Supporting Statement
For OMB Review and Approval of

Agency for Toxic Substances and Disease Registry (ATSDR)
A Prospective Birth Cohort Study Involving Environmental Uranium Exposure in the Navajo Nation

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SECTION B. Statistical Methods

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B.1. Respondent Universe and Sampling Methods

Respondent Universe & Background:

The study participants will be pregnant women who receive prenatal care or are willing to receive prenatal care from the following Navajo Area Indian Health Service (NAIHS) /NAIHS Public Law 93-638 (P.L. 93-638) contracted facilities: Northern Navajo Medical Center, Chinle Comprehensive Health Care Facility, Gallup Indian Medical Center, Tuba City Regional Health-Care Corporation, or Tséhootsooí Medical Center. NAIHS is one of 12 area offices of the Indian Health Service, a branch of the U.S. Public Health Service of the Department of Health and Human Services. The NAIHS is responsible for the delivery of health services through a combination of in-patient, out-patient and community health programs centered on six hospitals, seven health centers, and 15 health stations. Delivery of service is accomplished through eight service units. Navajo Nation administers care in some of the service units (i.e., Tuba City, Tséhootsooí , Kayenta) through P.L. 93-638 contracts administered by Navajo Nation Division of Health. NAIHS clinicians document approximately 3,000 Navajo live births per year in the greater NAIHS system, distributed among the participating hospitals as indicated Table B.1 below.

Table B.1: Navajo Area OB/GYN Statistics (2009) Self-Reported at Area and National Meetings (Source NAIHS clinical staff)

Service Unit	Chinle (NAIHS)	Tséhootsooí (PL-638)	Gallup (NAIHS)	Northern Navajo(NAIHS)	Tuba City (PL-638)	Total
Number of Births	541	459	664	763	519	2946

The five service units in the table comprise the hospitals where the majority of deliveries will occur for Navajo Nation, although high-risk deliveries may be referred to larger regional hospitals such as the University of New Mexico or the University of Arizona. These five service units will be the targets for recruitment in this study, with recruitment criteria requesting the participants to seek care and plan delivery at one of these hospitals. These service units also correspond well to the areas of highest anticipated risk. Kayenta also serves an exposed population, and will be included in the recruitment, although deliveries from that area will occur in a neighboring hospital such as Tuba City, Shiprock, or Chinle as Kayenta does not provide that services for labor and delivery.

A Navajo Community Health Assessment reported 3,003 live births in NAIHS in FY 2003 and noted that approximately 15-20% of live births on the Navajo Nation were born to adolescent mothers (<18 years of age) (Benally, 2005). In our discussions to solicit input from community members and clinicians, the necessity of including these minor mothers in the cohort has repeatedly been identified. Inclusion of mothers below the age of 18 in the research will require

consent from a parent for participation of the minor mom (or dad), assent from the minor for their participation, and the mom's consent for the participation of her unborn child (for which purpose she is seen as emancipated).

Case Definition: The intent of the study is to determine whether exposure to uranium contributes to either adverse birth outcomes in the case of parental exposure, or developmental delays associated either with parental exposure, in utero exposure, or exposure of the child during development. We anticipate all of these circumstances will be identified in the cohort, and will recruit women who have lived on Navajo lands for at least 5 years and fathers who have conceived a child, and are willing to have that child followed at birth, 2 months, 6 months, 9 months and up to1 year of age. Due to the infant mortality rate in this population, we may not obtain follow-up information on all children at each age point. Also, follow-up information may be limited due to miscarriages or participant fatigue. However, extensive training and outreach will be conducted to maximize participant response rate and minimize participant fatigue (See Part B.3).

Women will be identified through extensive outreach both in communities and at chapters, as well as through clinical settings. We will enroll women who have lived on Navajo Nation or within adjacent communities for at least 5 years; are willing to receive health care at one of the five participating clinical service units; and are willing to consent for follow-up of their child during a minimum of the first year of life. All participants will self-identify by contacting either dedicated staff in the clinics, or field staff working for NNDOH or the UNM Research Team. These staff will explain the opportunity to be in the study to eligible women they encounter during the course of various outreach activities, and administer screening eligibility questionnaires to interested individuals.

Fathers will be enrolled to the extent identified in order to assess environmental exposure, previous reproductive, and medical histories. Exposures of all three members of the family unit will be determined through surveys, environmental sampling and existing data assessment, and verified through biomonitoring.

Inclusion Criteria*:* Native American mothers from age 14 to 45 with confirmation of pregnancy will be recruited into the study. Inclusion criteria will be a willingness to receive prenatal care and deliver at one of the five health-care facilities participating in the study, to have resided in the Study Area for at least 5 years, and to be willing to have the child followed for up to 1 year. Following the mother's consent, the initial page of the intake survey will ask questions about the father, his knowledge of her participation, his willingness to participate, and a name for contact, if appropriate. The mother will continue to be included in the study should the father not be identified at that time. Efforts will be made to enroll eligible women as early in their pregnancy as possible (ideally, in the first trimester), but we will enroll women who volunteer for the study who are in their third trimester. The study will allow mothers who have multiple children during the study period to enroll multiple children.

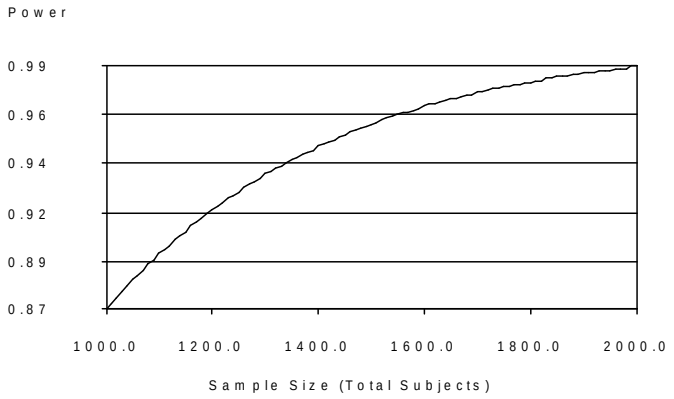
Fathers will be included in the study with consent regardless of age or residence. All babies born to qualifying mothers will also be included in the study with the mother's consent. If either parent is under 18, parental consent for their participation will also be necessary, as well as their assent as a minor. Mothers who turn 18 during the course of the study will be re-consented before any further data is collected from them. Because fathers will provide data only at enrollment, they will be re-consented when they reach 18. This will occur only in cases where the father is the primary caregiver.

Exclusion Criteria: Interested participants who do not have a clinical confirmation of pregnancy will be excluded from the study. Women who qualify for the study, but not willing to receive care and deliver at a participating medical facility, will be excluded. Women who are non-Native Americans will also be excluded from participating in the study. Women who are not willing to have their child followed for up to one year will be excluded. In addition, women not currently residing on the Navajo Nation or in immediately adjacent communities will be excluded. All exclusion criteria are consistent with the intent of the study to examine the reproductive and developmental toxicity of uranium exposures on the Navajo Nation. Living outside of the Navajo Nation, or not being pregnant, will not meet the intent. Previous cohort recruitment on the Navajo Nation resulted in fewer than 5 out of 1,304 being non-Native American, a number not sufficient to break out in an analysis. Finally, NAIHS and associated PL-638 health-care providers will be supporting the study through medical record reviews and provision of standard clinical care. Therefore, participants should be eligible to receive health care in those facilities.

B.2. Procedures for the Collection of Information

Target Enrollment Estimation Procedure: The target enrollment was estimated to be an average of 550 pregnant women per year, assuming a 10% pregnancy loss. This would result in 500 live-births (17% of the anticipated 3,000 Navajo births/year), for a period of 3 years giving a total anticipated sample size of 1,500 mother-infant pairs. Please refer to Chart 1 for statistical power at different sample sizes**.**

Chart 1: Power as a Function of Sample Size.



The projections of statistical power and sample size were based on several factors and assumptions. First, since there are multiple outcomes and exposures, with different underlying relationships, these calculations are only as an example of the study power that we would expect to achieve given different numbers of study participants (total number of subjects). We assume a comparison based on using the highest quintile (20%) of exposure, as the "exposed" group, and assume the exposure will double the risk of an adverse event, for example low birth weight defined as $<10_{th}$ percentile of weight for-length (probability of adverse event=10%). Given these assumptions, at a total sample size of 1,500, we have 95% power to detect an effect. If we enroll even 1,000 mother-infant pairs, we would still have 87% power to detect an effect of this magnitude. However, given that the effects may be more subtle, we acknowledge that these calculations likely represent a minimum target enrollment.

We are confident that we can enroll the required number of participants because we have a positive history of working with the NAIHS facilities and private hospitals on Navajo for many years, with direct experience working with Gallup and Shiprock-Northern Navajo Medical Center (NNMC) service units in recruiting participants for the medical monitoring as well as the DiNEH biological sampling programs. In addition, we anticipate our outreach plan will result in a similar willingness to participate in target service units, including Gallup and Shiprock-NNMC, each of which manages about 700-750 OB cases per year and has a significant exposed population. The Navajo population is stable; meaning those enrolled during early pregnancy will likely not be lost to the study by migration out of the study area. By partnering with the NAIHS and NNDOH, Public Health Nurses (PHNs) and Community Health Representatives (CHRs) who are already in place, we will be able to capitalize on data collected as part of routine prenatal care which will require record abstraction but not additional study visits. This will help to increase our efficiency.

Recruitment will be enhanced by dedicated Community Health and Environmental Research Specialists (CHERs) who are a part of the study team. The CHERs will provide follow-up contacts and additional data collection. Calculating the number of home visits possible for the CHERSs based on PHN and CHR estimates, and factoring in time for outreach and the number of births per year in the expanded catchment area, we estimate possible recruitment and follow-up for up to as many as 1,000 pregnant women per year. Given that we have been delayed by as long as a full year in finalizing the protocol and obtaining approval, that number may be overly optimistic at this point. We will work to achieve our original target goal in spite of the delayed start to recruitment, and we will utilize methods to maximize participant response rates and minimize participant dropout (Part B.3)

After the initial six months of recruitment, we will re-evaluate success and budgets, determine whether sufficient opportunity for carry-forward may remain and work with partner organizations to either revise strategies to increase enrollment, seek supplemental funding, or adjust targets as necessary for project success.

Recruitment of Study Participants: Sixty to seventy percent of Navajos stated that they would use traditional medicine for diagnosis of illness (Benally, 2005). However, clinicians, community advisors, and traditional medicine men with whom we have spoken have confirmed that nearly all Navajo mothers will also seek prenatal care in NAIHS (including PL-638 facilities). Therefore, prenatal clinics will provide the best opportunity to identify and recruit potential participants. Through outreach of our diverse team, we will hope to reach others who might not initially have sought prenatal care at the clinics. Within most NAIHS service units and PL-638 facilities, the prenatal clinics are staffed by midwives. The midwife programs are not centralized, but rather maintained independently within each service unit, and in some cases staffed in conjunction with family practice physicians. Obstetricians are on call and provide consultation or a higher level of care when complications are identified. The number of pregnant women who present at NAIHS facilities during the first trimester, however, appears to be low. Although one report suggests more than 80% of women has their initial visit in the first trimester (Benally, 2005), more recent data suggest that the percentage is much lower (USDHHS, 2008). Because those not seeking care in the first trimester may be at greater risk, this factor is a potential bias that must be addressed in the recruitment strategy by outreach to increase enrollment and prenatal care or controlled in the statistical analysis.

The number of prenatal visits varies dramatically, but more than one-half of pregnant women on Navajo are seen for NAIHS prenatal care more than nine times during their pregnancy. Public health nurses (PHNs), interpreter/drivers in the PHN program, and Community Health Representatives (CHRs) are often requested to contact those who miss appointments in efforts to get them to the clinics. Generally, these interventions are done in cases where specific concerns have been noted, or after repeated “no-shows”. Community Health and Environmental Research Specialists (CHERSs) will be working within the CHR administrative structure and therefore will be well integrated into that system. For women who miss their scheduled appointments, the CHERSs can conduct home interviews to ensure regular data collection at critical study time intervals as well as coordinate with CHRs to get participants to clinic appointments. Another role of the outreach will be to increase awareness of the need to meet appointments in both the mind of the mom as well as her extended family and friend’s network.

Study staff will introduce the project in tribal chapter meetings in partnership with the regional staff and CHERSs. This will be the initial step of the train-the-trainer model employed through all phases of community work in the project. At that time, all risks and benefits of participation in the cohort will be introduced. New CHERSs will partner with existing staff for this process as an element of training, and CHRs will also participate. We will work with NAIHS midwives and obstetricians in participating service units to distribute information on the study, and inform participants in the NAIHS medical monitoring program intakes about the work as well. Multiple venues for enrollment and consent will be available: chapter houses and WIC offices where project staff will have outreach information, at home visits attended by CHERSs and at hospital and clinics staffed by Navajo Nation Division of Health and NAIHS personnel.

Identification of interested women will be conveyed to the field staff by NAIHS referrals, by the CHRs, or through self-responses to information obtained at meetings, from printed materials, or radio announcements. Participants will be asked initially to complete a screening document to verify pregnancy and other eligibility criteria. This screening for eligibility can be administered by a broad range of participating agency staff, including CHRs, Women Infants and Children (WIC) personnel, Health Educators (HEs), and public health nurses (PHNs). If eligibility is confirmed, participants will be referred to Navajo Birth Cohort Study dedicated staff for consent to participate in the cohort study and for review of their medical records. Separate consents will be administered for the mother, the father, and in cases of minor parents, a parent of minor in addition to minor assents. Consents will be administered by dedicated research staff working either for NAIHS, Navajo Nation Division of Health (NNDOH), or University of New Mexico (UNM) Team.

Regardless of the site of administration for the consents, home visits will be scheduled to conduct surveys and obtain information of environmental quality in the home and surrounding area. These home intake visits will be done by CHERSs initially accompanied by field staff to ensure consistency and comfort with explaining and collecting environmental health data and information. Over time as CHERSs demonstrate the ability to work independently, they will conduct visits according to NNDOH home-visiting protocols accompanied by chapter CHRs when necessary. Participants' home locations will be determined by the CHERSs using hand-held GPS units. Through outreach in chapters, we anticipate other participants will be identified outside of the NAIHS clinics either through self-identification to the Research Team or through the CHERSs. Regardless of site of identification, all interested parties will receive the screening questionnaire to determine eligibility, followed by the appropriate consent document and accompanying NBCS fact sheet and timeline

B.3. Methods to Maximize Response Rates and Deal with Nonresponse

The research team includes a substantial number of community residents from the study area communities, as well as the collaborative. Although 60-70% of Navajos stated they would use traditional medicine for diagnosis of illness (Benally, 2005), clinicians, community advisors, and traditional medicine men with whom we have spoken have all confirmed that nearly all will also seek prenatal care in NAIHS (including P.L. 638 facilities) through the midwife programs in each service unit. Therefore, these prenatal clinics will provide the best opportunity to identify and recruit potential participants. Outreach will also be through meetings at chapter houses or public events, through word-of-mouth contact with Navajo Nation Division of Health Community Health Representatives or other Navajo Division staff members working in the communities, as well as the many community members working in IHS facilities or as part of the research team.

Within most service units, the prenatal clinics are staffed by midwives. The midwife programs are not centralized, but rather maintained independently within each service unit, and in some cases staffed in conjunction with family practice physicians. Obstetricians are on call and provide consultation or a higher level of care when complications are identified. The number of pregnant women who present at NAIHS facilities during the first trimester, however, appears to be low. Although one report suggests more than 80% of women has their initial visit in the 1st trimester (Benally, 2005), more recent indications are that the1st trimester number is a lower 61% (USDHHS, 2008). Because those not seeking care in the first trimester may be at greater risk, this factor is a potential bias that must be addressed in the recruitment strategy by outreach to increase enrollment and prenatal care or controlled in the statistical analysis. The number of prenatal visits varies dramatically, but more than one-half of pregnant women on Navajo are seen for NAIHS prenatal care more than nine times during their pregnancy. PHNs, interpreter/drivers in the PHN program, and CHRs are often requested to contact those who miss appointments in efforts to get them to the clinics. Generally, these interventions are done in cases where specific concerns have been noted, or after repeated “no-shows”. CHERSs will be working within the CHR administrative structure and therefore will be well integrated into that system. For women who do not appear for scheduled appointments, the CHERS can conduct home interviews to ensure regular data collection at critical study time intervals as well as coordinate with CHRs to get participants to clinic appointments. Another role of the outreach will be to increase awareness of the need to meet appointments in both the mind of the mom as well as her extended family and friend’s network.

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4. Test of Procedures or Methods to be Undertaken

Data will be analyzed using standard statistical software packages (R, SAS) by the UNM analytic team in collaboration with CDC's Geographic Research, Analysis and Services Program (GRASP) spatial analysts and Dr. Ettinger. The DiNEH Project modeling team has worked to develop analytically sound statistical models to incorporate multidimensional contributors to the observed outcomes. These models utilized geospatial methods and assessed appropriateness of Bayesian and determinist approaches, ensuring that all models are validated and utilize existing information to update assumptions and refine models as the datasets have grown in complexity and depth. Dr. Curtis Miller, a mathematician and statistician, and Glenn Stark, a PiBBS Fellow (Program for Interdisciplinary Biological and Biomedical Sciences), who has led this effort as part of his Ph.D. work have been included in this study team and will work closely with the GRASP team to establish appropriate geospatial models of the same rigor in analysis of these data. Dr. Ettinger has extensive training and experience in modeling health effects of environmental exposures and will be consulted on statistical analysis with the UNM analytic team.

Descriptive Statistics. Univariate and bivariate summary statistics and distributional plots will be examined for all variables to identify potential outliers and influential variables. Initial analyses will describe the distribution of the exposures and outcomes in the study sample. Outcomes that are not normally distributed will be appropriately transformed before analyzing their association with uranium exposure variables and other predictors. Bivariate correlations between variables will be calculated as appropriate. Nonparametric methods (e.g., Lowess) which make no assumptions about the functional form of the relationship between continuous variables, will be used to determine the interrelationships between the exposure variables (Schwartz, 1993), and will be used to test specific hypotheses about effects of predictor variables on outcomes, specifically in cases where transformations may not be effective to obtain normal distributions. Any potential outliers will be identified and verified for accuracy by study staff against the original data. When appropriate, regression diagnostics (such as use of residual plots and leverage plots to determine heteroscedasticity and curvilinearity), extreme studentized deviate (to detect outliers), Cook's D (to determine leverage of a particular covariate measurement on the model as a whole), and correlation matrices of the estimated coefficients (to check for co-linearity) will be examined. Regression analyses with these identified points set aside will also be considered as a sensitivity analysis to determine if a few values have substantial influence on the analysis.

Insuring data integrity. From our experience in modeling other data sets in Navajo Nation, we also anticipate the need to assess analytical problems presented by outliers, missing data, and failures of participants to understand questions. A variety of internal check routines will be set up to routinely check the data set for these potential problems and to ensure the most appropriate

solutions are applied prior to analysis. These may include simple subroutines to ensure the sum of years in prior and current addresses equals the age of the participant or mapping to ensure identified water sources are within driving radius of a residence. These may range, based on the situation, to imputation of data, re-contact of participants for clarification, dropping of specific participants from phases of the analyses where no statistically and scientifically justifiable resolution is available.

Covariate Selection. Potential confounding variables that may be associated with the exposures and outcomes will be identified from prior studies based on biological plausibility and will be included as covariates in multivariate models, as appropriate. We will use linear, logistic, or Cox proportional hazards regression, as appropriate, to examine bivariate associations between the key covariates and the outcomes as well as the key covariates and the exposure variables to assess potential for confounding. We will use the 10% "change-in- estimate" approach to identify statistically significant confounding variables. Some of the covariates will have strong correlations with each other and overly collinear variables will not be included jointly, to avoid producing unstable model results. If this is the case, the correlations will be noted in the reported analyses.

B.5. Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data

The following individuals are members of the study team and were consulted on statistical aspects of the design.

Name & Title	Contact information	Expertise	Study contribution
Robert Annett, Ph.D. Professor of Pediatrics Dept. of Pediatrics UNM School of Medicine	RAnnett@salud.unm.edu (505) 272-6854	Consultant on National Children's Study; pediatric neuropsychology	Survey instrument selection, analysis
Adrienne Ettinger, ScD, MPH Yale University, Assistant Professor,Department of Epidemiology & Public Health	Adrienne.ettinger@yale.edu (203) 785-6232	Designing, implementing epidemiologic birth cohort studies; participation in research design	Co-Investigator; Data analysis
Curtis Miller, Ph.D. UNM-CEHP Statistician and modeler	CMille02@salud.unm.edu (505) 272-4048	Statistics and modeling	Designed data collection; Data analysis
Candis Hunter, MSPH Agency for Toxic Substances and Disease Registry, Division of Health Studies	chunter@cdc.gov (770)488-1347	Environmental epidemiology	Power calculations and analysis

Janet Cragan, MD, MPH National Center on Birth Defects and Developmental Disabilities	jcragan@cdc.gov 404-498-3807	Child and Maternal Health	Power calculations and analysis
Myra Tucker, MPH National Ctr For Chronic Disease Prevention & Health Promotion Division Of Reproductive Health	mtucker@cdc.gov 770-488-6267	Reproductive and Maternal Health	Tribal health statistics, reproductive health outcomes