsample		Y	
Arsenous (III) acid	urine	"			
Arsenic (V) acid	urine	"			
Monomethylarsonic acid	urine	"			
Dimethylarsinic acid	urine	"			
Arsenobetaine	urine	"			
Arsenocholine	urine	"			
Trimethylarsine oxide	urine	"			
Antimony	urine	Heavy metal 6+, 1/3 subsample			
Barium	urine				
Cesium	urine	"			
Cobalt	urine	"			
Manganese	urine	"			
Molybdenum	urine	"			
Strontium	urine	"			
Tin	urine	"			

Chemical / Metabolite Name	Matrix	Eligibility	ROF		
			1	2	3
Thallium	urine	"			
Tungsten	urine	"			
Uranium	urine	"			
Copper[2]	serum	6+, 1/3 subsample		Y	Y
Selenium[2]	serum	"		Y	Y
Zinc[2]	serum	"		Y	Y
Phthalates					
Mono-ethyl phthalate	urine	"			
Mono-n-butyl phthalate	urine	"			
Mono-iso-butyl phthalate	urine	"			
Mono-benzyl phthalate	urine	"			
Mono-2-ethylhexyl phthalate	urine	"			
Mono-(2-ethyl-5-oxohexyl) phthalate	urine	"			
Mono-(2-ethyl-5-hydroxyhexyl) phthalate	urine	"			
Mono-(3-carboxypropyl) phthalate	urine	"			
Mono-(2-ethyl-5-carboxypentyl) phthalate	urine	"			
Mono-(2,6-dimethyl-6-carboxyhexyl) phthalate	urine	"			
Mono-(2,7-dimethyl-7-carboxyheptyl) phthalate	urine	"			
Polycyclic Aromatic Hydrocarbons					
2-Hydroxyfluorene	urine	6+, 1/3 subsample			
3-Hydroxyfluorene	urine	"			
9-Hydroxyfluorene	urine	"			
1-Hydroxyphenanthrene	urine	12+. 1/3 subsample			
2-Hydroxyphenanthrene	urine	6+, 1/3 subsample			
3-Hydroxyphenanthrene	urine	"			
4-Hydroxyphenanthrene	urine	"			
1-Hydroxypyrene	urine	"			
1-Hydroxynapthalene (1-Naphthol)	urine	"			
2-Hydroxynapthalene (2-Naphthol)	urine	"			
Organophosphate Insecticides: Diaklyl Phosphate Metabolites					
Dimethylphosphate	urine	6+, 1/3 subsample			
Dimethylthiophosphate	urine	"			
Dimethyldithiophosphate	urine	"			
Diethylphosphate	urine	"			

Chemical / Metabolite Name	Matrix	Eligibility	ROF		
			1	2	3
Diethylthiophosphate	urine	"			
Diethyldithiophosphate	urine	"			
Organophosphate Insecticides: Specific Pesticides and Metabolites					
Malathion dicarboxylic acid	urine	6+, 1/3 subsample			
3,5,6-Trichloro-2-pyridinol	urine	"			
2-Isopropyl-4-methyl-6-hydroxypyrimidine	urine	"			
para-Nitrophenol	urine	"			
2-(diethylamino)-6-methylpyrimidin-4-ol/one	urine	"			
3-Chloro-7-hydroxy-4-methyl-2H-chromen-2-one/ol	urine	"			
5-Chloro-1,2-dihydro-1-isopropyl-[3H]-1,2,4-triazol-3-one	urine	"			
Pyrethroid Pesticides					
cis-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid	urine				
trans-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid	urine	"			
3-Phenoxybenzoic acid	urine	"			
4-Fluoro-3-phenoxybenzoic acid	urine	"			
cis/trans-Dimethylvinylcyclopropane carboxylic diacid	urine	"			
cis-3-(2,2-Dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid	urine	"			
Other Pesticides					
Carbofuranphenol	urine	6+, 1/3 subsample			
N,N-diethyl-3-methylbenzamide (DEET)	plasma	12+, 1/3 subsample			
N,N-diethyl-3-methylbenzamide (DEET)	urine	6+, 1/3 subsample			
3-(diethylcarbamoyl) benzoic acid (DEET acid)	urine	"			
N-ethyl-3-methylbenzamide (Desethyl DEET)	urine	"			
N,N-diethyl-3-hydroxymethylbenzamide (Desethyl hydroxy DEET)	urine	"			
2,5-Dichlorophenol	urine	"			
Fungicides					
ortho-Phenylphenol	urine	"			
Ethylenethio urea (ETU)	urine	"			
Propylenethio urea (PTU)	urine	"			
Herbicides: Substituted Ureas					
Bensulfuron-methyl	urine	"			
Foramsulfuron	urine	"			
Halosulfuron	urine	"			
Nicosulfuron	urine	"			
Primisulfuron-methyl	urine	"			

Chemical / Metabolite Name	Matrix	Eligibility	ROF		
			1	2	3
Rimsulfuron	urine	"			
Sulfometuron-methyl	urine	"			
Sulfosulfuron	urine	"			
Chlorsulfuron	urine	"			
Oxasulfuron	urine	"			
Ethametsulfuron-methyl	urine	"			
Mesosulfuron-methyl	urine	"			
Metsulfuron-methyl	urine	"			
Prosulfuron	urine	"			
Thifensulfuron-methyl	urine	"			
Triasulfuron	urine	"			
Triflusulfuron-methyl	urine	"			
Other Herbicides					
2,4-Dichlorophenol	urine	"			
Atrazine	urine	"			
Diaminochlorotriazine	urine	"			
Desethylatrazine	urine	6+, 1/3 subsample			
Desethylatrazine mercapturate	urine	"			
Desisopropylatrazine	urine	"			
Hydroxyatrazine	urine	"			
Metolachlor mercapturate	urine	"			
Halogenated Phenolic Compounds					
2,4,5-Trichlorophenol	urine	"			
2,4,6-Trichlorophenol	urine	"			
Pentachlorophenol	serum	"			
Pentachlorophenol	urine	6+, 1/3 subsample			
5-Chloro-2-(2,4-dichlorophenoxy)-phenol (Triclosan)	serum	"			
Pentabromphenol	serum	"			
Perflourinated Compounds					
Perfluorooctanoic acid	serum	12+, 1/3 subsample			
Perfluorooctane sulfonic acid	serum	"			
Perfluorohexane sulfonic acid	serum	"			
2-(N-Methyl-perfluorooctane sulfonamido) acetic acid	serum	"			
Pefluorodecanoic acid	serum	"			
Perfluorobutane sulfonic acid	serum	"			

Chemical / Metabolite Name	Matrix	Eligibility	ROF		
			1	2	3
Perfluorononanoic acid	serum	"			
Perfluoroundecanoic acid	serum	"			
Environmental Phenols					
Bisphenol A	urine	6+, 1/3 subsample			
2-Hydroxy-4-methoxybenzophenone (Benzophenone-3)	urine	"			
2,4,4'-Trichloro-2'-hydroxyphenyl ether (Triclosan)	urine	"			
Methyl paraben	urine	"			
Ethyl paraben	urine	"			
Propyl paraben	urine	"			
Butyl paraben	urine	"			
Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans					
1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin (OCDD)	serum	12+, 1/3 subsample			
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD)	serum	"			
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)	serum	"			
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)	serum	"			
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (HxCDD)	serum	"			
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (PeCDD)	serum	"			
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	serum	"			
1,2,3,4,6,7,8,9-Octachlorodibenzofuran (OCDF)	serum	"			
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)	serum	"			
1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF)	serum	"			
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)	serum	"			
1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF)	serum	"			
1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF)	serum	"			
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)	serum	"			
2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF)	serum	"			
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)	serum	"			
2,3,7,8-Tetrachlorodibenzofuran (TCDF)	serum	"			
Polybrominated Dibenzo-*p*-dioxins and Dibenzofurans					
2,3,7,8-Tetrabromorodienzo-*p*-dioxin (TBDD)	serum	12+, 1/3 subsample			
1,2,3,7,8-Pentabromodibenzo-*p*-dioxin (PeBDD)	serum	"			
1,2,3,4,7,8-Hexabromorodibenzo-*p*-dioxin (HxBDD)	serum	"			
1,2,3,6,7,8-Hexabromodibenzo-*p*-dioxin (HxBDD)	serum	"			
1,2,3,7,8,9-Hexabromodibenzo-*p*-dioxin (HxBDD)	serum	"			
1,2,3,4,6,7,8,9-Octabromodibenzo-*p*-dioxin (OBDD)	serum	"			

Chemical / Metabolite Name	Matrix	Eligibility	ROF		
			1	2	3
2,3,7,8,-Tetrabromodibenzofuran (TBDF)	serum	"			
1,2,3,7,8-Pentabromodibenzofuran (PeBDF)	serum	"			
2,3,4,7,8-Pentabromodibenzofuran (PeBDF)	serum	"			
1,2,3,4,7,8-Hexabromodibenzofuran (HxBDF)	serum	"			
1,2,3,4,6,7,8-Heptabromodibenzofuran (HpBDF)	serum	"			
1,2,3,4,6,7,8,9-Octabromodibenzofuran (OBDF)	serum	"			
Dioxin-like Polychlorinated Biphenyls - cPCBs					
3,3',4,4'-Tetrachlorobiphenyl (PCB 77)	serum	12+, 1/3 subsample			
3,4,4',5-Tetrachlorobiphenyl (PCB 81)	serum	"			
3,3',4,4',5-Pentachlorobiphenyl (PCB 126)	serum	"			
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169)	serum	"			
Dioxin-like Polychlorinated Biphenyls - mPCBs					
2,4,4'-Trichlorobiphenyl (PCB 28)	serum	"			
2,3',4,4'-Tetrachlorobiphenyl (PCB 66)	serum	"			
2,4,4',5-Tetrachlorobiphenyl (PCB 74)	serum	"			
2,3,3',4,4'-Pentachlorobiphenyl (PCB 105)	serum	"			
2,3,3',4,4'-Pentachlorobiphenyl (PCB 114)	serum	"			
2,3',4,4',5-Pentachlorobiphenyl (PCB 118)	serum	"			
2',3,4,4',5-Pentachlorobiphenyl (PCB 123)	serum	"			
2,3,3',4,4',5-Hexachlorobiphenyl (PCB 156)	serum	"			
2,3,3',4,4',5'-Hexachlorobiphenyl (PCB 157)	serum	"			
2,3',4,4',5,5'-Hexachlorobiphenyl (PCB 167)	serum	"			
2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB 189)	serum	"			
Non-dioxin-like Polychlorinated Biphenyls					
2,2',5-Trichloro biphenyl (PCB 18)	serum	"			
2,2'3,5'-Tetrachloro biphenyl (PCB 44)	serum	"			
2,2',4,5'-Tetrachloro biphenyl (PCB 49)	serum	"			
2,2',5,5'-Tetrachlorobiphenyl (PCB 52)	serum	"			
2,2',3,4,5'-Pentachlorobiphenyl (PCB 87)	serum	"			
2,2',4,4',5-Pentachlorobiphenyl (PCB 99)	serum	"			
2,2',4,5,5'-Pentachlorobiphenyl (PCB 101)	serum	"			
2,3,3',4',6-Pentachlorobiphenyl (PCB 110)	serum	"			
2,2',3,3',4,4'-Hexachlorobiphenyl (PCB 128)	serum	"			
2,2',3,4,4',5' and 2,3,3',4,4',6-Hexachlorobiphenyl (PCB 138 & 158)	serum	"			
2,2',3,4',5,5'-Hexachlorobiphenyl (PCB 146)	serum	"			

Chemical / Metabolite Name	Matrix	Eligibility	ROF		
			1	2	3
2,2',3,4',5',6-Hexachlorobiphenyl (PCB 149)	serum	"			
2,2',3,5,5',6-Hexachlorobiphenyl (PCB 151)	serum	"			
2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153)	serum	"			
2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB 170)	serum	"			
2,2',3,3',4,5,5'-Heptachlorobiphenyl (PCB 172)	serum	"			
2,2',3,3',4,5',6'-Heptachlorobiphenyl (PCB 177)	serum	"			
2,2',3,3',5,5',6-Heptachlorobiphenyl (PCB 178)	serum	"			
2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180)	serum	"			
2,2',3,4,4',5',6-Heptachlorobiphenyl (PCB 183)	serum	"			
2,2',3,4',5,5',6-Heptachlorobiphenyl (PCB 187)	serum	"			
2,2',3,3',4,4',5,5'-Octachlorobiphenyl (PCB 194)	serum	"			
2,2',3,3',4,4',5,6-Octachlorobiphenyl (PCB 195)	serum	"			
2,2',3,3',4,4',5,6' and 2,2',3,4,4',5,5',6-Octachlorobiphenyl (PCB 196 & 203)	serum	"			
2,2',3,3',4,5,5',6-Octachlorobiphenyl (PCB 199)					
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (PCB 206)	serum	"			
2,2',3,3',4,4',5,5',6,6'-Decachloro biphenyl (PCB 209)	serum	"			
Hydroxylated Polychlorinated Biphenyls					
2,3,3',4',5-pentachloro-4-biphenylol (4-HO-CB107)	serum	"			
2,2',3,4',5,5'-hexachloro-4-biphenylol (4-HO-CB146)	serum	"			
2,2',3,4',5,5,6'-heptachloro-4-biphenylol (4-HO-CB187)	serum	"			
Polybrominated Diphenyl Ethers					
2,2',4'-Tribromodiphenyl ether (BDE 17)	serum	"			
2,4,4'-Tribromodiphenyl ether (BDE 28)	serum	"			
2,2',4,4'-Tetrabromodiphenyl ether (BDE 47)	serum	"			
2,3',4,4'-Tetrabromodiphenyl ether (BDE 66)	serum	"			
2,2',3,4,4'-Pentabromodiphenyl ether (BDE 85)	serum	"			
2,2',4,4',5-Pentabromodiphenyl ether (BDE 99)	serum	"			
2,2',4,4',6-Pentabromodiphenyl ether (BDE 100)	serum	"			
2,2',4,4',5,5'-Hexabromobiphenyl (BB 153)	serum	"			
2,2',4,4',5,6'-Hexabromodiphenyl ether (BDE 154)	serum	"			
2,2',4,4',5,5'-Hexabromodiphenyl ether (BDE 153)	serum	"			
2,2',3,4,4',5',6-Heptabromodiphenyl ether (BDE 183)	serum	"			
2,2',3,3',4,4',5,6'-Octabromodiphenyl ether (BDE 196)	serum	"			
2,2',3,3',4,4',6,6'-Octabromodiphenyl ether (BDE 197)	serum	"			

Chemical / Metabolite Name	Matrix	Eligibility	ROF		
			1	2	3
2,2',3,4,4',5,5',6-Octabromodiphenyl ether (BDE 203)	serum	"			
2,2',3,3',4,4',5,5',6-Nonabromodiphenyl ether (BDE 206)	serum	"			
2,2',3,3',4,4',5,6,6'-Nonabromodiphenyl ether (BDE 207)	serum	"			
2,2',3,3',4,5,5',6,6'-Nonabromodiphenyl ether (BDE 208)	serum	"			
2,2',3,3',4,4',5,5',6,6'-Decabromodiphenyl ether (BDE 209)	serum	"			
Hexabromobenzene (HBB)	serum	"			
Polychlorinated Naphthalenes					
1,2,3,4-Tetrachlorinated naphthalene (PCN 27)	serum	"			
1,2,3,5,7- and 1,2,4,6,7-Pentachlorinated naphthalene (PNC 52 & 60)	serum	"			
1,2,3,4,5,7- and 1,2,3,5,6,8-Hexachlorinated naphthalene (PNC 64 & 68)	serum	"			
1,2,3,4,6,7- and 1,2,3,5,6,7-Hexachlorinated naphthalene (PNC 66 & 67)	serum	"			
1,2,3,5,7,8-Hexachlorinated naphthalene (PCN 69)	serum	"			
1,2,3,4,5,6,7-Heptachlorinated naphthalene (PCN 73)	serum	"			
Toxaphenes					
Parlar 26 2-Endo,3-exo,5-endo,6-exo,8b,8c,10a,10c-octachlorobornane	serum	12+, 1/3 subsample			
Parlar 50 2-Endo,3-exo,5-endo,6-exo,8b,8c,9c,10a,10c-nonachlorobornane	serum	"			
Parlar 62 2,2,5,5,8c,9b,9c,10a,10b-nonachlorobornane	serum	"			
Volatile Organic Compounds (VOCs)					
1,1,1-Trichloroethane[3]	whole blood	12+, 1/2 subsample			
1,1,1,2-Tetrachloroethane[3]	whole blood	"			
1,1,2,2-Tetrachloroethane[3]	whole blood	"			
1,1,2-Trichloroethane[3]	whole blood	"			
1,1-Dichloroethane[3]	whole blood	"			
1,1-Dichloroethene[3]	whole blood	"			
1,2-Dibromo-3-chloropropane[3]	whole blood	"			
1,2-Dibromoethane[3]	whole blood	"			
1,2-Dichlorobenzene[3]	whole blood	"			
1,2-Dichloroethane[3]	whole blood	"			
1,2-Dichloropropane[3]	whole blood	"			
1,2,3-Trichloropropane[3]	whole blood	"			

Chemical / Metabolite Name	Matrix	Eligibility	ROF		
			1	2	3
1,3-Butadiene	Urine	6+, 1/3 subsample			
1,3-Dichlorobenzene[3]	whole blood	12+, 1/2 subsample			
1,4-Dichlorobenzene[3]	whole blood	"			
1,4-Dioxane[3]	whole blood	"			
2,5-Dimethylfuran[3]	whole blood	"			
Acrolein	urine	6+, 1/3 subsample			
Acrylonitrile	urine	"			
Benzene[3]	whole blood	12+, 1/2 subsample			
Benzene	urine	6+, 1/3 subsample			
Bromodichloromethane[3]	whole blood	12+, 1/2 subsample			
Bromoform[3]	whole blood	"			
Carbon Tetrachloride[3]	whole blood	"			
Chlorobenzene[3]	whole blood	"			
Chloroform[3]	whole blood	"			
cis-1,2-Dichloroethene[3]	whole blood	"			
Cumene[3]	whole blood	"			
Dibromochloromethane[3]	whole blood	"			
Dibromomethane[3]	whole blood	"			
Ethylbenzene[3]	whole blood	"			
Ethylene oxide	urine	6+, 1/3 subsample			
Furan[3]	whole blood	12+, 1/2 subsample			
Hexachloroethane[3]	whole blood	"			
m-/p-Xylene[3]	whole blood	"			
Methylene Chloride[3]	whole blood	"			
Methyl-tert-Butyl Ether (MTBE) [3]	whole blood	"			
n-Hexane[3]	whole blood	"			
Nitrobenzene[3]	whole blood	"			
Nitromethane[3]	whole blood	"			
o-Xylene[3]	whole blood	"			
Styrene[3]	whole blood	"			
Tetrachloroethene[3]	whole blood	"			
Toluene[3]	whole blood	"			
Toluene	urine	6+, 1/3 subsample			
trans-1,2-Dichloroethene[3]	whole blood	12+, 1/3 subsample			

Chemical / Metabolite Name	Matrix	Eligibility	ROF		
			1	2	3
Trichloroethene[3]	whole blood	"			
Vinyl chloride	urine	6+, 1/3 subsample			
Organochlorine Pesticides					
Hexachlorobenzene	serum	12+, 1/3 subsample			
beta-Hexachlorocyclohexane	serum	"			
gamma-Hexachlorocyclohexane	serum	"			
p,p'-DDT	serum	"			
p,p'-DDE	serum	"			
Oxychlordane	serum	"			
trans-Nonachlor	serum	"			
Mirex	serum	"			
alpha-Hexachlorocyclohexane (HCCH)	serum	"			
cis-Chlordane (or alpha)	serum	"			
trans-Chlordane (or gamma)	serum	"			
cis-Nonachlor	serum	"			
o,p'-DDE	serum	"			
Octachlorosytrene	serum	"			
Pentachloroanisole	serum	"			
trans,trans-9,12-Octadecadienoic acid	plasma	"			
trans-11-Octadecanoic acid	plasma	"			
Other					
Perchlorate[5]	urine	6+			
Thiocyanate[5]	urine	"			
Nitrate[5]	urine	"			

[0]All participants ages 3 and older

[1]All participants ages 1 year and older

[2] One-third sample ages 6 and older

[3] One-half sample ages 12 and older

[4] One-half sample of households

[5] one-third participants ages 6 and older

Laboratory Data Handling and Quality Control

Laboratory Data Quality Control:

- Most test procedures involve several operations and each operation is subject to errors. Both "precision control" (the agreement between replicate measurements) and "accuracy control" (the agreement between the mean estimate of a quantity and its true value) are evaluated to establish the quality of a laboratory. The goal of quality control is to ensure that the analytical values produced by a laboratory are sufficiently reliable and accurate for their intended purpose. A broader objective is to ensure that all laboratories produce analytical values that meet acceptable standards of precision and accuracy.

- A quality control program monitors laboratory performance to detect excessive random or systematic errors. Factors contributing to random error or imprecision include instability of the instrumentation, variations in the temperature, and variations in reagents and calibrators, handling techniques such as pipetting, mixing, and timing, and technical errors. Systematic error describes the error that is consistently low or high leading to inaccuracy. Examples of these factors include interfering substances or incorrect assignment of the amount of substance in the calibrator.

For the NHANES survey, the laboratories submit their quality control (QC) plan and data to the DHES staff along with the sample person results. The quality control data assures analytical accuracy and precision of the results. The quality control is linked to sample person results to see if changes in laboratory test distributions are due to instrument or method changes or represent changes in the population represented by the NHANES sample.

The quality control is reviewed by a Pathologist and Clinical Laboratory Scientist at DHES. The quality control plan is evaluated for conformity to laboratory standards (CLIA, CAP) and for appropriate statistical quality control rule systems. Internal (bench) QC data and external proficiency data are reviewed. In addition, NHANES uses "blinded" split and within-laboratory split replicates to evaluate the precision of the tests. The bench QC data is listed to check for consistency and completeness including review of dates of analyses, lot changes, and fixed, target means and standard deviations. The bench QC data is tested using Westgard rules such as 1:3S (exceeding three standard deviations from mean) to check for QC outliers. Bench QC is also analyzed quarterly to detect trends in the mean and standard deviations of the controls. In addition, external proficiency testing is reviewed when it is available for a test.

Summary reports are generated including blind QC analysis, 5% random repeat QC analysis, descriptive statistics of internal QC by quarter, and lot descriptive analysis. Shewhart charts (plots of QC values over time) of the internal QC data are reviewed for trends in the QC data. Sample person data is reviewed for descriptive statistics as a quality control technique. This includes checking sample person distribution statistics and generating a chart of sample person medians by stand.

The Pathologist or Clinical Laboratory Scientist help orient the lab to the NHANES survey and help set up the input of quality control to the NHANES collection system. In addition, they contact the laboratory when there are problems with quality control or sample person results. The Pathologist and Clinical Laboratory Scientist also visit the laboratory and conduct an inspection to assure that the laboratory is in compliance with CLIA standards. Quarterly status reports prepared by the laboratory are also reviewed.

Data Delivery and Editing:
The NHANES laboratories deliver the data to NHANES contractor (Westat). Westat then delivers the data to the NHANES analytic database at NCHS. Two systematic data QA/QC (quality assurance/quality control) processes, namely the inbound data QC review and the outbound data release production and QA, take place between the time the data arrive at NCHS and the time the data are released.

Inbound Data QC Review
After lab data have been delivered to NCHS, the QC review of the data are performed stand by stand for each lab. The project officer (PO) for the specific lab provides the specifications (specs) for the data review, and the Information Management Branch's lab data team provides the SAS programming support. Both the PO and the lab team will report any problems found and contact the lab and/or Westat if necessary. The general check points of this QC review include the completeness of the data delivery, missing data, duplicated records, correctness of the age and gender profiles, value outliers, age and gender mismatches between the data and MEC samples, limit of detection (LOD) fill values, correctness of the comment codes, etc. *Ad hoc* check points may also be created by the PO from time to time for certain labs. Ideally inbound data review should be done as soon as the data is delivered.

Data Release:
Outbound Data Release Production and Quality Assurance (QA) Review
NHANES data are released in two-year cycles. Data release production QA review may start as soon as the delivery of the 2-year data is complete and inbound data review for both the record level data and QC data is complete. Data release is a complicated and collaborated process. This process consists of the following steps: (1) the PO provides the release production editing specification, (2) the lab team produces the release data and the codebook, (3) two programmers other than the release production programmer independently perform the QA check on the dataset and the codebook, problems are corrected and additional QAs are performed until no problems are found by the QA reviewers, (4) the PO provides the release documentation and lab protocol files, (5) the lab team produces the frequent table and creates the self-extracting zip file that contains data, codebook, document and frequent table, (6) the lab team creates or updates variable list file (varlab) and filelist file, and (7) the lab team submits all release files for either the collaborator release or the public release. Collaborator release is a QC release that takes place prior to the public release. Unless there is a special agreement between NCHS and the collaborator, there is usually 90 days between the collaborator release and the public release. As a result of the collaborator response and/or new data issues identified, additional modification and QA on the release files may take place before the public release after the data have released to the collaborators.

Stored Specimens for Future Research

NHANES has maintained a specimen bank for future research, including genetic research, since 1991. Serum, urine and limited plasma samples are collected and stored from consenting adults as well as minors ages three years and older whose parents have given permission. DNA specimens are collected and stored from consenting participants ages 20 and older. Specimens in

the biobank are currently available from NHANES III (conducted from 1988–1994) and from NHANES 1999–present. DNA specimens are available from the second phase of NHANES III (1991–1994), NHANES 1999–2002, and NHANES 2007–present.

Specimens are stored in two Specimen Banks. Surplus samples that were initially used for laboratory assays included in the surveys, have since been stored at -70 °C and have been through at least two freeze-thaw cycles. They are stored at a commercial repository under contract to NCHS. In addition, on average, eight vials of sera were also stored in vapor-phase liquid nitrogen at the CDC and ATSTR Specimen Packaging, Inventory and Repository (CASPIR) Repository in Lawrenceville, GA. These specimens have not undergone a freeze-thaw cycle. The CASPIR Repository is considered a long-term repository for the NHANES specimens. NCHS is making both of these collections available for research proposals. The research proposals that can use the surplus specimens will receive higher priority. Proposals that request the specimens in CASPIR need to justify the use of the unthawed specimens.

The NHANES Stored Specimen Program offers investigator-driven research opportunities, with laboratory testing conducted in the investigator's own facility. Investigators must apply to use the NHANES specimens (http://www.cdc.gov/nchs/nhanes/genetics/stored_specimens.htm). Though participants consented to storing samples of their blood for future testing only research projects that include results that are judged not to have clinical significance for participants will be accepted. Clinical significance is defined by the following criteria:

- The findings are valid and done by a CLIA-certified laboratory, and
- The findings may have significant implications for the subjects' health concerns, and
- A course of action to ameliorate, or treat the concerns is readily available.

To determine if this limited resource should be used in the proposed projects, a Technical Panel evaluates the public health significance and scientific merit of the proposed research. Scientific merit is judged as to the scientific, technical or medical significance of the research, the appropriateness and adequacy of the experimental approach, and the methodology proposed to reach the research goals. All proposals for use of NHANES samples are also evaluated by the NCHS Ethics Review Board (ERB) for any potential human subjects concerns. The NCHS ERB will review the proposal even if the investigator has received approval by their institutional review panel.

Rules governing the use of DNA samples are outlined in a separate protocol previously submitted to the ERB, Protocol # 2009-06 "Plan for Making DNA from the NHANES Available for Genetic Research." NHANES is currently developing a new protocol, which will be submitted to the ERB before December 31, 2011, making DNA available to researchers.