

Centers for Birth Defects Research and Prevention

The National Birth Defects Prevention Study Protocol

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Centers for Birth Defects Research and Prevention

The National Birth Defects Prevention Study Protocol

1. GENERAL DESCRIPTION AND OVERVIEW

Birth defects are the leading cause of infant mortality in the United States, accounting for 21% of all infant deaths in 1995. In addition, birth defects are the fifth leading cause of years of potential life lost and contribute substantially to childhood morbidity and long-term disability. Although several human teratogens have been identified, most birth defect cases have an unknown etiology. Surveillance systems can be used extensively to identify birth defects risk factors, as well as to identify unusual patterns of birth defect occurrences. However, because individual birth defects are relatively rare, it has been difficult in the past to conduct a study large enough to provide the necessary power to evaluate the causes of specific defects.

The Centers for Birth Defects Research and Prevention (CBDRP) is a collaborative effort between the CDC's National Center on Birth Defects and Developmental Disabilities (the CDC's Metropolitan Atlanta Congenital Defects Program) and nine birth defects surveillance registries across the United States (Arkansas, California, Iowa, Massachusetts, North Carolina, New Jersey, New York, Texas and Utah). This collaborative effort provides a unique and unprecedented opportunity to evaluate risk factors for birth defects. Major strengths include: 1) large population-based birth defects registries including populations with diverse environmental; 2) improved case definition (classifying birth defects into subgroups that are etiologically and pathogenetically more homogeneous) and specified criteria for case inclusion; 3) an interview instrument which was developed collaboratively and administered at all sites obtains information on relevant exposures and potential confounders; 4) large sample size which provides unprecedented power to evaluate potential risk factors for specific birth defects; and 5) the use of biologic markers for exposure and susceptibility.

1.A. Summary of the National Birth Defects Prevention Study

In 1998, Congress passed legislation that directed the Centers for Disease Control and Prevention (CDC) to establish the Centers for Birth Defects Research and Prevention (CBDRP). The Birth Defects Prevention Act of 1997 was originally introduced in 1992 and passed in 1998 (Attachment 1). Money was appropriated in 1996 for CDC to initiate some of the activities described in the bill, which included the funding of the CBDRP.

In 1997, cooperative agreements for \$800 thousand dollars per year for a period of five years were awarded to seven states (Attachment 2) to establish the CBDRP and support their collaboration in activities aimed at the prevention of birth defects. Specifically, these awards were designed to: 1) bolster ongoing surveillance activities (including the integration of prenatal diagnoses into surveillance registries); 2) develop, implement, and evaluate local studies (including research, special services, and program evaluation); and 3) contribute 400 interviews per year (300 case interviews and 100 control interviews) to the National Birth Defects Prevention Study (NBDPS). For fiscal year 2004, each center received approximately \$900,000.

The NBDPS is a case-control study of birth defects risk factors and is based on the existing birth defects surveillance registries in the nine CBDRP. Interviews have been conducted with 24,263 women as of July 2006, including 17,676 mothers of infants with birth defects and 6,587 mothers of infants without birth defects. A competitive renewal process for additional 5-year cooperative agreements occurred in June of 2002. Two new Centers, North Carolina and Utah received funding as a result of this recompetition. The North Carolina and Utah Centers began data collection in the Fall of 2003. Data collection for the new Centers includes births occurring after December 31, 2002. Two Centers (New York and New Jersey) did not receive funding in June of 2002. New Jersey does not currently collect new data. The New York received full funding in September 2004 and began collecting new data.

1.B. History and Purpose of Birth Defects Risk Factor Surveillance

The Atlanta Birth Defects Risk Factor Surveillance Project (BDRFS), which was initiated in 1993, is a surveillance-based approach to evaluating risk factors for birth defects (CDC Protocol #1104, OMB #0920-0010). BDRFS was based on the Metropolitan Atlanta Congenital Defects Program (MACDP)(CDC Protocol #1955), which has been in existence since 1968. MACDP is a population-based, multiple source case ascertainment birth defects surveillance system for the five county metropolitan Atlanta area (Clayton, Cobb, Dekalb, Fulton, Gwinnett).

The Atlanta BDRFS studied selected MACDP case-infants and a random sample of control infants. The BDRFS protocol (CDC IRB #1104) which was in effect for births occurring between January 1, 1993 and early 1997, had three main components: 1) parental interviews; 2) improved birth defects classification; and 3) biologic specimens for use in evaluating biologic markers of exposure and susceptibility. Each year, maternal interviews were conducted with about 300 mothers of case-infants and 100 mothers of control-infants. In addition, biologic samples were requested on approximately half of these mother-infant pairs. (More than 400 biologic specimens from Atlanta BDRFS participants were banked).

The goals of the Atlanta BDRFS were to: 1) gain new information on causes of birth defects; 2) evaluate factors already suspected of influencing the occurrence of birth defects;

3) develop new surveillance methods; 4) maintain a biologic specimen bank that could be used in the future to generate and test hypotheses (evaluating biological markers of exposure and susceptibility risk factors for birth defects); and 5) develop and test methods in birth defects surveillance and research which could be exportable to other birth defects surveillance systems.

In 1993, CDC funded two five-year cooperative agreements with Iowa and California to conduct the BDRFS using their own surveillance systems. Among the three participating sites, 1,995 interviews were completed (1,213 case mothers and 782 control mothers). Several specific analyses have been published (e.g. obesity and birth defects, fertility treatment and craniosynostosis). Substantial experience was gained during the five year BDRFS effort. This experience provided a strong framework for the development of the NBDPS and the

development of this revised protocol, which includes births occurring after October 1, 1997. The BDRFS experience also provided experience for the oversight of NBDPS activities, the design of the computer assisted telephone interview, the development of tracking systems, and the development of a pooled relational database.

2. NBDPS INVESTIGATORS AND COLLABORATORS

2.A. CDC Investigators

2.A.1. Centers for Birth Defects Research and Prevention

This activity involves collaboration between the National Center on Birth Defects and Developmental Disabilities (NCBDDD) of the CDC, and the state-based CDRP. Jennita Reefhuis, PhD, Birth Defects Epidemiology Team, is the Project Officer for the CDRP and Ms. Tineka Yowe-Conley is the Study Coordinator. Dr. Reefhuis and Ms. Yowe-Conley are primarily responsible for the direction and administration of the CDRP. As project officer, Dr. Reefhuis is responsible for directing and providing technical assistance to the CDRP in the development of the NBDPS protocol, evaluating study conduct, and oversight of the individual cooperative agreements with the CDRP. Dr. Reefhuis is responsible for insuring that all IRB and OMB requirements are met. In addition, Dr. Reefhuis is the lead scientific consultant to the NBDPS. Dr. Reefhuis has responsibilities for providing technical assistance to the CDRP including study design, protocol development, data storage, and data management. As study coordinator, Ms. Yowe-Conley is responsible for the day-to-day management of the study and for coordinating activities among the Centers, and preparing and submitting all IRB, OMB and Certificate of Confidentiality applications.

Mr. Chris Cospers, Data Manager/Programmer, Mr. John Sims, Programmer, and Justin McCarthy, Programmer, are responsible for security, transfer and maintenance of NBDPS-

related data. They, also design, program, and implement custom applications to assist in the execution of the study. In addition, they support, instruct, and coordinate the data pooling efforts of CDC contractors and data managers from the nine CDBRP.

Mary Jenkins, PhD is responsible for coordinating the biologics component of the NBDPS. Dr. Jenkins is responsible for overseeing the collection, storing and analysis of biologic specimens for CDC and the NBDPS, including the submission of individual one page genetic research descriptions to the CDC Institutional Review Board (IRB) for review.

Stuart Shapria, MD is responsible for providing technical assistance related to case definition and birth defect classification and for clinical review of potential NBDPS participants.

2.A.2. CDC Site for the NBDPS

The CDC CDBRP in Atlanta, one of nine study sites, is an activity which involves the collaboration of NCBDDD and the Division of Environmental Health Laboratories at the National Center for Environmental Health. As Principal Investigator of the Atlanta NBDPS, Dr. Reefhuis is responsible for the study protocol, study conduct, interview instrument, and scientific aspects of the study design, data management, and analysis. In addition, Dr. Reefhuis is responsible for meeting human subjects requirements, supervising the activities of the CDC NBDPS staff, and collaborating with the other 8 Centers for Birth Defects Research and Prevention.

Dr. Mary Jenkins is responsible for the collection, storage and analysis of biologic specimens for CDC and the submission of individual one-page genetic research descriptions to the CDC IRB for review. She is responsible for coordinating the efforts between the NCBDDD and the Division of Environmental Health Laboratory Sciences. Dr. Shapria determines the eligibility of all of the cases included in the CDC NBDPS and collaborates with the clinicians at all of the CDBRP.

Dr. Jan Cragan and James Kucik have primary responsibility for MACDP and its related projects as well as contractual agreements with Battelle Centers for Public Health Research and Evaluation (Battelle/CPHRE). Ms. Carolyn Sullivan and TBA share responsibilities for record management and coordination of the Atlanta study, including case identification, and management of the study tracking system.

Dr. Peg Gallagher, Division of Environmental Health Laboratory Sciences, is the lead scientist responsible for assessment of the biologic specimens that are collected in the CDC NBDPS as well as for the Centralized Laboratory (biologic specimens from all Centers to be stored at the CASPIR facility). Ms. Cynthia Sturchio is responsible for the coordination activities of the Centralized Laboratory.

In addition to the above investigators, there may be a variety of other CDC investigators involved at any one time with this surveillance and research project. Some of these include:

i) The National Center on Birth Defects and Developmental Disabilities:

Sonja Rasmussen, M.D., M.A.

Adolfo Correa, M.D., Ph.D.

Suzanne Gilboa, Ph.D.

2. B. CBDRP Investigators

2.B.1. Arkansas

University of Arkansas for Medical Sciences
Arkansas Department of Health

Charlotte A. Hobbs, M.D., Ph.D.
Principal Investigator
Assistant Professor of Pediatrics
11219 Financial Center Parkway
Suite 250
Little Rock, AR 72227

2.B.2. California

March of Dimes/California Birth Defects Monitoring Program
California Department of Health Services

Gary Shaw, Dr., P.H.
Principal Investigator
March of Dimes/California Birth Defects Monitoring Program
California Department of Health Services
1917 Fifth Street
Berkeley, CA 94710

2.B.3. Iowa

Iowa Birth Defect Registry, University of Iowa

Paul Romitti, Ph.D
Principal Investigator
Dept. of Epidemiology/Iowa Birth Defects Registry
C21-E GH
200 Hawkins Dr
Iowa City, IA 52242

2.B.4. Massachusetts

Massachusetts Department of Public Health
Boston University Slone Epidemiology Unit
Brigham and Women's Hospital

Marlene Anderka, M.P.H.
Principal Investigator
Massachusetts Dept of Public Health
250 Washington Street
5th Floor
Boston, Massachusetts 02108-4619

2.B.5. North Carolina

University of North Carolina, Chapel Hill
North Carolina Department of Health and Human Services

Andy Olshan, PhD
Co-Principal Investigator
UNC School of Public Health
2103 McGavran-Greenberg Hall
CB#7435
Chapel Hill, NC 27599-7435

Bob Meyer, PhD
Co-Principal Investigator

North Carolina Birth Defects Monitoring Program
1908 Mail Service Center
Cotton Building
Raleigh, NC 27699-1908

2.B.6. New Jersey

New Jersey Department of Health and Senior Services

Marge Royle, PhD
Principal Investigator
Program Manager, Early Identification and Monitoring
New Jersey Department of Health
And Senior Services
P.O. Box 364
Trenton, New Jersey 08625-0364

2.B.7. New York

New York Department of Health

Charlotte Druschel, M.D.
Principal Investigator
New York Department of Health
2 University Place, Room 160
Albany, New York 12203-3313

2.B.8. Texas

Texas Birth Defects Monitoring Division,
Texas Department of Health

Mark Canfield, Ph.D.
Co-Principal Investigator
Texas Department of Health
1100 West 49th Street
Austin, Texas 78756

Peter Langlois, Ph.D.
Co-Principal Investigator
Texas Department of Health
1100 West 49th Street
Austin, Texas 78756

2.B.9. Utah

Utah Department of Health
University of Utah

Marcia Feldkamp, PA, MSPH
Principal Investigator
Utah Birth Defects Network
44 N. Medical Drive
P.O. Box 144697
Salt Lake City, UT 84114-4697

The principal investigators at each CBDRP work collaboratively with CDC scientists on scientific aspects of study design and analysis, including development of the study protocol, interview instrument design, and study conduct. In addition, they are responsible at their individual sites for: 1) meeting human subjects research and IRB requirements; 2) data storage and management; 3) clinical review of potential cases; 4) statistical aspects of study design and analysis; 5) collecting, processing, storing, and analyzing DNA from buccal swab specimens; and 6) coordinating a variety of laboratory components of the study. (This may include assessments of some biologic markers of exposure in selected participants, and/or the development and testing for possible environmental toxicants in biologic specimens.)

Collaborators at the Boston University Slone Epidemiology Unit have additional responsibilities, including provision of an up-to-date drug dictionary on a quarterly basis to be used for coding of reported prescription and nonprescription drug use.

2.C. Other Collaborators

In an effort to further understand birth defects risk factors, there may be a variety of additional investigators involved at any one time with this study. Such collaboration is essential to the success of this project because it allows scientists with differing expertise to work together, substantially improving the ability to better understand birth defects risk factors. Attachment 2 lists other researchers and project-related staff currently involved with the CBDRP.

In addition to the collaborators from the CBDRP, a number of individuals employed by Battelle/CPHRE will have important roles in this project, providing interview services for

several NBDPS sites, including Atlanta and New York. Battelle/CPHRE will have specific responsibilities, including: 1) maintaining the interview instrument; 2) tracing, contacting, and interviewing study subjects; 3) developing and updating study tracking systems; 4) coding of interview data; 5) providing monthly reports; 6) collecting biologic specimens; and 7) providing complete, clean, and edited data in a timely fashion.

Primary Battelle Memorial Institute/Survey Research Operations Personnel:

Diane Burkom, M.A.
Battelle/CPHRE
6115 Falls Road, Second Floor
Baltimore, Maryland 21209
(410) 377-5660

Alison Woomert, Ph.D.
Battelle/CPHRE
100 Capitola Drive, Suite 301
Durham, North Carolina 27713
(919) 544-3717

Charles Knott
Battelle/CPHRE
100 Capitola Drive, Suite 301
Durham, North Carolina 27713
(919) 544-3717

Cathy Murphy
Battelle/CPHRE
100 Capitola Drive, Suite 301
Durham, North Carolina 27713
(919) 544-3717

3. METHODS AND MATERIALS

3.A. Description of State-Based Birth Defects Surveillance

All of the nine CDBDRP have population-based birth defects surveillance systems (Arkansas, California, Georgia, Iowa, Massachusetts, North Carolina, New York, Texas and Utah) that have legislative authority to collect information on infants with major congenital

malformations. A description of the surveillance system in each state can be found in Attachment 3 (A copy of the document providing this authority to the CDC is included in Attachment 3A). Each program monitors all births occurring to residents in a defined geographic area having at least 35,000 births each year. These birth defects surveillance programs include information on live born and stillborn infants diagnosed with at least one major birth defect within the first year of life, with diagnoses ascertained up to 5 years of life. Similar methods of multiple source case ascertainment are used at each site; most cases are registered through regular visits to local hospitals by members of the CBDRP surveillance staff, where records such as log books and patient's charts in nurseries, maternity units, and pediatric wards are reviewed to obtain clinical information and basic demographic data. Cases are also identified from the records of local cytogenetic laboratories, prenatal diagnosis clinics, genetic clinics, and vital records. Certificates of live birth, infant death, and stillbirth are supplied by state health departments.

Data are abstracted at each CBDRP onto a case record, which has been designed to meet specific surveillance needs at the site. However, all CBDRP case records include the same basic demographic information, specific written diagnoses, 6-digit diagnostic codes, birth related information, cytogenetic data, complications of birth, prenatal data, pregnancy history, family history and other risk factor information.

The use of prenatal diagnosis for birth defects has become increasingly prevalent over the past decade. In many instances, because of a diagnosis during pregnancy of a serious birth defect or chromosomal abnormality, the pregnancy is electively terminated. For surveillance systems to have complete population-based ascertainment of birth defects, it is now necessary to obtain information from prenatal diagnosis clinics and on elective terminations of pregnancy. It is important to include prenatally diagnosed cases in any epidemiologic study of birth defects; without such inclusion criteria, an increasingly substantial number of case-infants will not be included in the study, which will likely make interpretation of study results very difficult. All CBDRP plan to include prenatally diagnosed cases, to the fullest extent possible.

3.B. The National Birth Defects Prevention Study Methods

The National Birth Defects Prevention Study (NBDPS) is a collaborative case-control study of birth defects risk factors, which is based on the existing birth defects surveillance registries of the nine CBDRP. The primary goal of this study is to improve the understanding of the causes of birth defects. It is anticipated that information obtained from this study is likely to be ultimately useful in the prevention of birth defects.

Using a computer assisted telephone interview (CATI), NBDPS interviews have been conducted with more than 24,263 women as of July 2006, including 17,676 mothers of infants with birth defects and 6,587 mothers of infants without birth defects. Because data (without personal identifiers) from all nine CBDRP is being pooled electronically for analysis (see sections 3.B.5. and 6.B), scientists within the CBDRP are committed to using a unified approach. It is critical to the overall success of the study to limit the introduction of site-specific differences, which may lead to biases, differential response rates, and other potential compromises to the overall quality of the data. To minimize data comparability problems, identical procedures and materials will be used to the fullest extent possible by all participating institutions. CBDRP scientists have been working closely together since the Fall of 1996 to design and implement the NBDPS using a standardized study protocol. A letter from the CDC original project officer (Mr. Edmonds) stating the importance of identical study procedures was sent to all CBDRP and included in individual human study subjects submissions (Attachment 4).

Committees were formed with representatives from each CBDRP to design the collaborative case-control study. The **standards committee** designed the protocol for the study including standard forms and procedures for identifying, contacting, and interviewing study participants. The **questionnaire and methods committee** revised the Birth Defects Risk Factor Surveillance (BDRFS) mother interview instrument and arranged to have the questionnaire placed into a CATI format. The **clinicians committee** (geneticists and clinicians from each CBDRP) decided on the case definitions for the 30 birth defects categories included in the study and developed guidelines to assist with case identification and review, medical record

abstraction, and coding. The **biologic committee** designed the protocol for the collection of biologic and environmental samples and developed a plan for banking and sharing the biologic specimens. The **data sharing committee** (Section 3.B.5.a) has the ongoing task of deciding how the data will be equitably shared for analysis. This committee is responsible for review of all protocols for data analysis as well as addressing human subjects issues, data access, collaboration, and authorship.

The efforts of these committees and the efforts of the CBDRP scientists are described in more detail on the following pages of the NBDPS Protocol. As further described in this document, each site proposes to use to the fullest extent possible, the following identical approaches:

- 1) case definition - (Sections 3.B.1. and 4.A., Attachments 5 and 6)
- 2) control selection - (Sections 3.B.2. and 4.B)
- 3) letters of introduction - (Sections 4.C.1 and 4.C.2, and Attachment 18)
- 4) tracing procedures - (Section 4.C. and Attachment 15)
- 5) contacting procedures - (Sections 4.C. and 4.D., Attachment 22 and 23)
- 6) telephone interview - (Section 3.B.3 and Attachment 8 and 22)
- 7) informed consent - (oral and written) (Section 5, Attachment 10 and 22)
- 8) collection, processing and storing of biologic samples -
(Sections 3.B.4. and 6.D., Attachments 9 and 25)
- 9) calculation of participation rates (Section 3.B.5.c., Attachment 14)
- 10) replication, editing and use of data (Sections 3.B.5, 6.B., and 6.C).

3.B.1. Case Definition

Clinicians at each CBDRP worked together to develop the NBDPS case definition. Infants are eligible for inclusion if they have one or more defects from the list of selected birth defects included in Attachment 5. In addition to selecting birth defects, which are of unknown or uncertain etiology, these defects were selected for one or more of the following reasons. The defect is:

- a) considered to be a major defect (affecting survival, requiring substantial medical care, or resulting in marked physiological or psychological impairment);
- b) usually identifiable in the first 6 weeks of life (may be extended for some defects); and
- c) consistently classifiable.

In addition, other criteria may be considered, including:

- a) the defect is either common (and thus of public health importance) or rare (and thus unlikely to be studied in smaller studies);
- b) the pathogenetic mechanism of the specific defect is similar to other included defects; or
- c) there are specific etiologic hypothesis(es) which require additional study.

Cases can be: 1) live born infants; 2) stillborn infants greater than 20 weeks gestational age or 500 grams; or 3) prenatally diagnosed and terminated fetuses at any gestational age or weight.

3.B.1.a. Clinical Review

Numerous studies have documented extensive etiologic heterogeneity in birth defect cases with similar anatomic problems. For example, neural tube defect cases that have no associated defects have different epidemiologic characteristics and familial recurrence risk patterns from cases that have other defects. To provide a sound epidemiologic framework to study specific defects, the presence of associated defects, and accurate clinical descriptions of defect types can and should be used in classifying birth defect cases into subgroups that are etiologically and pathogenetically homogeneous.

To accomplish this goal, clinical staff review the abstracted medical records of case-infants which are ascertained each year by the individual CBDRP to determine if that case-infant meets the specified case definition and inclusion criteria. The clinicians use a standard clinical review and classification protocol, which was developed over the past year by CBDRP clinicians

(Attachment 6) working closely together. To evaluate case eligibility, the clinicians use a system of communication (including a clinician's listserv, e-mail and fax) between CBDRP to have questions and issues rapidly resolved. Phone conferences and meetings are also scheduled as needed.

General inclusion and exclusion criteria include:

- 1) certain types of birth defects cases which have been ascertained solely through prenatal diagnosis will be included (including the method of diagnosis, as noted by clinical reviewer);
- 2) cardiac defects will be included if the diagnosis is based on echocardiography (at least);
- 3) cases with the following known etiology are excluded: chromosomal/micro deletion disorders and single gene disorders; and
- 4) cases with teratogenic syndromes and recognized phenotypes of unknown or uncertain etiology are included.

3.B.1.b. Clinical Database

A clinical database which has been developed by the collaborating clinicians and the Programmers/Data Managers (Attachment 7) provide additional detailed information to aid in classification of cases during analysis. The information contained in the clinical database is obtained through the abstraction of medical records. The database includes physical attributes of the infant (e.g. weight and head circumference), verbatim diagnoses (from medical records) of birth defects, exams used to determine diagnoses, relevant cytogenetic/molecular tests, family history information, and autopsy results, if available. The clinical database from each CBDRP are be compiled at CDC without personal identifiers and linked by study identification number (ID) to the interview database. The clinicians also review each case and classify the infant into appropriate categories for analysis. For example, infants may have one major defect (isolated case) or 2 or more major birth defects in different systems (multiple case).

3.B.2. Control Selection (See also Section 4.B.)

Control-infants from each CBDRP will be selected randomly either from vital records (birth certificates) or from hospitals of birth. Using birth certificates to identify controls is only an option in the few states where vital records are recorded electronically in a timely manner (generally within weeks of delivery). The CBDRP which use birth certificates for the selection of controls are:

- 1) Iowa: access to these certificates is obtained through a signed research agreement with the Iowa Dept of Public Health;
- 2) Massachusetts: access to these certificates is obtained through 24A/B and departmental IRB approval;
- 3) New Jersey: authority, through the Health Department, is to select controls from the Electronic Birth Certificate system.
- 4) Georgia: as an agent for the Georgia Dept. of Human Resources, the Centers for Disease Control and Prevention has legal authority for the collection of health information, as provided in Chapter 12 of the Official Code of Georgia (OCGA). With this authority, CDC routinely reviews medical records of births in the five-county metropolitan Atlanta area to determine if birth defects are present and to abstract information, as necessary to conduct the Metropolitan Atlanta Congenital Defects Program. In addition to having this authority, the protocol for selecting controls in this manner has been in place since the beginning of this study in 1993 (CDC protocol #1104). Original IRB approval of this method was granted on May 14, 1992 and the most recent approval of protocol #1104 was granted on April 6, 2006 (exp. May 17, 2007). Beginning with births in January 2000, we began selecting some controls from vital records, and beginning with 2001 births all controls have been selected from vital records.
- 5) North Carolina

6) Utah

The CBDRP which use hospital data for the selection of controls are:

- 1) New York: uses the CDC's control selection protocol to select controls from hospital birth records; the Health Department Commissioner or his/her agents has statutory authority to carry out studies;
- 2) California: has a legal mandate to obtain information on infants without malformations to serve as controls;
- 3) Arkansas: used the CDC's control selection protocol to select controls from hospital birth records from 1998-1999. The authority to do this was established through a legislative act in 1985. This legislation states that the purpose of Arkansas Reproductive Health Medical System (ARHMS) is to "collect and analyze data from a number of sources to describe trends in reproductive endpoints". All hospitals with patient records containing information pertaining to reproduction and development are required to share information in those records with the ARHMS. Beginning with births in January 2000, Arkansas began randomly selecting controls from birth certificates.
- 4) Texas: uses the CDC's control selection protocol to select controls from hospital birth records. Texas has a state law that allows them to get controls from hospitals.

In anticipation of a 70% participation rate (based on the experience from the BDRFS study), each CBDRP selects randomly from the population (from either vital records or hospital birth logs) approximately 150 eligible controls each year for inclusion in the study. A randomly selected birth is not eligible for inclusion in the study if the chosen infant:

- 1) is actually a case or has major birth defects ascertained within one of the CBDRP birth defect surveillance systems;
- 2) is not a resident of the geographic area covered by one of the CBDRP population-based registries at the time of delivery;
- 3) is adopted or in foster care;
- 4) has a deceased mother; or
- 5) is a stillborn.

Whether hospital records or birth certificates are used as the source for control-infants, the records are reviewed to insure the selected birth is not a case-infant and to abstract information for the purpose of follow up and contact. When birth certificates are used as a source for controls, only parental and physician contact information will be obtained.

3.B.3. Interview Instrument

Mothers of all case and control infants who agree to participate in the NBDPS are interviewed by telephone in a search for birth defects risk factors. This interview provides the framework for the NBDPS, providing critical information, which is used in all aspects of the study. Building on interviews of over 2,500 mothers of infants with and without birth defects from the original BDRFS study (OMB 0920-0010; expires 5/31/2009, extension application currently under review at OMB), a one-hour computer assisted telephone interview (CATI) was programmed for the NBDPS (Attachment 8).

In summary, the BDRFS telephone interview was modified by: 1) updating the instrument to evaluate possible new and emerging birth defects risk factors (e.g. new prescription and nonprescription drugs, diet); 2) rewording some questions to improve the quality of exposure information obtained; 3) deleting some questions and sections which proved to be less fruitful than originally expected; and 4) expanding other sections to provide necessary increased detail.

The NBDPS interview instrument contains sections on pregnancy history (including prenatal diagnosis), maternal conditions and illnesses, family history, lifestyle and behavioral

factors (including alcohol use and substance abuse), nutrition, multivitamin use, environmental exposures, and occupational history.

The interview instrument has undergone an evaluation in 2003, and a revised interview instrument was approved in 2005. The order of the questionnaire was changed to make the flow more comfortable for participants. We also added a few questions on maternal stress, dieting, diarrhea, and paternal smoking. We shortened the sections on drinking water, illicit drug use, occupation, and pregnancy to keep the total questionnaire length approximately the same. The revised CATI was implemented for births beginning January 1, 2006.

The primary language of the NBDPS interview instrument is English. However, the interview has been translated to Spanish, and 6% of interviews have been completed in Spanish. In addition, letters of correspondence and consent forms have been translated for Spanish speaking participants.

3.B.4. Biologic and Environmental Samples

3.B.4.a. Background

Although interview instruments are a major tool in the search for causes of birth defects, they have limitations for evaluating genetic susceptibilities to disease and limitations for evaluating certain exposures because of problems of recognition and recall. In a concentrated effort to improve our understanding of the etiology of birth defects, particularly in the area of gene-environment interactions, we are collecting biologic samples for use in the evaluation of biologic markers of exposure and susceptibility.

Collaborating CBDRP scientists are collecting cheek cell samples (buccal brushes) on all case-infants and control-infants, and their parents. The CBDRP researchers bank specimens collected as part of the NBDPS, storing the samples in a manner which will permit efficient retrieval and optimum stability for later use in studies related to birth defects etiology. (See Section 3.B.4.c.)

In general, use of banks for the storage of biologic specimens is becoming increasingly important for epidemiologic research for several reasons. Major expenditures in time and money are spent in sample collection. The ability to use banked specimens to test new hypotheses or to utilize new techniques is advantageous to the research efforts to understand the causes of birth defects. In addition, by maintaining biologic specimen banks, which have the capacity to allow for research tests as new hypotheses or improved technologies emerge, the potential contributions of study participants are maximized. This approach is the most reasonable, provided participants are informed of the intent to bank their specimens and the intent to use their specimens for such research studies.

The use of a biologic specimen bank that can be built over time and maintained indefinitely is particularly important for testing hypotheses regarding risk factors for birth defects. Serious birth defects occur in about three percent of all births; individual defects are much rarer (incidence of individual defects ranges from approximately 1 in 1000 live births to 1 in 10,000 live births). Because of the rarity of individual defects, many years of data collection are required to obtain enough cases of a specific type of birth defect to complete a particular etiologic study. The length of time between obtaining the biologic specimen and the availability of adequate numbers for a specific candidate gene analysis is likely to be a minimum of two to five years, and may well be much longer; the banking of biologic specimens, therefore, is a lengthy process.

Of note: In previous CDC IRB reviews of the Atlanta BDRFS, there has been much discussion surrounding issues related to genetics research. This protocol incorporates the approaches proposed by the NBDPS and approved by the CDC IRB in previous reviews of the Atlanta Birth Defects Risk Factor Surveillance Project (CDC Protocol #1104).

3.B.4.b. Collection and Use of Buccal Cells for NBDPS Participants

The collection of buccal cells provides a relatively simple, inexpensive, and convenient means of obtaining DNA samples for use in PCR-based evaluations of genetic differences at specific gene loci. Buccal cell samples are collected on a sterile brush by rotating it on the inner

cheek. Because the procedure is simple and noninvasive, participants can collect the samples, using materials sent to them at home, and return the samples by mail.

Scientists with the CBDRP collect cheek cell specimens from NBDPS participants to be used to analyze DNA markers. Each CBDRP collects cheek brushes from each case and control infant and their mother and father.

3.B.4.c. Banking of Biologic Samples

After the cheek cell samples have been collected, each CBDRP retains at their site, one brush. The other brush is sent to the CDC Centralized Laboratory for processing. Once processing is complete at the Centralized Laboratory, samples are sent to the CDC storage facility (CASPIR) where they will be stored and identified only by study ID number.

It is the expectation of scientists within the CBDRP that a portion of the DNA will be banked for very long-term research studies, perhaps even decades in the future, when the technologies available are likely to be able to make use of these in ways that can only be imagined now (perhaps, for example, by carrying out sequencing of the entire human genome in each individual sample). These samples will be stored indefinitely unless a request is received from the participant to destroy them.

CBDRP scientists plan to share aliquots of these samples, without personal identifiers, to carry out collaborative research studies, as approved by appropriate internal and external review of the proposed research. (Attachment 12). Quality control DNA studies have begun among the CBDRP and a protocol for how the samples are made available to CBDRP scientists has been developed. On Center has withdrawn samples from CASPIR and begun some genotyping. As previously mentioned, sharing of samples with collaborating investigators is done without personal identifiers, unless specific permission has been obtained from human subjects committees at the participating institutions. Only researchers within the CBDRP will have access to these materials. Investigators retain control of biological materials obtained at their CBDRP, unless the participant requests that these materials be destroyed (Attachment 10).

Of note: There is no commercial value in these samples and profits from any materials associated with this study are not expected. The samples will not be used for commercial purposes. Neither researchers nor study participants will receive profits from the donated materials.

3.B.4.d. Evaluation of Genetic Susceptibilities to Birth Defects

An extremely important use of the NBDPS biologic specimen bank relates to the evaluation of genetic susceptibilities to birth defects using the "candidate gene" approach. Candidate genes are genes which are thought to play a role in the normal embryology and pathophysiology of different organ systems (e.g. growth factor genes, steroid receptors, and homeobox genes); genetic variation in some candidate genes may be involved in the pathogenesis of birth defects.

Increasing numbers of genes are being mapped and sequenced, and with the use of polymerase chain reaction (PCR), it is becoming increasingly possible to evaluate the role of genetic differences at specific gene loci and their interaction with specific exposures in the etiology of birth defects. Molecular DNA technology is moving at a tremendously fast pace, and more potential genes and genetic markers are available almost daily. Therefore, it is difficult, if not impossible, to know which new genes or genetic markers will be available for study even in the very near future, or which of the available genes would be selected in our research efforts to better understand birth defects risk factors; individual research tests may or may not be performed on specific samples.

For each of the major classes of congenital anomalies, a number of candidate genes warrant further research. Because of the known association between periconceptional folate supplementation and prevention of neural tube defects, genes involved in folate metabolism, such as the methylenetetrahydrofolate reductase (MTHFR) and methionine synthase genes, as well as the alpha and beta folate receptors, will be studied. Since micro deletion of the chromosome 22q region has been identified in some cardiac defects, microdeletion studies using microsatellite markers from this region are of interest in the study of congenital heart defects. Studies on clefting conditions might include a number of candidate genes thought to potentially

play a role in the causality of cleft palate, including transforming growth factor alpha (TGFA), transforming growth factor beta 3 (TGFb3), retinoic acid receptor (RARA), the proto-oncogene BCL3, and the thyroid hormone receptor alpha 1 (THRA1). For congenital anomalies involving the eyes, genes recently identified such as crystallin (associated with cataracts), the PAX6 gene (associated with aniridia) and the PITX2 gene (involved with Rieger syndrome, a multi system condition which includes iris abnormality as a feature) might be studied. Limb abnormalities could be studied with the HOX-D13 and several T-box transcription factor genes that have been associated with limb abnormalities. These examples emphasize the broad range of different genes, which may be included in our research study to identify genes, which may confer an increased susceptibility to a particular birth defect.

In addition, a number of disorders have been recognized as being associated with very specific gene mutations, and although not a primary goal of this project, research studies of these could make significant contributions to the range of mutations seen in those disorders. Examples include sonic hedgehog, associated with holoprosencephaly; the craniosynostosis syndromes associated with mutations in FGFR1, FGFR2 and FGFR3; achondroplasia and the FGFR3 mutation; Stickler syndrome and mutations in COL2A1 and likely others. In addition to these evaluations, several other genetic factors have already become established in existing birth defect literature as focal points of biomarker research. Other candidate genes that could be explored include the GSTM1-null mutation and variants of other carcinogen-metabolizing genes such as cytochrome P450 (CYP) mixed function oxidases (e.g. CYP 1A1, 1A2, 2E1).

It is apparent that the number of potential genes of interest is quite long and differs depending on the specific birth defect to be studied. In addition, many of the genes or genetic markers of potential interest have yet to be identified. Because it is not possible at this time to specify all genes to be studied in our quest to better understand birth defects, we plan to approach the human subjects committee with individual one-page research plans for each gene or genetic marker to be studied in our specimens. A brief proposal (one to two pages) providing information on the specific gene/gene marker to be studied will be submitted (Attachment 11);

the proposal will include justification for its study, potential clinical relevance of the information, and plans to deal with clinically significant findings (if any). A list of approved proposed genes for study can be found in Attachment 12.

3.B.4.e. Biologic Markers of Exposure to Environmental Teratogens

In addition to maintaining a biologic specimen bank for the purposes of conducting research to evaluate candidate genes, it is important to maintain a specimen bank to allow better laboratory identification of possible teratogenic exposures, as new techniques become available.

Biologic markers of exposure are useful in reducing the effects of both differential and nondifferential exposure misclassification in epidemiologic studies. Differential misclassification occurs when the ability to recall exposure events varies among subgroups, a classic problem in the epidemiologic study of birth defects. Such misclassification, which is primarily due to recall bias, can lead to spurious associations between putative exposures and birth defects.

Nondifferential misclassification occurs when the proxy for exposure is imperfect but uniform across subgroups (case and control subjects). Because misclassification due to poor proxy measures (e.g., job titles) can jeopardize the epidemiologist's ability to detect true underlying risks, such nondifferential misclassification may play a substantial role in limiting the progress towards understanding birth defects etiology. In addition, biomarkers of exposure can be useful in quantifying exposure levels, which is an important epidemiologic tool when assessing dose-response relationships.

Because cheek cell samples are designed for the analysis of DNA only and not well suited for the evaluation of environmental exposures, other biologic samples would be required for this purpose. Although not included at this time in the general NBDPS Protocol, CBDRP scientists are evaluating and considering the use of other biologic samples such as blood, urine or saliva, to be used in evaluating environmental factors and gene-environment interactions.

3.B.4.f. Environmental Sampling

Some CBDRP investigators plan to use environmental sampling to quantify residential exposures, as appropriate and feasible. As an example, levels of DBP in tap water and in biologic samples (blood and urine) were evaluated in a small subgroup of these participants as a local study (1). Researchers in metropolitan Atlanta are currently evaluating the potential association between DBP in drinking water and birth defects by linking existing water source and treatment data obtained from water utilities in metropolitan Atlanta with the Metropolitan Atlanta Congenital Defects Program.

Because laboratory refinements in exposure assessment are occurring rapidly, having adequately collected and stored biologic samples for future evaluation is important. An example of how new laboratory techniques will be helpful in the study of birth defects, is the use of a high-resolution magnetic sector spectrometer to evaluate individual volatile organic chemicals in blood. Using this instrument, scientists in EHLS have recently developed a unique analytical method that enables the determination of parts per trillion levels of 31 volatile organic chemicals (including DBP) in 10 mL of blood. This technique has been used in one local study (2).

3.B.4.g. Centralized Geocoding of NBDPS Residence Data

During the interview portion of the study, information is collected on residence addresses from three months before conception to date of index birth. Geocoding this information (i.e. assigning geographic coordinates) will be extremely valuable for studies of environmental health as well as other topics. For example, geocoding would allow studies of distance to points such as factories, toxic waste dumps, nuclear power plants, and health care facilities. It would also allow studies that require assigning residences to areas such as aquifers, geological regions (e.g. high radon areas), areas of water treatment utilities, plumes from pollution sources, census tracts and their variety of socioeconomic variables. The surveillance systems of some of the Centers are already geocoding case-infants as part of their routine surveillance of the patterns of birth defects occurrence.

The NBDPS plans to conduct geocoding for all interviewed cases and controls from all Centers for Birth Defects Research and Prevention, and some Centers have already begun this process on their local data. However, we plan to centralize the geocoding for all Centers to improve the consistency of coding across centers, and ensure that all data from all centers can be coded. Centralized geocoding will result in:

1. **Increased consistency and quality control:** Although there are several automated techniques for geocoding, it is expected that their accuracy will vary within and between Centers (e.g. accuracy is highest in urban areas). Also, a certain percentage of addresses for each Center will require interactive (manual) geocoding; one group doing that will maximize consistency across Centers.
2. **No delays in coding data from a particular center due to lack of resources:** While all Centers have the capability to geocode their NBDPS data (in-house or through contract), five Centers have stated they will need additional funding to implement it.

Current IRB approval for each Center states that in order to protect confidentiality of subjects, no identifying information will leave the Center as part of routine NBDPS data collection. Thus the residential history data are not included in the monthly CATI replication to CDC. Some Centers have stated that even if data are geocoded centrally, all the geocoded and original address data must be returned to them. After the centralized geocoding is complete, all geocoded data will be returned to the center of origin; a centralized repository of the geocodes will NOT be maintained at CDC.

The Geospatial Research, Analysis, and Services Program of ATSDR has offered to geocode all NBDPS residence data from all Centers, at no cost to the NBDPS. This group, external to the NBDPS, does not need any information about the NBDPS participants besides the actual address. They will only know that at that address a child was born in the past 7 years, and that the mother participated in the NBDPS interview.

Objective

The purpose of this project is to complete centralized geocoding while maintaining subject confidentiality.

Proposed Procedures for Geocoding

Each Center will obtain any necessary IRB approval from their own institutions.

1. Each Center obtains residence data from their data manager for all cases and controls with estimated dates of delivery from the start of the study (births after 10/1/1997) up to the designated cutoff date for each data batch . For example, the first data batch will include study IDs with a date of delivery of 10/1/1997 through 12/31/2003. This includes all maternal residential addresses from 3 months before conception through date of delivery.
2. Each Center locally cleans the data (make sure spelling is correct for cities, etc.).
3. Each Center replaces the NBDPS identification number with a new ID number (per instructions, which will be independent of year of birth or case/control status), and must save the key relating the two numbers.

4. Each Center sends the cleaned residence history data without the original NBDPS identification number to a CDC/NBDPS contact person.
5. The CDC/NBDPS contact person batches the data from all 10 Centers and sends to the CDC/ATSDR geocoders.
6. The CDC geocoders complete the geocoding and return the data with the geocodes to the CDC/NBDPS contact person.
7. The CDC/NBDPS contact person splits the data by Center and sends data to each appropriate Center.
8. Each Center links back to the NBDPS identifier.
9. No data will be retained by the NBDPS team or the ATSDR team.

For each specific project, exposure assessment may be done locally according to precise instructions or may be sent to a central location for more consistent results. If the latter, the project will be sent for approval to the CDC IRB and the local center IRB. New ID number will again be used in order to protect confidentiality and to blind the exposure assessment personnel regarding case/control status.

Protection of Human Subjects

Geocoding staff would have access to address data of NBDPS subjects, but be unable to link with NBDPS data to determine case/control status or any other information. They will sign and be bound by the NBDPS confidentiality and data use oath.

3.B.4.h. Evaluation of Gene-Environment Interactions

Individual susceptibility (biomarkers of susceptibility) to the effects of environmental agents may vary depending on specific genetic or other host factors (e.g., nutrition or immune function). If the effect of an exposure on the occurrence of birth defects depends on the interaction between

the exposure and genetic susceptibility, then neglecting to study such interaction may lead to underestimating the magnitude of the association between the exposure and the outcome.

As previously mentioned (Section 3.B.4.d.), it is increasingly possible to evaluate the role of genetic differences at specific gene loci and their interaction with specific exposures in the etiology of birth defects. Candidate genes that could be explored, include the GSTM1-null mutation and variants of other carcinogen-metabolizing genes such as cytochrome P450 mixed function oxidases. Together, these genetic variants of enzymes involved in detoxification reactions may play a significant role in the metabolism of DBP in tap water and other environmental exposures. Since evidence suggests that these genetic variants may modify susceptibility to a range of adverse health effects, analyzing polymorphisms within these genes should be studied to evaluate their possible link with birth defects. One example is the previously mentioned MTHFR gene, in which evidence suggests that genetic polymorphisms within MTHFR can combine with environmental exposures to place a particular subpopulation at greater risk for having children with neural tube defects.

3.B.4.i. Future Access to Genetic Information

We intend to inform study participants of general study progress and research findings, as studies are completed. For this purpose, a roster of participants is being maintained and updated periodically. The first participant newsletter was mailed to participants in December 2000. Additional newsletters that describe the status and completed work of studies using the NBDPS are mailed to past participants on approximately a yearly basis.

We originally did not intend to provide participants with individual study results. However, we now plan to allow subjects to request their own results, if desired, for any clinically significant tests. The consent form has been revised to clarify this as follows: studies that will be done on the collected biologic samples are not meant to test medical status. Since all studies

will be done in research labs, there is no plan to notify participants of study results. Research labs do not have the same quality control standards as clinical labs. Research labs may also use less expensive techniques, which can make the tests less reliable than those from a clinical lab. However, a few of these studies may have clinical importance. For any tests that have clinical importance, summarized results will be published in a peer-reviewed journal and also the study newsletter. After the summarized results are published, participants will be able to request individual test results that may be of clinical importance. Participants are also advised to contact their healthcare professional if they have any questions about whether or not genetic tests could be useful.

If NCBDDD receives a request for the results of individual genetic tests carried out in the NBDPS, we will comply with the Privacy Act and respond in the following way:

1. Most of the cheek cell samples will be stored for future use. If someone requests genetic test results and we have not done anything with their sample yet, we will inform them that their sample has not yet been included in any studies of clinical significance. We will reiterate what was included in their written consent about the cheek cells being used for future research and that results from these studies are not meant to test individual medical status, and that only results from tests that have clinical significance can be reported. We will tell them that if they are concerned about genetic factors that may be associated with birth defects, we suggest that they discuss this with their health care provider. If they do not have a health care provider, we may be able to refer them to a qualified physician or counselor in their area.
2. If someone requests genetic test results and we have done studies of clinical significance using their sample, we will either have a clinical geneticist call them back or send them a letter telling them that based on the testing that was done on their cheek sample it appears that they do or do not carry a specific genetic marker. We

can tell them the name and location of the genetic marker and some basic information in lay terms. We will explain to them the limitations of the testing that was done (as described above) and offer to assist them in locating a clinical geneticist or genetic counselor if needed. Each CBDRP site will have a clinical geneticist available to answer questions. We will explain to the participant that if they are concerned about genetic factors that may be associated with birth defects, regardless of the results, we suggest that they discuss this with their health care provider, and will help them locate a provider if needed.

We have developed a fact sheet that can be sent to anyone requesting information on the genetic testing done as part of the NBDPS. The fact sheet explains the nature of the testing that will be done on the cheek cells, the limitations of the technology being used; the fact that the results so far have no clinical implications at this time, and that the research is being conducted to generate hypotheses for future study.

3.B.5. Analytic Approach

Using the diagnostic information included in the clinical database, individual defects will be categorized into appropriately homogeneous groups, including the use of isolated and multiple defects. Analysis of risks from a given exposure will be carried out within broad categories, such as all vascular disruption defects and be narrowed to a given defect such as gastroschisis.

Because controls are population-based and randomly selected, all controls can be utilized for any of the subgroup analyses which involve interview information. Additionally, other cases can be compared to the case group of interest in certain analyses, when appropriate.

In some cases, analysis will be hypothesis driven (e.g. the further evaluation of a previously described association between fever and neural tube defects) and in other cases, analysis will be conducted in the search for new risk factors for individual defects (e.g. the evaluation of associations between specific birth defects and newly available prescription drugs). Univariate

analysis will be used to look for individual risk factors for specific defects. Multivariate logistic regression will be the major analytic tool used to evaluate confounding and look for best-fit models to explain the observed outcomes.

An important analytic tool will be to look for evidence of gene-environment interaction in the analysis. Genetic information will be obtained using DNA-based polymorphisms; individuals will be classified according to the presence or absence of specific susceptibility alleles, as well as whether they have those alleles in single (heterozygotes) or double dose (homozygotes). Evidence for interaction will be sought in logistic regression modeling using specific interaction terms.

3.B.5.a. Sharing Data

In early discussions among the CBDRP principal investigators, it was decided that the data should be compiled, edited, and coded centrally to ease the difficulties associated with combining data during analysis. It was agreed that CDC was the best place to accomplish this with the assistance of a data manager whose funding comes from all the collaborating CBDRP. The data managers, while located at CDC, assist all CBDRP with the tasks related to combining study data (see Section 6).

The Data Sharing Committee has two representatives from each CBDRP. Each CBDRP has two votes. The committee has established guidelines for access to the compiled interview and biologic data and is responsible for ensuring that the data is shared equitably among the CBDRP. Any researcher interested in using the pooled data for analysis submits a letter of intent and later a more detailed proposal to the committee for review. The committee considers the scientific merit of the proposals and encourages collaboration among the researchers where possible. The committee has also established guidelines for authorship, acknowledgments, and other issues related to the publication of studies using the collective data (Attachment 13A). The committee will also insure that all proposed research complies with human subjects requirements. Additional IRB review will be required for: 1) any research involving

collaborators outside the CDC/CBDRP group; or 2) any studies which fall outside of the scope of the current protocol.

3.B.5.b. Sample Size and Power

A birth defect is a structural abnormality present at birth. Most, but not all, are included within the range 740.0 to 759.9 of the International Classification of Diseases Ninth Revision (ICD-9). Birth defects, as a group, are relatively common, occurring in 3-5% of all births. Individual birth defects, however, are relatively rare. Conditions within this category include a heterogenous group of outcomes with differing morphogenesis and they cannot be appropriately evaluated as a group. In the past, it has often been difficult to conduct epidemiologic studies because of the relatively small numbers of specific birth defects. Pooling data from the nine CBDRP maximizes sample size and provides unprecedented power to evaluate potential risk factors for specific birth defects (Attachment 14).

3.B.5.c. Study Participation Rates

Calculation of participation rates is needed to monitor and evaluate the study progress and interpret study outcome. The targeted participation rate is 75% for both cases and controls. So that rates are comparable across sites, it is important for participation rates to be calculated at each CBDRP using the same methods. Eligibility and participation rates are calculated using the definitions included in Attachment 14. Participation rates are calculated for several subcategories of study participants: cases, controls, specific birth defect groups, and by CBDRP.

Participation rates are frequently monitored throughout the study. Specific reasons for not participating are noted (e.g. mother speaks neither English or Spanish, the mother is deceased). Because of the ongoing nature of the NBDPS, calculation of the overall participation rate at any point in time results in artificially low rates. This is because, while the denominator (identified cases and controls) may be timely, the numerator may lag because some eligible participants will

still be in the tracing and contact phase of the study and they have yet to be interviewed. For this reason, additional rates will be calculated to enable us to best estimate how the study is progressing. Since we are aiming to complete maternal interviews within 6 months of the infant's birth, participation rates which are calculated for a period ending 6 months prior should be expected to be more complete. We also complete participation rates for completed birth periods birth periods meaning infants born \geq 24 months ago since all interviews must be completed by 24 months post EDD. The combination of running monthly participation rates (which will be artificially low) and the rates for completed years give both a long and a short view of the study progress.

4. PARTICIPANTS

Births occurring on or after October 1, 1997 are eligible for inclusion in the NBDPS in all CBDRP except Arkansas, New Jersey, North Carolina and Utah. (The starting date for Arkansas and New Jersey is January 1, 1998 and the starting date for North Carolina and Utah is January 1, 2003. As described in Subpart D of 45 CFR Part 46, this project fulfills the requirements for investigations involving children in that it involves only minimal risk and presents an opportunity to understand and prevent a serious problem affecting the health and welfare of children.

4.A. NBDPS Case Selection

As previously described in Sections 3.A., each of the CBDRP programs conducts population-based birth defects surveillance, monitoring all births occurring to residents in a defined geographic area having at least 30,000 births each year. Following a clinical case review, using specified inclusion and exclusion criteria (Section 3.B.1, Attachment 6) case-infants are selected from among the population of infants ascertained within the individual CBDRP surveillance programs (Attachment 3). Infants who have been diagnosed with at least one of the selected defects (Attachment 5) are eligible for inclusion in the NBDPS. Approximately 300 eligible case-infants will be enrolled in the NBDPS study each year at each Center.

4.B. Selection of Control-Infants

Each of the nine CBDRP conducts interviews with approximately 100 mothers of control-infants every year. As described in Section 3.B.2., controls are randomly selected from among all births occurring in the defined geographic area of each CBDRP, using one of two methods. Researchers in Iowa, Georgia, Massachusetts and New Jersey, select their controls randomly from electronic birth certificate files; Arkansas, California, New York and Texas use hospital data to select controls using a stratified random sample, weighted by the number of births occurring in each of the birth hospitals in that area. Using a random numbers generating program, a list of potential controls is generated, stratified by hospital and month of delivery, with over sampling to allow for about 70% participation rates.

Regardless of which method is used, each birth has the same probability of being selected from within each geographic area (from among the approximately 35,000 to 75,000 yearly births occurring to residents of that specific geographic area). Once a birth has been randomly selected, identifying information is abstracted, including names of the infant, mother and father, address, and date of birth.

4.C. Procedures for Tracing and Contacting Participants

The investigators (and their contractors) have in place an extensive procedure for tracing individuals (Attachment 15) to insure that lost-to-follow-up rates are minimized as much as possible. Once potential participants have been located, one of two initial contact procedures may be used, depending on the CBDRP. Two different procedures exist, either because of specific state legislation or because of specific constraints placed on individual CBDRP by their respective IRBs. Some CBDRP (Iowa, New York, and Massachusetts) first contact the physician of record, prior to contacting the patient. (See Section 4.C.1.) Others (Arkansas, California, Georgia, North Carolina, New Jersey, Texas and Utah) contact the patient directly without contacting their physician. (See Section 4.C.2.)

4.C.1. Initial Contact - Physician

For the states with physician contact as a first step, physicians are sent a letter with return receipt requested. The letters inform them of the plan to interview one or more their patients and ask if there is any reason their patient(s) should not be contacted (example - Attachment 16). If a reply is not received within 21 days, contact is initiated with the mother (through Battelle/CPHRE) by an introductory letter (Section 4.C.2., Attachment 18) which explains the purpose of the study and requests their participation.

Occasionally, CBDRP researchers find that not enough specific information from the medical records is available to determine whether or not the birth defect meets the CBDRP case definition and inclusion criteria. When this situation occurs, a letter is sent to the appropriate physician (according to hospital records) asking for assistance (Attachment 17). The letter is followed with a phone call to determine if the physician has additional information on the specific diagnosis, which they are willing to share with us. Once all available classification information has been obtained, if the infant meets the CBDRP case definition, enrollment in the study is initiated.

4.C.2. Initial Contact - Prospective Participant

Letters of introduction (Attachment 18) are mailed to participants. Initial letters differ, depending on whether the participant is the parent of a case infant or a control infant, and whether there is a known pregnancy termination, fetal death, or infant death. Included with the letter of introduction is a \$20 money order, a fact sheet on rights of human subjects (Attachment 18A), a calendar (Attachment 19), food frequency list (Attachment 20), and a study information pamphlet (Attachment 21).

4.C.3. NBDPS Pamphlet

The study pamphlet (Attachment 21) which serves several purposes was designed by members of the CBDRP standards committee and revised in 2003. The pamphlet addresses a number of IRB issues raised by several states and provides general information to participants, physicians and other interested parties. The pamphlet is included with the letter of introduction to potential NBDPS participants, because more information is provided in the pamphlet than might

be reasonably included in an introductory letter. The pamphlet includes detailed study information, answers to anticipated questions, and addresses anticipated concerns.

4.D. Telephone Contact - Study Participants

Ten days after the initial letters of introduction have been sent, a follow-up telephone call is made by specially trained interviewers at each CBDRP site to: 1) explain the study; 2) obtain oral informed consent; and 3) set up a convenient time for the conduct of the telephone interview. The NBDPS CATI Interviewer Instructions Manual is included in Attachment 22. A complete description of telephone scripts is included in Attachment 23.

Following the introduction, the interviewer establishes with the participant the expected date of delivery (EDD) and an estimated date of conception (DOC). If the respondent knows the due date, then the CATI instrument automatically calculates the DOC and which pregnancy months correspond with the calendar months. If the mother does not know the due date, the interviewer will use the previously determined EDD, which was abstracted from medical records or calculated, as necessary following the protocol for determining the estimated date of conception (Attachment 24).

Once the calendar is established, the interview is conducted (Attachment 8), following the question-by-question interviewer instructions (Attachment 22). Interviews are targeted for completion within 6 months of delivery, with a maximum time to interview of 24 months after EDD (or delivery for full term infants). No interviews will be conducted in the first 6 weeks after EDD (or delivery for full term infants). If pregnancies have been electively terminated, interviews will be delayed until six weeks after the EDD, to avoid routinely obtaining interview information earlier for such cases, resulting in potential bias.

For some CBDRP sites, when telephone interviews are not possible (e.g. the participant does not have a phone or has been difficult to locate through routine tracing), in-person interviews may be conducted.

4.D.1. Letter of Thanks

Immediately following completion of the interview, subjects are sent the interview thank you letter and a copy of the participant newsletter (Attachment 27).

4.E. Request for Cheek Cell Samples

After the interview thank you letter is sent, a buccal (cheek) cell collection kit is sent to the mother to take a sample on her child (if living) and both parents. The collection kits include a letter describing the buccal collection, informed consent forms, simple instructions, a \$20 incentive, materials for completing the specimen collection, and prepaid U.S. mail packets for specimen return. If after several weeks the completed buccal kit is not returned, the interviewers will call the mother to see if she has any questions about how to complete the kit. If there is still no response, a final letter is sent to the mother encouraging her to complete the kit and stating that if we don't receive the kit 2 weeks hence, we will assume she is not interested in participating.

4.F. Incentives for Cheek Cell Samples

CBDRP sends participants \$20 with the cheek cell sample (Attachment 26).

4.G. Incentive for Completion of the Entire Study

Following the completion of study participation, a letter of thanks is sent to the mother (Attachment 27). Mothers who complete both the interview and the cheek cell sample kit are sent an additional \$20 money order along with the letter of thanks and the newsletter. This third incentive is being offered at three Centers (New York, Atlanta and North Carolina). It may be expanded to all sites at a later date. All Centers will send a thank you letter following receipt of the buccals, even if they aren't offering the third incentive.

4.G.i. Recollection of buccal cell samples that fail laboratory quality control analyses (Approved 6/3/2004)

The Centralized Laboratory located at CDC performs quality control analyses of NBDPS buccal cell brushes. The majority of samples pass the quality control analyses but occasionally the laboratory is unable to obtain reliable information from some samples. This may be due to a

variety of reasons, such as too little genetic material or contamination of the sample during transit or processing.

On June 3, 2004 we received approval to request additional samples from subjects whose buccal cell samples do not pass these quality control analyses. We approach the subject initially by phone and then by a follow up letter. The following documents were added to the NBDPS protocol.

- Buccal Re-Collect Phone Script (Attachment 25D)
- Buccal Re-Collect Letter (Attachment 25E)
- Buccal Re-Collect Thank You Letter (Attachment 25F)

Currently, NBDPS subjects who receive the original buccal cell packet are compensated with a \$20 money order for collecting cheek cells from the mother, father and baby. Additionally, at three Centers (CDC, NY and NC), subjects receive a second \$20 money order once their samples are received at the local Center. We follow this same scheme when issuing the re-collection packets (i.e. a total of two \$20 money orders will be given to subjects who complete the re-sample process).

4.G.1.i. Dry Brush Recollection Pilot Study

Collaborators in Iowa and Georgia will be asking to recollect buccal brushes from families that previously submitted buccal cells using a method that did not allow cytobrushes to dry during transport, referred to as the “wet” brush method. The kits used for the pilot study will contain cytobrushes that are allowed to dry during transport, referred to as the “dry” brush method. Previous studies show that brushes allowed to dry during transport result in better quality and quantity DNA (the NBDPS switched from “wet” brush collection to “dry” brushes in August of 2003). In addition, collaborators in

Georgia will request samples from those families who participated very early in the study (1997-1998) and were never asked to collect buccal cells.

Targeted subjects have birth years between 1997 and 2001. The case families to be included have children with spina bifida or longitudinal limb defects. Inclusion criteria for both cases and controls are that the child is still living, they previously contributed a buccal sample (except the GA families from 1997 and 1998), and they have not been asked to resubmit buccal samples previously (In June of 2004 the NPBDS received approval to request additional “dry” brush samples from subjects whose buccal cell samples do not pass quality control analyses). There will be a 1:1 ratio of cases: controls that is proportional to the total number of eligible cases per birth year.

There are 180 total families that will be asked for a dry brush recollects (pending confirmation of live status by comparison with the National Death Index). The subjects include: 82 from Georgia: 41 controls and 41 cases (30 spina bifida including 12 who never contributed buccals; 11 longitudinal limb defects including 2 who never contributed buccals) and 98 from Iowa: 49 controls and 49 cases (36 spina bifida; 13 longitudinal limb defects).

4.G.3. Biologics Focus Groups

We have received CDC IRB approval to conduct focus groups to assess the barriers to participation in the collection of biological specimens by mothers on themselves, their infants, and young children (Attachment 33). Those collaborating on this project are interested in conducting multiple well-designed focus group discussions to assess the attitudes of both mothers who participate and mothers who do not participate in the collection of biological specimens.

The goal is to increase the effectiveness of studies that currently collect biological specimens from infants and their families but with less than optimal response rates, such as the National Birth Defects Prevention Study (NBDPS), and studies that are working to implement the use of biological specimens, such as the Pregnancy Risk Assessment Monitoring System (PRAMS).

4.H. Benefits and Risks to Subjects

Some subjects may be uncomfortable responding to some of the questions (e.g. substance and alcohol use). Respondents (once having agreed to participate) are reminded that they maintain the option of not answering any individual question. It is our experience, however, that mothers of newborns are enthusiastic about participating in studies which elucidate the causes of birth defects. In addition, the interviewer will communicate to participants that we do not know whether there is a link between birth defects and some of the health behaviors and exposures, which are the subject of this research.

The procedure of cheek cell sampling causes little to no discomfort and has a minimal possibility of infection. Risks associated with the genetic research conducted on these samples are minimal because the anticipated genetic research conducted within NBDPS is not, in general, of a sensitive nature. The research performed will relate to highly polymorphic genetic variants. In addition, the risks of disclosure have been minimized through the records handling precautions (Section 6) and the removal of personal identifiers from interviews and biologic specimens.

There are no other immediate risks or benefits to the study subjects. It is our expectation that science and society in general will benefit if we are better able to understand the causes of birth defects; this information may lead to improved intervention and prevention strategies for birth defects.

5. INFORMED CONSENT PROCEDURES

The investigators believe that these surveillance and research activities present no more than minimal risk, and thus are consistent with regulations concerning the protection of human subjects.

5.A. Oral Informed Consent for Interview

For the interview, no written informed consent will be obtained. Oral consent to an interview is obtained prior to conducting the interview. (See page 2, telephone script, Attachment 23.) Any questions a woman may have about the study are answered, and verification of the study may be obtained, if necessary, from the principal investigator at each CBDRP site.

5.B. Written Informed Consent for Genetic Research

Written informed consent is obtained for each participant that agrees to provide cheek cell samples. The standard version (written for Metropolitan Atlanta) is included in Attachment 10. The written informed consent document has been subjected to a number of reading level evaluations; it is at approximately the ninth grade reading level. It includes the following information: the purpose of the study, the procedures, risks and benefits, information on confidentiality, costs, compensation, the participant's right to refuse or withdraw, control, ownership, and commercial value of biologic materials, and phone numbers for questions about the research and about their rights as a human subject.

The written informed consent document informs participants that CBDRP scientists intend to conduct genetic research within the CDC laboratory and/or laboratories within the Centers for Birth Defects Research and Prevention which is directly related to birth defects research. The consent form explicitly states:

“These samples will be used to study genes that may play a role in why some babies have birth defects. They will only be used to study birth defects and for no other purpose.”

Occasionally mothers return cheek cells samples without the signed informed consent form. These mothers are then sent another consent form with a request for the appropriate signatures. If, after repeated attempts, the mother does not return the signed consent form, and can no longer

be contacted, her family's cheek cells will be included in the study. By returning the cheek cell samples these mothers have in fact given implied consent.

In addition, the informed consent documents (both written and oral) include information regarding the Certificate of Confidentiality and a description of the circumstances under which study information will be shared (Attachments 10, 23, 28).

6. RECORDS MANAGEMENT

6.A. Medical Records Data

The birth defects case records, which include identifying information, are stored permanently in locked file cabinets at each CBDPR site. Information is entered electronically and all data files are password-protected. Access to data files that include personal identifiers (such as names, addresses, telephone numbers, Social Security numbers, and hospital chart numbers) are restricted to staff members who have a need to work with the data and who know the password and data set name. Analytic data are accessible to collaborating scientists who have signed the Confidentiality and Data Use Oath (Attachment 13A, last two pages).

At each site, all data stored on disks are protected by a computer security system that limits access to designated staff that has a legitimate need to access this information because of their official duties involving records processing (updating, correcting, and changing records). These safeguards conform to the privacy act system of records number 09-20-0136, published on page 37718 of the Federal Register on September 25, 1984.

Note: For the Atlanta NBDPS, the MACDP and the original BDRFS are covered by a federal Assurance of Confidentiality (308(d)), which requires all employees, contractors, and students to sign annual confidentiality pledges. All data collected within MACDP, including that used by the Birth Defects Risk Factor Surveillance Project are covered by this Assurance. The coverage extends to all historical data and will extend to all future data. Because of the nature of the collaborative effort, a Certificate of Confidentiality has been obtained for the sites participating in the NBDPS (Section 7; Attachment 28).

6.B. Interview Data

The interviews are administered using a custom Microsoft Access database created by the Boston University Slone Epidemiology Unit and Battelle/CPHRE. The CATI database contains all of the screens to guide the interviewer through the entire interview while collecting the data directly into the database. Personal identifiers that are part of the case interview are stored in a separate database from the interview data. The interview database contains all of the interview's coded responses and is linked to personally identified information only by a nine-digit study identification code. The interview database is configured for replication, a process that will allow the transfer of information to the CDC where the interviews will be collected and maintained centrally. (See Section 3.B.5.a.)

The CDC has dedicated a server to the NBDPS, which receives incoming interview data from the individual CBDRP sites. This server is located in the secure NCBDDD IRM office. The NBDPS Server runs Windows 2000 Advanced Server and has password protected access. The resources of the NBDPS Server are only accessible locally at the server. At the CDC, Chris Cosper and Dr. Honein, the CBDRP Co-Project Officer, are currently the only users with local access to the NBDPS Server. Data coordinators at all CBDRP sites send data monthly via the Secure Data Network (SDN).

The process by which individual CBDRP will update information to the NBDPS Server is known as replication, a process that allows multiple copies of the same database to be synchronized so the resulting two databases will be identical. Each CBDRP site keeps only its own data, and CDC maintains a repository of the combined data from all sites.

At monthly intervals, the individual states will use the NBDPS replication tool to transfer their data to the central database via the SDN.

6.C. Clinical Data

The clinical database at each CBDRP contains characteristics of each case and control infant but does not contain personal identifiers. Access to the database is limited to those clinicians and researchers who have direct responsibility for maintaining the database. All employees, contractors and collaborators at any of the CBDRP sites with access have signed pledges of confidentiality. We also track the analytic database to ensure all data is returned at the conclusion of the analysis.

The clinical database will follow the same replication and security process as the interview instrument database (Section 6.B.) It will be linked to the interview database only by the nine-digit study identification number.

6.D. Biologic Specimen Data

Biologic samples obtained as part of this project are stored in a secure manner without identifiers (with the exception of study identification number) in appropriate storage facilities at the individual CBDRP and the CDC central biologic specimen repository, as described in Section 3.B.4.c. Banking of Biologic Specimens. A tracking system has been developed at CDC to record the location and history of biologic specimen collection for this study and it includes specific information on the use of individual study specimens. Each CBDRP uses this biological tracking system and again the data is replicated to CDC where the compiled database is maintained.

The NBDPS Server is backed up weekly and taken offsite periodically by the NCBDDD IRM team. Although these data are only identifiable by a nine-digit identification number, it is still maintained with appropriate security measures. The data extracts transferred to CDC do not include names, addresses or other personal identifiers.

7. CERTIFICATE OF CONFIDENTIALITY

As previously mentioned, a Certificate of Confidentiality has been obtained (Attachment 28) for the CBDRP and the NBDPS. All CBDRP have cooperative agreements with CDC and have individual IRB approval to conduct the NBDPS in their geographic area. All have provided letters of support for the Certificate of Confidentiality (Attachment 29).

Investigators within the CBDRP are not considered outside investigators. It is understood, however, that before outside collaborators (other than CBDRP scientists or their affiliates) are permitted access to personally identifiable data from the NBDPS, special permission must be sought from participants and permission must be granted for any such specified use.

Potential participants are given the following information about the Certificate of Confidentiality:

All information that we gather in this study will be kept private. This is assured under Section 301(d) of the Public Health Service Act (42 U.S.C. 241(d)). The Certificate of Confidentiality prevents study staff from being forced under a court order or other legal action to identify you or anyone else in this study. Records may be reviewed by officials checking on the quality of the research. This protection lasts forever (even after death) for any persons who were subjects in the research during any time the certificate was in effect. Information about you may be shared with other participating sites and other researchers when and if research review committees have approved it. The shared data will not contain any information that could identify any individual.

8. INTERNAL AND EXTERNAL REVIEWS AND APPROVALS

Documents supporting internal and external reviews and approvals are included in Attachment 30. All CBDRP sites have received approval by their individual IRBs. The original CDC IRB approval for the Atlanta BDRFS was granted on May 14, 1992, with the original authorization to give an Assurance of Confidentiality granted on May 8, 1992. The most recent CDC IRB approval for the Atlanta NBDPS was granted on February 17, 2006 with an expiration

date of February 17, 2007. The Certificates of Confidentiality for the NBDPS were awarded to the original CBD RP sites on August 2, 1999 (Attachment 30) and will expire August 31, 2009.

References

1. Miles AM, Singer PC, Ashley DL, Lynberg MC, Mendola P, Langlois PH, Nuckols JR. Comparison of trihalomethanes in tap water and blood. *Environ Sci Technol*. 2002 Apr 15;36(8):1692-8.
2. Lynberg M, Nuckols JR, Langlois P, Ashley D, Singer P, Mendola P, Wilkes C, Krapfl H, Miles E, Speight V, Lin B, Small L, Miles A, Bonin M, Zeitz P, Tatkod A, Henry J, Forrester MB. Assessing exposure to disinfection by-products in women of reproductive age living in Corpus Christi, Texas, and Cobb county, Georgia: descriptive results and methods. *Environ Health Perspect*. 2001 Jun;109(6):597-604.