



**Centers for Birth Defects Research and Prevention
Birth Defects Study To Evaluate Pregnancy exposures (BD-STEPS)**

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Centers for Birth Defects Research and Prevention (CBDRP)

BD Study To Evaluate Pregnancy exposures Protocol

1. GENERAL DESCRIPTION AND OVERVIEW

Birth defects are the leading cause of infant mortality in the United States, accounting for 20% of all infant deaths in 2007 (1). In addition, birth defects contribute substantially to childhood morbidity and long-term disability. Although several human teratogens have been identified, for most infants with birth defects, the etiology is unknown. Surveillance systems can be used to identify some birth defects risk factors for which information is included in the surveillance system, as well as to identify unusual patterns of birth defect occurrences. A number of case control studies of birth defects have been conducted, but because individual birth defects are relatively rare, it has been difficult to conduct a study large enough to provide the necessary power to evaluate risk factors for specific defects.

The Centers for Birth Defects Research and Prevention (CBDRP) is a collaborative effort between the U.S. Centers for Disease Control and Prevention's (CDC's) National Center on Birth Defects and Developmental Disabilities (NCBDDD) and a number of other Centers located within universities or public health departments across the United States with access to data from birth defects surveillance registries. This collaborative effort provides a unique and unprecedented opportunity to evaluate risk factors for individual birth defects. Major strengths include: 1) large population-based birth defects surveillance systems including diverse populations and diverse environments; 2) detailed, standardized case definition (classifying

birth defects into subgroups that are as etiologically and pathogenetically homogeneous as possible) and specified criteria for case inclusion; 3) an interview instrument that is uniformly administered allows collection of information on relevant exposures and potential confounders; 4) large sample size that provides unprecedented power to evaluate potential risk factors for specific birth defects; and 5) the use of biologic markers for exposure and genetic susceptibility. For births from October 1997 through December 2011, the CDBRP collaborated on the National Birth Defects Prevention Study (NBDPS), a case control study of risk factors for birth defects (2). Beginning with births in January 2014, the CDBRP will collaborate on the BD Study To Evaluate Pregnancy exposureS (BD-STEPS), which will extend and expand upon the previous study.

1.A. Executive Summary

The purpose of the BD Study To Evaluate Pregnancy exposureS (BD-STEPS) is to identify modifiable maternal exposures in early pregnancy that may increase the risk for having a pregnancy affected by certain major, structural birth defects. BD-STEPS builds upon experience from previous collaborative projects of the CDBRP, namely the Birth Defects Risk Factor Study (3, 4) and the National Birth Defects Prevention Study (2, 5, 6). Collaborators in the BD-STEPS study sites will use data from existing population-based birth defects surveillance systems to identify children with at least one of 17 major birth defects. Mothers of these case infants and mothers of randomly selected live born control infants will be contacted and invited to participate in a maternal interview covering multiple topics, with a focus on the key areas of: (1) diabetes, obesity, and physical activity; (2) other chronic maternal medical conditions; (3) infertility; and (4) medication use. During the maternal telephone interview, consent will be requested to access certain medical records for purposes of future validation research. After

the interview, mothers will receive saliva collection kits, and consent will be requested for residual dried blood spots from newborn screening. Targeted follow-up on-line questionnaire modules will be used to obtain more detailed information on specific exposures.

BD-STEPS will start data collection for infants with a date of birth on or after January 1, 2014 in each of the defined study regions.

1.B. History of CDC-Sponsored Birth Defects Case-Control Studies

The Atlanta Birth Defects Risk Factor Surveillance Project (BDRFS), which was initiated in 1993, was a surveillance-based approach to evaluating risk factors for birth defects (CDC Protocol #1104, OMB #0920-0010). BDRFS was conducted on the foundation of 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 metropolitan Atlanta area (7). 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 and substantial experience was gained during the five year BDRFS effort (4, 8).

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

Cooperative agreements for a period of five years were awarded to five states in 1997 (California, Iowa, Massachusetts, New York, and Texas) to establish the CBDRP and support

their collaboration in activities aimed at the prevention of birth defects. Data collection included births occurring on or after October 1, 1997. Two additional sites were funded in 1998 (Arkansas and New Jersey); data collection at these sites began January 1, 1998. 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, linkage to 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). A competitive renewal process for additional five-year cooperative agreements occurred in June of 2002. Two new Centers, North Carolina and Utah, received funding as a result of this re-competition. The North Carolina and Utah Centers began data collection in the fall of 2003. Data collection for the new Centers included births occurring after December 31, 2002. Two Centers (New York and New Jersey) did not receive continued funding in June of 2002. New Jersey did not resume new data collection; the New York Center received full funding again in September 2004 and began collecting new data. Another competitive renewal process for an additional 5 years occurred in December 2008; five centers were funded in December 2008 (Arkansas, California, Massachusetts, North Carolina, and Utah), and 3 centers (Iowa, New York and Texas) were funded in June 2009 after a 6 month break in funding. Data collection for the NBDPS was halted for infants with estimated dates of delivery on or after January 1, 2012, and all interviewing will be halted on March 31, 2013. NBDPS interviews have been conducted with 42,552 women as of August 2012, including 31,027 mothers of infants with birth defects and 11,525 mothers of infants without birth defects.

1.C. Certificate of Confidentiality

The CBDRP has had a Certificate of Confidentiality for the NBDPS since 1999. A Certificate of Confidentiality will be obtained for the CBDRP for the BD-STEPS collaboration. The Certificate specifies that only investigators who are part of the CBDRP collaboration may access individual level data from NBDPS and BD-STEPS that includes Personally Identifiable Information or PII (e.g. date of birth of mother and baby).

Potential participants will be given the following information about the Certificate of Confidentiality (or something very similar):

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. However, you should understand that the investigators are not prevented from reporting information obtained from you to authorities in order to prevent serious harm to yourself or others. Saliva samples will be stored without your names, but are linkable. Information about you may be shared with other participating sites and other researchers when and if it has been approved by research review. The shared data will not contain any information that could identify any individual.

As an example, the Certificates of Confidentiality for the NBDPS were awarded to the original CBDRP on August 2, 1999 (Attachment 2) and will expire on January 31, 2014.

1.D. Institutional Review

The original CDC Institutional Review Board (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 January 12, 2012 with an expiration date of January 29, 2013. All CDRP that collaborate on BD-STEPS have cooperative agreements with CDC (with the exception of the CDC site for the CDRP) and all local IRBs will defer to a central study IRB held by the CDC IRB, consistent with current recommendations (9) unless prohibited by state law. Once grantees have been identified, documents supporting internal and external reviews and approvals will be included as an attachment to the final protocol.

2. INVESTIGATORS AND COLLABORATORS

2.A. *CDC Investigators*

2.A.1. **CDC's Centers for Birth Defects Research and Prevention (CBDRP)**

This activity involves collaboration between the National Center on Birth Defects and Developmental Disabilities (NCBDDD) of the CDC, and the state-based CBDRP. Jennita Reefhuis, PhD, Birth Defects Epidemiology Team, is the Project Officer for the CBDRP and Kimberly Newsome, MPH, BSN, is the Study Coordinator. Dr. Reefhuis and Ms. Newsome are primarily responsible for the direction and administration of the CBDRP. As project officer, Dr. Reefhuis is responsible for directing and providing technical assistance to the CBDRP in developing the BD-STEPS protocol, evaluating study conduct, and overseeing the individual cooperative agreements with the CBDRP. Dr. Reefhuis is responsible for insuring that all IRB and OMB requirements are met. In addition, Dr. Reefhuis is the lead scientific consultant to BD-STEPS. Dr. Reefhuis has responsibility for providing technical assistance to the CBDRP including study design, protocol development, data storage, and data management. As study coordinator, Ms. Newsome is responsible for the day-to-day management of the study, coordination of activities among the Centers, and preparation and submission of all IRB, OMB and Certificate of Confidentiality applications. Data Manager/Programmer, Chris Cospers and a contracted programmer are responsible for security, transfer and maintenance of BD-STEPS-related databases. 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 CBDRP.

Mary Jenkins, PhD is responsible for coordinating the biologics component of BD-STEPS. Dr. Jenkins is responsible for overseeing the collection, storage and analysis of biologic specimens for CDC local BD-STEPS and all study sites of BD-STEPS.

Stuart Shapira, MD is responsible for providing technical assistance related to case definition and birth defect classification and for clinical review of potential BD-STEPS participants.

Margaret Gallagher, PhD in the Division for Laboratory Sciences (DLS) at the National Center for Environmental Health (NCEH), is the lead scientist responsible for the Central Laboratory, receipt and storage of saliva kits, DNA extraction, and quality assurance. Shannon O'Brien is responsible for the coordination of activities of the Central Laboratory.

2.A.2. CDC Site for BD-STEPS

The CDC site for the CBDRP in Atlanta is a collaboration between NCBDDD and the DLS at NCEH. As Principal Investigator of the Atlanta BD-STEPS site, Sarah Tinker, PhD is responsible for the study protocol, study conduct, interview instrument, and scientific aspects of the study design, data management, and analysis. In addition, Dr. Tinker is responsible for meeting human subjects' requirements, supervising the activities of the CDC BD-STEPS staff, and collaborating with the other CBDRP.

Dr. Mary Jenkins is responsible for the collection, storage and analysis of biologic specimens for CDC site for the CBDRP. She is responsible for coordinating the efforts between the NCBDDD and the DLS at the NCEH. Drs. Stuart Shapira and Fernando Arena, MD, PhD determine the eligibility of all of the cases included in the CDC site of BD-STEPS and collaborate with the clinicians at all of the CBDRP.

Dr. Jan Cragan, MD, MPH has primary responsibility for MACDP and its related projects. The local Data Manager and Local Study Manager share responsibilities for record management and coordination of the CDC BD-STEPS study, including case identification and data entry.

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:

Elizabeth Ailes, PhD

Cheryl Broussard, PhD

Amanda Burke, MPH

Tiffany Colarusso, MD

Krista Crider, PhD

April Dawson, MPH

Suzanne Gilboa, PhD

Margaret A. Honein, PhD, MPH

Jodi Jackson, PhD

Richard Olney, MD

Cora Peterson, PhD

Kara Polen, MPH

Hilda Razzaghi, PhD

2.B. External CDRP Investigators

The principal investigators at each of the CDRP 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 for: 1) meeting human subjects research and IRB requirements; 2) data storage and management; 3) providing clinical review of potential cases; 4) tracking and tracing of study subjects and mailing study materials; 5) statistical aspects of study design and analysis, and 6) sample storage, management, and analysis for BD-STEPS samples requested from the biorepository.

2.B.1. TBD

After the grants are awarded Principal Investigators and other key staff at each of the BD-STEPS sites will be described in this section.

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.

In addition to the collaborators from the CDRP, a number of individuals employed by the interviewing contractor will have important roles in this project as they will be conducting interviews of all study subjects. This contracted organization will have specific responsibilities, including: 1) maintaining the interview instrument; 2) interviewing study subjects; 3) ensuring a functional link between STEPS-TRACK and any of their call center electronic tracking systems; 4) providing monthly reports on the number of interviews and other metrics; 5) making follow-up

and reminder calls to study subjects for collection of biologic specimens; and 6) providing complete, clean, and edited data in a timely fashion. The interviewing contractor will also provide the coding of interview data.

Primary Personnel: TBD.

2.D. BD-STEPS Coordinating Council

The Principal Investigators from each of the CBDRP, including the CDC site, will comprise the BD-STEPS Coordinating Council. The Coordinating Council is the decision-making body for BD-STEPS. They will meet monthly by conference call and at least once per year in person.

2.E. BD-STEPS Data Sharing Committee

Each of the CBDRP will provide two members for the Data Sharing Committee. This committee will review letters of intent, proposals, abstracts, and manuscripts that involve previously unpublished data collected by BD-STEPS. The data covered by these guidelines include all interview, clinical, and biologic data associated with this study.

3. SUBJECT IDENTIFICATION AND RECRUITMENT

3.A. Case Identification

3.A.1. Birth Defects Surveillance

All of the CDRP have population-based birth defects surveillance systems 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 CDC is included in Attachment 4). Each program monitors all births occurring to residents in a defined geographic area having between 30,000 and 80,000 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 2 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 CDRP surveillance staff, where records such as log books and patients' 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 of the CDRP onto a local surveillance case record, which has been designed to meet specific surveillance needs at the site. However, all BD-STEPS case records include the same basic demographic information, specific written diagnoses, six-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 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 CDBDRP plan to include prenatally diagnosed cases, to the fullest extent possible.

3.A.2. Case Definition

Infants are eligible for inclusion if they have one or more defects from the proposed list of 17 birth defects included in Attachment 5. In addition to selecting birth defects with unknown or uncertain etiology, these defects were selected for 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 six weeks of life (may be extended for some defects); and
- c) Consistently ascertainable and classifiable.

In addition, other criteria may be considered, including:

- a) The defect is common (and thus of public health importance);
- 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.A.3. Eligibility Clinical Review

Clinical staff at each of the CBDRP will review the abstracted medical records of case-infants which are ascertained by that 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 that is based on the NBDPS clinical criteria (6). To evaluate case eligibility, the clinicians use a system of case-notes in the clinical database shared between the CBDRP, which allows questions and issues to be rapidly resolved. Phone conferences and meetings for the clinicians involved in the study will be scheduled as needed.

Clinical 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 will be excluded: chromosomal/micro-deletion disorders and single gene disorders; and
- 4) Cases with teratogenic syndromes and recognized phenotypes of unknown or uncertain etiology will be included.

3.B. Control Identification

Control-infants from each of the CBDRP will be selected randomly from live-born infants without a major birth defect, identified either from vital records (birth certificates) or from hospitals of birth, and represent the birth population from which the case infants were identified. Using birth certificates to identify controls is only an option in states where vital records are recorded electronically in a timely manner (generally within weeks of delivery).

1. Georgia: as an agent for the Georgia Department of Human Resources, CDC 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 a three-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 BDRFS in 1993 (CDC protocol #1104). Original IRB approval of this method was granted on May 14, 1992 and the most recent approval of protocol #2087 was granted on March 2, 2009 (exp. January 29, 2013). All BD-STEPS controls will be selected from vital records as long as access remains timely.
2. For each of the included sites a description will be given on how they select controls.

In anticipation of a 70% participation rate (based on the experience from the NBDPS), each of the CBDRP will select randomly from the population (from either vital records or hospital birth logs) approximately 75 eligible controls each year for inclusion in the study.

Whether hospital records or birth certificates are used as the source for control-infants, the records are reviewed to ensure that, given the available information, the selected control-infant does not have a birth defect. Records are also reviewed to abstract information for the purpose of follow up and contact.

3.C. Exclusion Criteria for Cases and Controls

There are certain criteria for both case and control mothers that result in ineligibility to participate in BD-STEPS. Case and control infants are not eligible if:

- 1) The mother is not a resident of the geographic area covered by one of the CDRP population-based registries at the time of delivery;
- 2) The mother is deceased;
- 3) The mother is incarcerated;
- 4) The mother participated previously in the NBDPS or BD-STEPS;
- 5) The mother cannot complete the interview in either English or Spanish;
- 6) The mother is younger than 15 (or younger than 18 if required by law); or
- 7) The infant is adopted or in foster care.

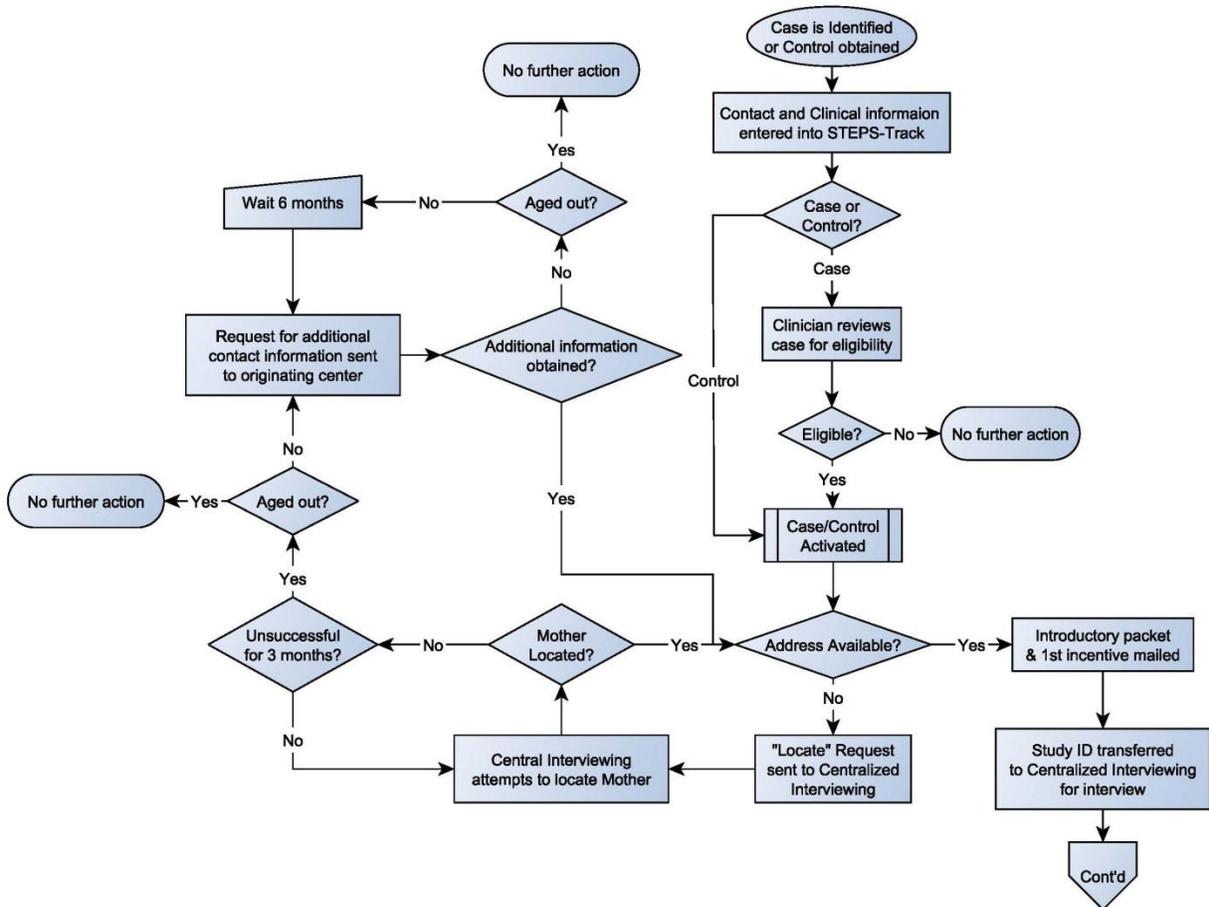
3.D. Tracking Database (STEPS-TRACK)

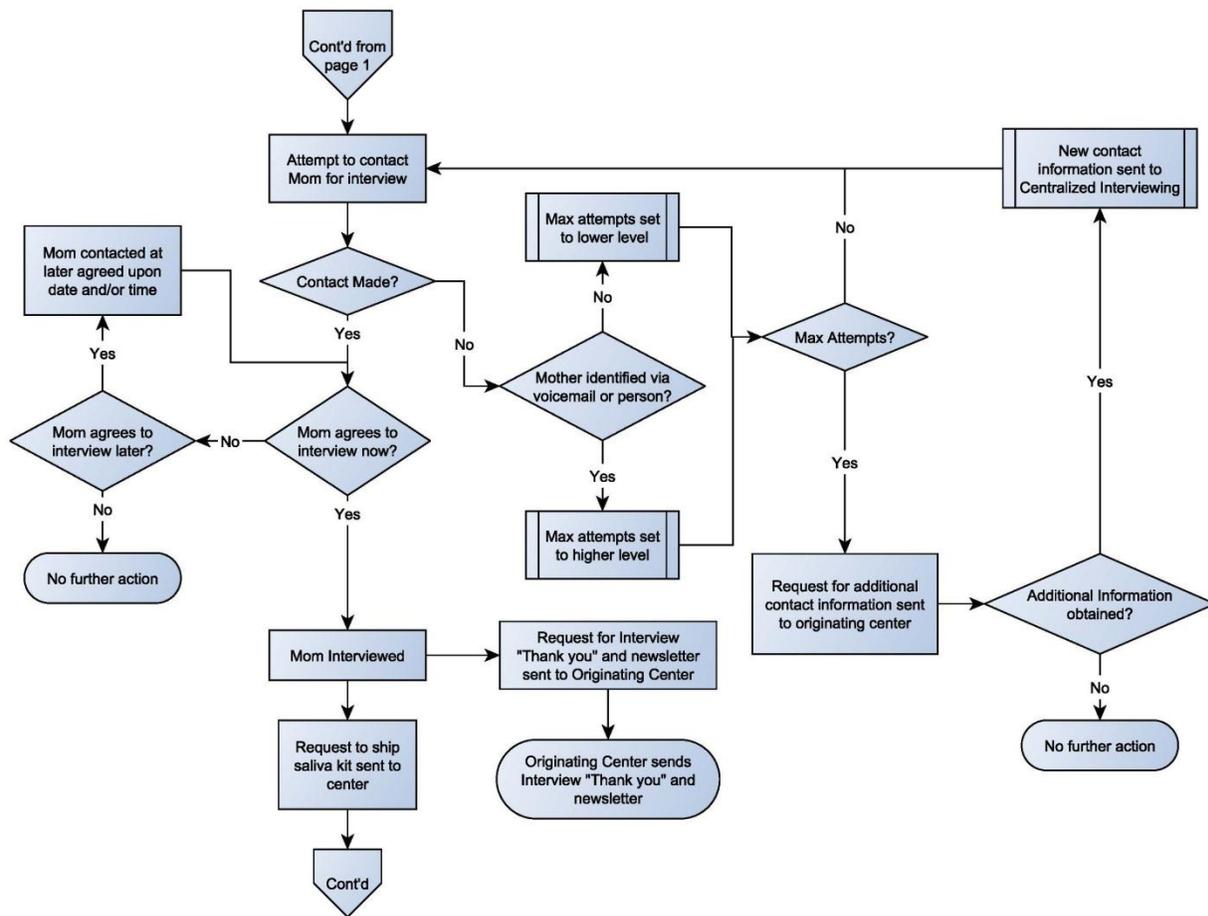
3.D.1. Data Flow Chart

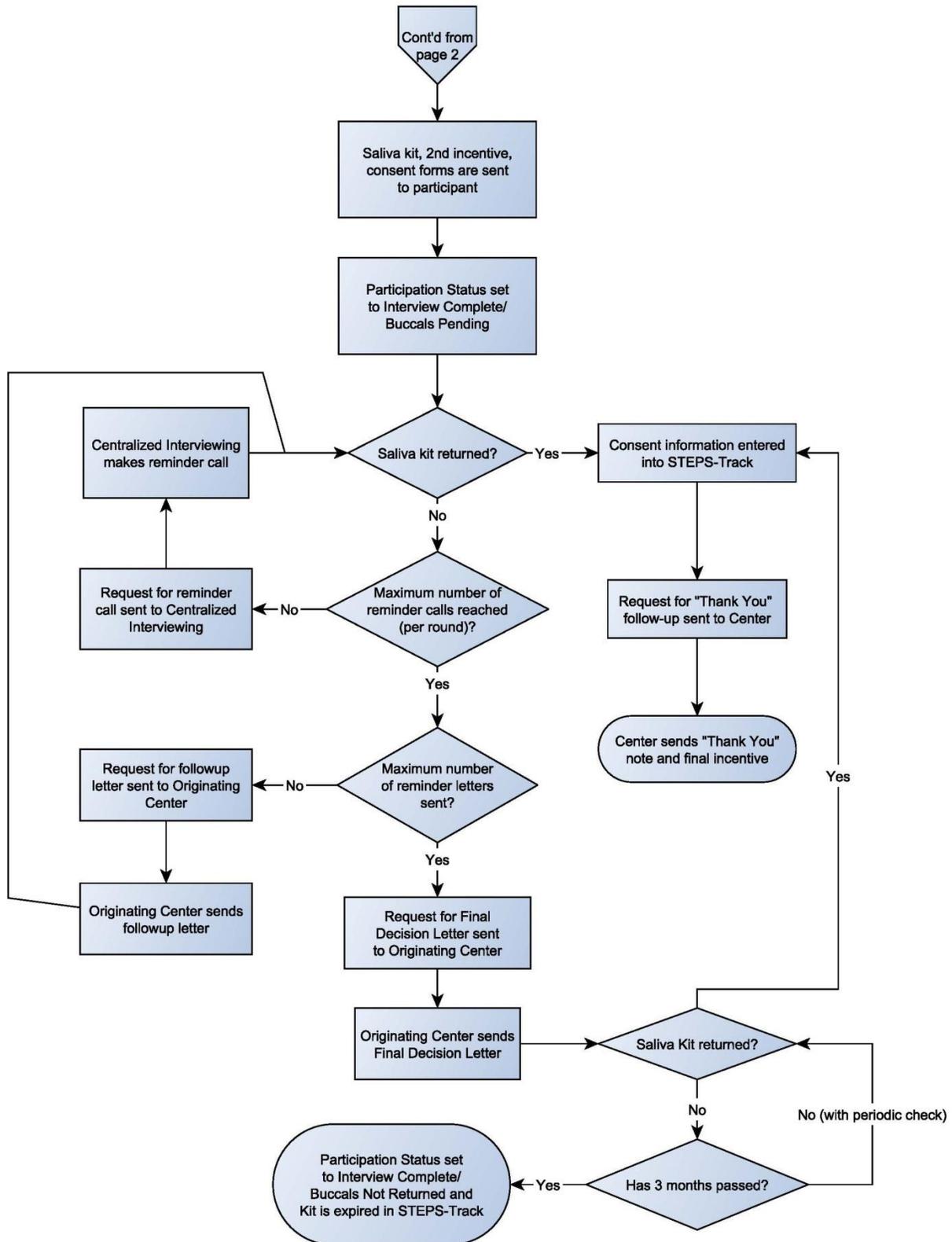
STEPS-TRACK is a web-based database developed by CDC that will facilitate the flow of information on eligible participants. Access to the data will be limited; sites will only have access to their own data. The central interviewing site will have access to the information for all of the sites after appropriate agreements between the contracting facility and the local sites

are in place. CDC will only have access to the CDC data. The flow of data through STEPS-TRACK is presented in Figure 1.

Figure 1. Flow of information in STEPS-TRACK







3.E. Recruitment

3.E.1. Initial Mailing and Incentive

Letters of introduction (Attachment 6) are mailed to prospective participants by the local site after a participant has been identified, and at least six weeks after the estimated date of delivery. 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 gift card, a fact sheet on rights of human subjects (Attachment 7), a calendar (Attachment 8), and a study information pamphlet (Attachment 9).

3.E.2. Tracking and Tracing

Address information from the data abstraction or vital records is used for the initial mailing. After two weeks, the interviewers start calling the available phone numbers (see Figure 1). If no confirmation is received that the available numbers reach the intended woman, an additional request for contact information is sent to the local center. If no additional information is found, the central interviewing facility will use an online tracing provider to find additional contact information.

3.E.3 Scheduling interviews

When the woman is reached by the central interviewing facility, an interview can be completed at that time or scheduled for a later time. Interviewing will be available at a range of hours at each of the local sites. Women can also call the toll-free number to arrange for an interview after they receive the intro packet or email the interviewing facility to schedule an interview.

4. INTERVIEW

4.A. Centralized Interviewing Staff

BD-STEPS interviewing for all the sites will be done by one central CDC-funded contract interviewing facility, which will increase consistency and efficiency. Contact information for the subjects will be sent from the individual CBDRP to the interviewing facility via the secure STEPS-TRACK system. Interviews will be conducted via the telephone using a system that allows eligible participants to see a local phone number displayed on their caller ID display for each of the sites (Voice over Internet Protocol, VoIP).

4.B. Interview

4.B.1. Oral 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 of telephone script, Attachment 10). In case of un-emancipated minor mother the interview will be postponed until she is 18, if possible, otherwise consent will be asked of the maternal grandmother of the infant. 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.

4.B.2. Interview Instrument

Mothers of all case and control infants who agree to participate in BD-STEPS are interviewed by telephone. This interview provides the framework for BD-STEPS, providing critical information, which is used in all aspects of the study. Building on interviews of over 40,000 mothers of infants with and without birth defects from the original NBDPS (OMB 0920-0010; expires 04/13/2015), an approximately 45-minute computer assisted telephone interview (CATI) will be

programmed for BD-STEPS (Attachment 11). The primary language of the BD-STEPS interview instrument is English. However, the interview has been translated to Spanish (12% of NBDPS interviews were completed in Spanish). In addition, letters of correspondence, telephone scripts and consent forms have been translated for Spanish-speaking participants. Being able to complete the interview in English or Spanish is a criteria for inclusion in the study; translated interviews are not allowed.

4.B.2.1. Questions Retained from NBDPS

A large portion of the BD-STEPS interview will be maintained from the NBDPS to make pooling of the CDBRP's NBDPS and BD-STEPS data possible; pooled data will facilitate the analysis of rare exposures and the examination of trends over time. The BD-STEPS interview instrument contains sections on pregnancy history, family history, multiple births, fertility, maternal conditions and illnesses (including diabetes, genitourinary infections, and fevers), medication and herbal use, emotional stress, physical activity, obesity, alcohol and tobacco use, residential history, occupational history, and demographic characteristics (including race/ethnicity, acculturation status, and education).

4.B.2.2. Innovative Questions Introduced in BD-STEPS

Innovative questions were added to the BD-STEPS telephone interview in response to some of the findings from NBDPS and to new findings in the literature. Changes include:

- Adding questions about maternal diseases and their treatment including thyroid disease, asthma, autoimmune disease, transplant receipt, cancer, depression, and anxiety;
- Updating the instrument to evaluate possible new and emerging birth defects risk factors (e.g. new medications);

- Adding questions about exposures not explored before that have biological plausibility and public health importance (e.g. dental procedures and transplant receipt);
- Expanding sections to provide increased detail (e.g. indication and dose for specific medications).

4.B.2.3. Online Modules (Targeted Follow-up)

With increasing access to and use of the internet, online questionnaires are an increasingly functional platform for obtaining data. As a pilot project within BD-STEPS, specific online questionnaire modules will be developed that are administered to only a select group of BD-STEPS participants identified through information reported in the CATI. For these pilots the likely initial topics will include occupational exposures among nurses (because of the high likelihood of internet access among this group and the specific, potentially teratogenic exposures that depend on the details of the job, such as anesthetic gases) and confirmation of specific medication exposures (because of the opportunity to show pictures of pills, pill bottles and boxes).

4.B.3. Additional Oral Consents

At the end of the interview, additional oral consent will be requested of the mother. This will be a tiered consent(10) where the mother can say no to any of the individual requests. Consent will be asked for:

- Medical records. Consent will be asked for access to review medical records at a future time. Medical records contain specific information that might be hard for women to recall, and medical record review allows validation of exposures reported by the mother

in the CATI. Initial topics for which medical records might be requested include fertility treatments and diabetes management.

- Future contact. In the future, we would like the ability to contact some of these mothers again for additional information (e.g. access to care) or long term outcomes (e.g. co-morbidities). Asking for consent when we talk to the mothers will enable us to attempt to contact them in the future.
- Global Unique Identifier. If possible it would be immensely helpful to create a Global Unique Identifier (GUID)(11) among different birth defects researchers. Especially in a field with outcomes as rare as birth defects it would be helpful to link between different studies, and GUIDs might assist in this effort.

4.C. Thank You Letter

Immediately following completion of the interview, subjects are sent the interview thank you letter, which is mailed by the local site (Attachment 12).

4.D. Newsletter

With the thank you letter we will send a copy of the participant newsletter, which contains information about BD-STEPS and the progress and findings of the study to date (Attachment 13). Through the newsletter and possibly a BD-STEPS web site, we intend to inform study participants of general study progress and research findings, as analyses are completed. For this purpose, a roster of participants will be maintained and updated if change of address information is received. In the newsletter, study participants are asked to provide us with updates to their contact information. Newsletters that describe the status and completed work of studies using BD-STEPS data will be mailed to past participants on approximately a yearly

basis. The CBDRP will send newsletters to those participants for whom we have reliable contact information.

DRAFT

5. BIOSPECIMEN COLLECTION, PROCESSING, AND STORAGE

5.A. Saliva Collection Kits

In an 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. Individual susceptibility (biomarkers of susceptibility) to the effects of environmental agents may vary depending on specific genetic or other 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 inaccurate estimates of the magnitude of the association between the exposure and the outcome. After the interview thank you letter is sent, a saliva collection kit is mailed to each family. STEPS-TRACK contains a biologics portion that generates labels and tracks shipping and receiving of each biospecimen collection kit. The tracking system includes coded study and specimen identifiers. Saliva collection kits are mailed to each mother and include collection devices for her child (if living), herself, and the child's father. The collection kits include a letter describing saliva collection, informed consent forms, a pen, instructions, a \$20 incentive, devices for completing the specimen collection, a Frequently Asked Questions form, and prepaid U.S. mail packets for specimen return. After several weeks, if the completed saliva collection kit is not returned, the interviewers will call the mother and the local site will send a reminder letter to see if she has any questions about how to complete the kit (see Figure 1). If there is still no response after two rounds of reminder calls and letters, a final letter is sent to

the mother encouraging her to complete the kit and stating that if the kit is not received within 2 weeks, we will assume she is not interested in participating.

5.A.1. Written Consents

5.A.1.1. Saliva Samples (Mother, Father, and Infant)

Written informed consent is obtained for each participant who agrees to provide saliva samples. The standard version (written for Metropolitan Atlanta) is included in Attachment 14. It includes the following information: the purpose of the study, the procedures, risks and benefits, information on confidentiality, compensation, research laboratories, the participant's right to refuse or withdraw samples, control, ownership, commercial value of biologic materials, and phone numbers for questions about the research or the participant's rights as a human subject.

The written consent document informs participants that the CBDRP scientists intend to conduct genetic research that is directly related to birth defects in the CDC Central Laboratory and/or other CBDRP laboratories. 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.”

If parents cannot read the written consent forms or if they returned a kit without a signed consent form and prefer verbal consent, they are read a verbal consent form by an interviewer.

If mothers return saliva samples without the signed informed consent form, they are sent another consent form with a request for the appropriate signatures. After repeated attempts, if the mother does not return the signed consent form, her family's saliva samples will be

included in the study. By returning the saliva samples, these mothers have implied their consent to participate.

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, 14, 15).

The consent form also states the following:

“For any tests that have clinical importance, we will publish summarized results in the study newsletter. This newsletter is sent to all participants”.

If we receive a request for the results of individual genetic tests carried out in BD-STEPS, we will comply with the Privacy Act and respond in the following way:

We will reiterate what was included in their written consent, which stated that results from these studies are not meant to test individual medical status and were completed in research labs so we do not plan to return any individual results. We will explain the limitations of the testing that was done (e.g. testing was completed in non-CLIA certified labs) and explain that tests of clinical importance will be published as summarized results in the study newsletter. We will also suggest that they discuss any genetic tests that they think would be useful to them with their health care provider, and will offer to assist them in locating a provider.

We have developed a fact sheet that can be sent to anyone requesting information on the genetic testing done as part of BD-STEPS (Attachment 16). The fact sheet explains the nature of the testing that is completed on the saliva samples, the limitations of the technology being

used, why individual test results will not be reported, how a participant can withdraw from the study, and other relevant information.

5.A.1.2. Newborn Dried Blood Spot (Infant)

Newborn screening is completed by collecting blood from infant heel sticks on filter paper soon after the infant's birth. Some state health departments store these bloodspot samples in a manner that would allow retrieval if parental consent was obtained. CBDRP in states that retain dried bloodspots from newborn screening in a manner that allows them to be linked to BD-STEPS data will include an additional consent form to ask for permission to retrieve them to use for epigenetic, genetic or exposure biomarker analyses. These samples will be stored at the local sites.

5.A.2. Incentive

Each of the BD-STEPS sites includes a \$20 gift card with each saliva collection kit. Mothers who ask for additional kits during saliva collection follow up calls will receive a maximum of one additional kit that contains another \$20 gift card.

5.B. Central Laboratory

The saliva kits will be sent directly from each participant to the CDC Central Laboratory for processing and quality control analyses. To ensure that Central Laboratory staff have no access to participants' personally identifiable information, CDC staff not affiliated with the Central Laboratory will mark consent forms (saliva and dried blood spot) as received in STEPS-TRACK and will be responsible for storing them in a secure manner. Central Laboratory staff will scan barcodes on labels affixed to the saliva collection devices into STEPS-TRACK and BioLab (the laboratory information management system). Through STEPS-TRACK, each local site will

receive notification of saliva kits from their site that were received by Central Laboratory staff.

Biologic samples obtained as part of BD-STEPS are stored in a secure manner without identifiers (with the exception of study identification number) in secure storage facilities at the CDC Central Laboratory until they are shipped to the biorepository.

5.C. Final Thank You and Incentive

Following notification via STEPS-TRACK that kits and consent forms were received at the Central Lab, each of the BD-STEPS sites will send a final letter of thanks and an additional \$20 gift card to each participating mother (Attachment 17).

5.D. Quality Control

Quality control data for each sample are stored in a laboratory information management system that was developed at CDC (BioLab). Access to BioLab will be limited to CDC Central Laboratory staff, data managers, and programmers who are responsible for the database. The server is backed up weekly and taken offsite periodically by CDC's ITSO team. These data are only identifiable by a nine-digit study identification number and are maintained using stringent security measures.

5.D.1. Recollects

The majority of samples pass the quality control analyses, but occasionally the Central Laboratory is unable to obtain enough DNA to provide reliable data, or the data from quality assessments are inconsistent. These indeterminate quality control results are potentially due to contamination of the sample during transit or processing, non-paternity or non-maternity, or sample mix-ups by study participants or during processing in the laboratory. Following verification that no donor sperm, eggs, or embryos were used that would explain the

inconsistent quality control results, additional samples are requested from families if one or more of the family members' saliva samples do not pass the quality control analyses. We approach the mother initially by phone, followed by a letter. Examples of recollect phone scripts and letters are included as Attachments 18A and 18B. A \$20 gift card is included with the recollection kit, and another \$20 gift card is sent to participants with a final thank you letter once recollected samples are received at the CDC Central Laboratory. Each family is asked to recollect samples no more than one time.

5.E. Central Biorepository

Use of biospecimen storage banks is becoming increasingly important for epidemiologic research for several reasons. Major expenditures in time and money are spent in sample collection. Maintaining biospecimen banks, which provide the opportunity for additional research as new hypotheses or improved technologies emerge, allows the potential contributions of study participants to be maximized. This approach is particularly important for testing hypotheses regarding risk factors for birth defects. Because individual defects are rare, many years of data collection are required to obtain enough samples to complete a valid etiologic study for a specific birth defect. The length of time between obtaining the specimen and having adequate numbers for a specific analysis is likely to be a minimum of three to five years, and may well be much longer. Participants will be informed of the intent to bank their specimens for birth defects research.

Once processing is complete at the Central Laboratory, samples are sent to a centralized storage facility operated by an external contractor. The samples are stored in a manner that permits efficient retrieval and optimum stability; they can be identified only by the study

identification number. The biorepository will release specimens only on the authorization of the CDC Collection Custodian (Mary Jenkins, PhD).

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 samples in ways that can only be imagined now. These samples will be stored indefinitely unless a request is received from the participant to destroy them.

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 and neither researchers nor study participants will receive profits from the donated materials.

6. BD-STEPS Data Analysis

6.A. Data Sharing

The Data Sharing Committee has two representatives from each of the CDRP. Each of the CDRP has two votes. The NBDPS Data Sharing Committee established guidelines for access to the compiled interview and biologic data and is responsible for ensuring that the data are shared equitably; these same guidelines will be used for BD-STEPS (Attachment 19). Any of the CDRP researchers 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 will use guidelines established in NBDPS for authorship, acknowledgments, and other issues related to the publication of studies using the collective data (Attachment 19); the committee will also insure that all proposed research complies with human subjects' requirements. Additional IRB review will be required for any studies that fall outside of the scope of the current protocol.

6.B. Data Preparation

The interview data from the local CDRP will be combined into databases by the CDC programmers for use in statistical analyses by analysts at each of the CDRP. Data will be released annually based on completed cohorts defined by expected date of delivery for each calendar year. There will be several data cleaning steps that will be implemented before release of the data.

6.B.1. Clinical Data Classification

Numerous studies have documented extensive etiologic heterogeneity in birth defect cases with similar anatomic appearance and/or proximity. To provide a sound epidemiologic framework to study specific defects, the presence of associated defects, and accurate clinical descriptions of defect types should be used in classifying birth defect cases into subgroups that are etiologically and pathogenetically homogeneous. All BD-STEPS cases are classified by clinical geneticists, or, where appropriate, pediatric cardiologists. Each case is classified as to whether it occurs in isolation or in addition to other defects. Heart defects are additionally classified as to whether the heart defect is simple or complex.

6.B.2. Interview Data Coding

The interview instrument will contain open-ended questions that will be coded by the CDC-contracted interviewing facility into variables that will be easier to analyze. Examples of such open ended questions include occupational information and family history.

6.B.3. Geocoding of Address Data

During the interview portion of the study, information is collected on residential addresses at the estimated time of conception. Geocoding this information (i.e. assigning geographic coordinates) will be extremely valuable for studies of environmental health as well as other topics through linking of spatially-coded exposure data. For example, geocoding will allow studies to assess whether proximity to certain types of locations, such as factories, toxic waste dumps, nuclear power plants, or health care facilities are associated with risk of the studied defects. It will also allow participants to be assigned, based on their residences, to their drinking water source, geological regions (e.g. high radon areas), areas of water treatment

utilities, plumes from pollution sources, and census tracts, for which a variety of associated socioeconomic variables are available.

The CBDRP's will conduct centralized geocoding for all interviewed cases and controls, which will maximize consistency in geocoding across Centers. The residential history data will not be centrally available, and some states will require that all the geocoded and original address data 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 in the past to geocode all NBDPS residence data from all Centers, at no cost, and they will be approached again. This group, external to BD-STEPS, does not need any information about BD-STEPS participants besides the actual address. They will only know that at that address a child was born whose mother participated in BD-STEPS interview.

6.C. *Data Analysis Databases (Tools)*

The combined data from all BD-STEPS sites will be released after a calendar year is completed and the classification and coding have been completed. Data will be released in a Microsoft Access database to all the participating BD-STEPS sites. Analysts are required to complete training on the use of the data analysis tools and to have received approval from the Data Sharing Committee of their analytical proposal prior to beginning data analysis.

6.D. *Biologics*

6.D.1. External Quality Assessment

To ensure that each lab actively involved in genetic and epigenetic analyses of BD-STEPS specimens is proficient in their respective laboratory techniques independent of the source material or extraction procedure, an external quality assessment (EQA) that was developed for the NBDPS will be amended and will be used for laboratories planning to conduct research using BD-STEPS samples. EQA protocols are included as Attachments 20A and 20B.

6.D.2. Biorepository Sample Requests

Specimens stored in the BD-STEPS biorepository will be used to evaluate genetic susceptibilities to birth defects using candidate genes, genome wide associations, copy number variants, epigenetics, sequencing, and other emerging technologies. The CBDRP scientists will share aliquots of these samples, without personal identifiers, to carry out collaborative research studies, following the approval of proposed research projects by the Data Sharing Committee (Attachment 19). As previously mentioned, sharing of samples with collaborating investigators is done without personal identifiers, and only the CDC Collection Custodian can make a biorepository sample request. The CBDRP investigators submit sample requests to the CDC, and CDC CBDRP staff process requests to ensure that each of the CBDRP receives their allotted portion of samples that passed quality control. Only researchers within the CBDRPs will be allowed to request these biospecimens. Investigators retain control of biological materials obtained at their CBDRP, unless the participant requests that these materials be destroyed (Attachment 14 and 16).

6.D.3. Genetic Analyses Database

The NBDPS has established a centralized database to store results from genetic analyses that will also be used to store genetic results from BD-STEPS. Centralized storage of genetic data will facilitate data sharing between CDRPs. Genetic data that have coded identifiers are submitted to CDC via the Secure Data Network. These data are compiled and maintained by the CDC in a commercial off-the-shelf database that has been certified through CDC's Certification and Accreditation process. The combined data from all completed analyses will be released annually to BD-STEPS analysts.

6.E. Publications

BD-STEPS study sites are expected to write several publications per year analyzing NBDPS or BD-STEPS data or combining the NBDPS and BD-STEPS data. In the author list "Birth Defects Study to Evaluate Pregnancy Exposures" will be included as an author if pooled data are included and the CDC funding will be acknowledged in the funding section.

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