

**National Health and Nutrition Examination Survey**  
**OMB No. 0920-0950**  
(Expires November 30, 2021)

**Nonsubstantive Change to conduct  
NHANES Developmental Projects**

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This is a request for nonsubstantive changes to the National Health and Nutrition Examination Survey (NHANES) (OMB No. 0920-0950, Exp. Date 12/31/2019), conducted by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC). The currently approved package includes a proposal to conduct developmental studies to support NHANES data collection. An infant blood pilot test was initially proposed and approved in 2017 but not fielded. The project was delayed as improved biospecimen collection methods were being developed by NHANES laboratory staff. This submission requests approval to pilot test blood collection, via venipuncture, from 100 study participants less than 12 months of age.

The project was delayed after NHANES laboratory staff suggested different methodology was being developed that would provide better biospecimen sample collection. This submission requests approval to pilot test blood collection, via venipuncture, from 100 study participants less than 12 months of age. The burden hours for this project have already been accounted for within the Developmental Projects & Special Projects line in the most recently approved revision. Findings from these studies will be reported to OMB and uploaded to Reginfo.gov.

#### A. Justification

##### Circumstances Making the Collection of Information Necessary.

NHANES is conducted annually. It includes a household interview, physical measures and additional interviews done in the NHANES Mobile Examination Centers (MECs). There may also be follow-up interviews or components (such as a second dietary interview) that take place after the MEC exam. A major advantage of continuous NHANES data collection is the ability to address emerging public health issues and provide objective data on more health conditions and issues by changing/modifying survey content. Though collected annually, NHANES data are released in two-year cycles. Some survey content stays the same across multiple cycles of NHANES. However, new survey content may be added, existing content may be modified or some content may be dropped at the beginning of each two-year survey cycle.

There is great value in testing new methodologies before they are implemented in the main survey. Testing allows NHANES staff to determine how long the protocol will take and how well received the procedure will be among our participants. The results of such testing also allow the NHANES program to make changes or adjustments to improve the methodology without affecting the results from the main study. Finally, it also provides hands on training opportunities for NHANES survey staff responsible for collecting the data. Testing is a vital step in making sure NHANES is effective and efficient in its use of resources. Such measures promote improved data quality once the data is collected in the actual survey. Since data collection is continuous, methodology studies must be conducted during ongoing NHANES data collection. This nonsubstantive change request captures one such methodological study, the burden hours for which have already been approved on line 6 (Developmental Projects & Special Studies) of the burden table within the current package (OMB No. 0920-0950, Exp. Date 11/30/2021).

## 1. Purpose and Use of the Information Collection

### Proposed Project – NHANES Blood Collection Study in Infants under 12 Months of Age: 2019-2020 Pilot Study

The Division of Health and Nutrition Examination Surveys (DHANES) would like to conduct a pilot study among infants in the NHANES 2019-2020 cycle. The relationship of early child nutrition and of environmental exposures to health outcomes throughout the lifespan is an important public health priority. Infancy (birth to <12 months) is a vulnerable life stage nutritionally because of the demand for nutrients to support growth and development. It is also a vulnerable life stage when exposures to environmental chemicals or toxicants may negatively influence growth and development. Exposures during infancy may also have the potential to impact gene expression and the trajectory of chronic disease progression (Perera and Herbstman, 2011).

Early child nutrition affects taste preferences, dietary behaviors, and the development of dietary patterns. Traditionally, the Dietary Guidelines for Americans focused on individuals ages 2 years and older (HHS and USDA, 2015). The Agricultural Act of 2014 mandated that beginning with the 2020-2025 edition, the Dietary Guidelines include comprehensive dietary recommendations for children from birth to 24 months (HHS and USDA, 2015). As a result, nationally representative data on infant dietary measures and exposures are needed for advancing the science base needed to inform current and future Federal nutrition programs, policies, consumer information, future Dietary Guidelines. Similarly, it is important to better understand levels of exposure to environmental toxicants to inform programs and policies.

The following tests were requested by the key collaborators from the Federal Data Consortium on Pregnancy and B-24 Months. Funding is provided by the following agencies: US Department of Agriculture (USDA), Department of Health and Human Services (HHS), Environmental Protection Agency (EPA) and Department of Defense (DOD). This proposal addresses the opportunity for a pilot study to lay the foundation for moving towards regularly collecting blood from all infants in NHANES.

#### Proposed Measures and Activities:

- Infant health questions
  - a. Safety Exclusion Questionnaire, Follow-up Questionnaire for Venipuncture Refusal (Attachment 1b)
- Complete blood count (CBC)
- Metal panel (lead, cadmium, total mercury, speciated mercury, manganese, and selenium)
- Folate levels
- Iron status markers (ferritin, transferrin receptor, hemoglobin from CBC)
- Inflammation markers (c-reactive protein, and alpha-1-acid glycoprotein)
- Retinol level
- Per- and polyfluoroalkyl (PFASs) substances
- Vitamin D status

- a. Update measurement list to add vitamin D (Attachment 1c), which is not being done in other age groups

Currently, NHANES only collects and analyzes blood from participants 1 year of age and older. Thus, no national data are currently available on the blood levels of relevant analytes for infants. NHANES collects data on dietary exposures of infants and new questions were added to the NHANES 2019-20 cycle that may support resulting data obtained from collecting and testing blood in infants. If it is determined that this component can be added to a future NHANES, blood measures of nutritional status and environmental chemical exposures can be combined with NHANES questionnaire and dietary data. This will enhance the already available information on NHANES concerning infants and help to advance public health initiatives that serve our youngest Americans. Several factors must be taken into consideration before this pilot could be added to the main NHANES survey, including the additional oversampling of infants required to achieve the appropriate sample size for making national estimates, the additional cost, time and effort associated with increased oversampling and the benefit vs. burden to the infant and parent/guardian.

The proxy (parents /guardians) of up to 100 eligible NHANES participants from birth to less than 12 months, will be asked permission for their infant to participate in the pilot. Parents or guardians who do not give permission will be asked to complete a short questionnaire outlining the reasons for refusal. This pilot study will establish the feasibility of collecting and analyzing blood in infants who participate in NHANES at the MEC. DHANES considers the proposed venipuncture protocol to be both safe for the sampled participants and suitable for the MEC environment. The amount of time needed for this project is estimated at 15 minutes per participant. Participation is voluntary. This pilot would take place in the NHANES mobile examination center (MEC) at multiple locations until the necessary sample size is obtained. Select findings will be reported to participants (see attachment 1d). This study would begin, as soon as feasible, once approval has been received.

The objectives of this project are to:

- Determine if parents/guardians will allow us to draw blood from their infant
- Understand reasons for refusal
- Determine if the phlebotomists are able to collect blood on infants in a reasonable time period and obtain the volumes required for this protocol, and
- Assess if valid laboratory testing can be performed from a small volume of blood after shipment from different areas of the U.S.

A target of 100 infants (estimated to take 10-12 MEC locations) in NHANES 2019-2020 will be included in this pilot. Based on blood collection from 1-2 year olds in the current NHANES we are aiming for a response rate of 60 percent among parents/guardians who agree to the blood draw. Once a parent/guardian agrees to the blood draw, there may be some infants not able to have blood collected (ineligible or unable to have blood drawn). Our final analytic sample will be those infants that are eligible and have blood successfully collected.

We will evaluate the pilot after parents of the first 20 infants have been asked to participate, and at every 20 cases thereafter. We will determine if the response rate warrants continuing or if

minor changes are needed (pending approval by the ERB and OMB (if needed)) to improve the response rate. For every 20 cases, along with checking response rates, we will evaluate, how parents/guardians receive this testing, occurrence of any adverse events, and any operational effects at the MEC. A system will be developed to capture qualitative feedback from the phlebotomist and/or observer for each encounter with the parent/guardian and infant during the phlebotomy exam so a comprehensive understanding of the experience can be evaluated (i.e., even if the parent agrees to have the blood drawn from the infant, we want to know if it was a bad experience with the infant being very upset and/or parent unhappy). We will evaluate the qualitative feedback documentation in real time and to determine if there is a need to stop the pilot, due to disruptions in the flow of MEC exams or other reasons.

The success of the pilot will be determined using the following criteria: 1) approximately 60%\* of parents/guardians of eligible participants agree to have blood drawn via venipuncture (based on current response and completion rates from NHANES participants ages 1-2 years, which is currently 68%); 2) of the 60% who agree to phlebotomy, approximately 85% to have successful phlebotomy using venipuncture and a successfully analyzed sample by the testing laboratory. All infants less than 12 months will be analyzed as a whole, and we will look at results by age. Ideally that would be in 3 months age ranges (0-3, 3-6, 6-9, 9-12) or <6 months and 6 months and older.

\* Currently, the response rate for blood collection in participants 1-2 years old is 68% with a 91% successful blood draw rate, in 3-5 year olds, the response rate is 75% with a 95% successful blood draw rate in this group, and in 6-11 year olds, the response rate is 81% with a 96% successful blood draw rate in this group.

To determine the above criteria, we will measure or collect:

- Percent of participants less than 12 months of age
- Percent of eligible participants whose parents or guardians agree to the exam
- Percent of eligible participants who have blood successfully drawn
- Percent of eligible participants who have some but not all blood collected (partial phlebotomy exam results)
- Percent of completed venipunctures that had adequate blood volume to perform all testing stated in protocol
- Percent of blood samples that had processing or aliquoting issues in the MEC
- Reasons for not participating
- Reasons for not completing the exam
- Length of time to complete the exam
- The percent of participants that had all lab tests completed

#### Justification of Laboratory Tests

Venipuncture blood will be used to perform laboratory tests that will provide information on important aspects of the health and nutritional status of infants. Infancy is a vulnerable life stage nutritionally because of the demand for nutrients to support growth and development. It is also a vulnerable life stage when exposures to environmental chemicals may negatively influence growth and development. The relationship of early child nutrition and other exposures to health outcomes throughout the lifespan continues to grow as an important public health priority.

Collaborating federal agencies, supporting this pilot, have chosen analytes based on their public health relevance to attain national estimates among infants. Early child nutrition affects taste preferences, dietary behaviors, and the development of dietary patterns. Inadequate intake of nutrients, poor diet behaviors, and unhealthy weight gain in early childhood lead to numerous developmental and long-term health problems. Measurements of environmental toxins such as lead or mercury may help to better understand exposure among infants either from the environment or through their mother.

Analytes were also chosen that could be tested on a small blood volume that can safely be drawn from infants. Measures of nutritional status include CBC, iron status markers, folate, and vitamin D. Measures of exposures to environmental chemicals include lead, methylmercury, and per- and polyfluoroalkyl substances. The selected analytes were chosen based on the public health relevance of each test as it relates to the growth and development among infants.

#### Iron Status and Inflammation Markers

The biologic markers to assess iron status and inflammation are: 1) serum ferritin, 2) soluble transferrin receptor (sTfR), 3) hemoglobin (Hgb), 4) alpha-1-acid glycoprotein (AGP), and 5) C-reactive protein (CRP). The prevalence of iron deficiency was estimated to be 15.1% among children 12-23 months of age in NHANES 2003-2010 (Gupta et al., 2017). Prevalence of iron deficiency anemia was estimated to be 2.7% among children 12-35.9 months of age in NHANES 2007-2010 (Gupta et al., 2016). Many factors can be contributed to low iron at birth, such as maternal anemia, maternal hypertension or diabetes during pregnancy (Baker et al., 2011).

The iron status of infants is of public health concern due to the adverse consequences of iron deficiency during this period of rapid growth and development. Recent efforts to link blood measures of iron status as well as iron supplementation to changes in health outcomes during infant development have identified a lack of data in the United States. Addressing these data gaps requires fundamental information in several areas including the current iron status of this vulnerable group. Knowledge about the degree of iron deficiency further helps to inform decisions about supplementation and fortification. In addition, the availability of data on iron status of surveyed individuals allows targeted research to elucidate factors that may influence status and health outcomes in infancy such as inflammation, obesity, and ethnic variations.

Serum ferritin is useful in evaluating iron metabolism and in determining the body's iron reserves, whereas sTfR best describes functional iron status after iron stores have been depleted. Both serum ferritin and sTfR are influenced by inflammation; thus, the proper interpretation of iron status involves adjusting these data with indicators of inflammation, such as AGP and CRP. AGP may be elevated during infections as well as acute and chronic inflammatory processes, such as Crohn's disease. CRP may be elevated with trauma, infection, or inflammation.

#### Retinol Level

Retinol is a test for vitamin A deficiency. Vitamin A is essential for proper health, growth and development, by promoting growth of healthy cells and protecting against infections. Breast milk continues to be the best source of vitamin A in an infant's diet. Vitamin A deficiency can lead to loss of appetite, eye problems, increased risk of infections, frequent diarrhea, iron deficiency anemia and failure to thrive.

### Folate Status

Folate is the naturally-occurring form of the vitamin and can be found in foods. Folic acid is the synthetic form of the vitamin that is used in most supplements and in fortified foods. Folate is essential for infant brain development. Low levels may result in anemia. Folate is consumed by infants from human milk and or from folic acid fortified infant formula and, once complementary foods are introduced, other fortified foods. To better understand the effects of these exposures, folate status (serum folate) from infant blood can be assessed. Although there is little information available on the extent of folate deficiency in children, several studies have suggested suboptimal folate status (Molloy et al., 2008). Monitoring intake/exposure among infants and assessing nutritional status is crucial in informing research on long-term health outcomes including obesity, diabetes, and cognitive development. Working towards having a population-based sample that describes these parameters is crucial to support public health. Measuring blood folate concentrations in infants, including RBC and serum folate, is also relevant in assessing the impact of FDA's 2016 approval for voluntary fortification of corn masa flour with folic acid (FR Notice April 15, 2016; Hamner et al., 2009).

### Vitamin D Status

The American Academy of Pediatrics recommends, beginning at birth, that all infants consume 400 IU of vitamin D per day. Human milk is not a good source of vitamin D; therefore, for infants consuming human milk either as the sole source or a partial source of nutrition, vitamin D supplementation is recommended (Wagner and Greer 2008). For infants who are not consuming enough vitamin D fortified formula (i.e., <1,000 mL/day), vitamin D supplements are recommended. Monitoring if these recommendations are adhered to or not is a public health concern. In addition, the updated regulations of the Food and Drug Administration for the Nutrition Facts label requires vitamin D to be listed on food packaging; thus, monitoring vitamin D status is a priority (FDA 81 FR 33741).

### Heavy Metal Exposures

Infants can be exposed to toxicants like lead through food and human milk in addition to environmental exposures. Lead exposure can impact normal growth and development of infants as well as impact longer-term outcomes (CDC "Facts on Lead" webpage). Children are at particular risk for lead toxicity. Approximately one-half million children in the United States 1-5 years of age have blood lead levels above 5 micrograms per deciliter (CDC reference level for public health action, <https://www.cdc.gov/nceh/lead/default.htm>). Data are currently not available through NHANES for children under 1 year of age. Substantial evidence shows that chronic, low level exposure during infancy (and childhood) can result in lower IQ, persistent behavioral issues, and impaired cognition and memory (<https://www.cdc.gov/nceh/lead/prevention/default.htm>). Lead is of interest to study as higher levels are associated with lower iron, ferritin and hemoglobin levels that are also included in this pilot study.

Mercury is a metal that occurs in the environment from both natural and anthropogenic sources. The three chemical forms of mercury are elemental or metallic mercury (Hg<sup>0</sup>), inorganic mercury (mercurous and mercuric cations), and organic mercury, which includes ethyl mercury and methylmercury (MeHg) as well as many other organic forms. Methylmercury is a highly

toxic substance; a number of adverse health effects associated with exposure, with the most being neurotoxicity, particularly in developing organisms (EPA, CASRN 22967-92-6). Methylmercury is by far the most common form of mercury in the food chain, and almost all-human exposure to methylmercury is through the consumption of fish, except for infants whose exposure is through human milk and, once introduced as a complementary food, through seafood. Nearly all seafood (fish and shellfish) contains at least trace amounts of methylmercury, although certain types of fish are high in methylmercury. Among one-5 year olds in NHANES, about 50-60% have a detectable blood total mercury (methyl, ethyl, and inorganic) and methylmercury.

The 2015-2020 Dietary Guidelines for Americans and FDA/EPA Advice on What Pregnant Women and Parents Should Know about Eating Fish both provide dietary guidance for children 2 years of age and older regarding seafood consumption and health. Although this advice includes guidance for lactating mothers to encourage a healthy diet while also limiting potential methylmercury exposure to the infant through human milk, there is little guidance in the United States on seafood consumption for infants (and children in the second year of life) with the exception of guidance related to potential allergenicity. This poses a major gap in guidance to support public health.

#### Other Environmental Exposures

Per- and polyfluoroalkyl substances (PFASs) are synthetic chemical substances of varying carbon chain length with varying functional groups that are used in hundreds of manufacturing and industrial applications including fire resistance and oil, stain, grease, and water repellency (EPA 822-R-16-003 and EPA 822-R-16-002). Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are examples of PFASs found in drinking water. Evidence suggests these chemicals are persistent in the environment, are found at very low levels both in the environment and in the blood of the general U.S. population, can remain in people for a very long time, and cause developmental and other adverse effects in laboratory animals. Evaluating levels of PFASs in infants is crucial in monitoring exposures during a vulnerable time-period of infant growth and development. It is possible that serum concentrations in infants could be quite low. Attempting to analyze PFASs through the pilot study would help determine if detection frequency may be an issue, informing future decisions regarding analysis of PFOAs in infant populations.

#### Technical Training

The phlebotomists are certified and qualified to collect these samples. Classroom refresher training and practice will be provided by NCHS and the contractor. This training will include a training video from the Center of Phlebotomy Education and practice on unique equipment - Nita Newborn™ (an anatomically correct model of a 4 lb, 16" newborn). The training may include procedures such as the use of pediatric blood drawing supplies, pre-warming the blood draw site, specific holding and arm stabilization techniques, and using intense transillumination devices for infant venipuncture.

Summary of details and proposed data collection forms for this project are provided in Attachment 1a and 1b. The NHANES Health Measurements list has been updated to include the



tests for this pilot in this age group (see attachment 1c). For an example of the Report of Findings, see attachment 1d. Attachment 1e contains examples of information that may be used to help gain participant cooperation.

9. Explanation of any payment or gift to respondents.

Participants in the pilot studies described in this non-substantive clearance request will not receive any additional incentives. They will simply receive the already approved incentives given to regular NHANES participants.

12. Estimates of Annualized Burden Hours and Cost

The Blood Collection – Infants Pilot Study is budgeted for 15 minutes. The maximum number of respondents would be 100 infants. The maximum burden is 25 hours (100 respondents\*15/60 hours = 25 hours). This time was already budgeted and approved in line 6 (“Developmental Studies & Special Projects) of the original submission. No additional burden is sought.

ANNUALIZED BURDEN HOURS AND COSTS

Type of Respondent	Form	Number of Respondents	Number of Responses per respondent	Average Burden per Response (in hours)	Total Burden Hours
Blood Collection – Infants Pilot Study Participants	Blood Collection – Infants Pilot Study Form	100	1	15/60	25
Total		100			25

15. Explanation for Program Changes and Adjustments. The project described in this submission does not change the burden hours from the previously approved clearance. The burden hours in this submission are captured in the “Developmental Studies & Special Projects” line of the burden table currently approved for NHANES.

List of attachments:

- 1a. Blood Collection – Infants Description
- 1b. Blood Collection – Infants Form
- 1c. Blood Collection – Infant Measurement List
- 1d. Blood Collection – Example Report of Findings

## 1e. Blood Collection – Gaining Cooperation