

CHAMACOS, A Longitudinal Birth Cohort Study: Lessons from the Fields

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ABSTRACT

In the year 2000, the US Congress passed the Children's Health Act directing the nation to conduct a national birth cohort study of environmental influences on children's health and development. In anticipation of the National Children's Study, lessons can be learned from current smaller birth cohort studies. For example, NIEHS and EPA have funded 12 Centers for Children's Environmental Health Research, a number of which are conducting longitudinal birth cohort studies to understand the environmental impact on children's health. The Center at the University of California, Berkeley, known as CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas) is a community-university partnership studying pesticide and allergen exposures to pregnant women and their children, and the potential effects of these exposures on growth, neurodevelopment, and respiratory disease. This paper describes the research activities of CHAMACOS and some lessons that have been learned, including the importance of building a strong community infrastructure. Although the challenges of collecting longitudinal data may be greatest in minority or impoverished communities, these communities are most at risk for exposure to environmental hazards and should be represented in the National Children's Study.

Key Words: children, longitudinal birth cohort, organophosphate, pesticide, exposure, neurodevelopment, growth, asthma, environment,

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farmworker, Latino, CHAMACOS.

INTRODUCTION

In the year 2000, the United States Congress passed the Children's Health Act (United States Congress, 2000). This Act directed the nation to conduct a national birth cohort study of environmental influences, both harmful and helpful, on the health and development of children. A mandate of the National Children's Study is to examine the chemicals that children are exposed to in their environments, and to determine whether these exposures are related to adverse short- and long-term health outcomes. Currently, the National Institute for Child Health and Human Development, along with a consortium of federal agencies, including the National Institute of Environmental Health Sciences (NIEHS), the United States Environmental Protection Agency (US EPA), and the Centers for Disease Control and Prevention (CDC), is coordinating the planning and organization of this study. A number of advisory committees and working groups are developing hypotheses and study designs and working to address the myriad issues involved in mounting such a massive and complex undertaking. The study proposes to follow approximately 100,000 children in the U.S. from prenatal development into adulthood. The environment will be viewed and approached on a broad scale, encompassing the many chemical, physical, social, and behavioral factors that affect health and development.

Birth Cohort Studies in the U.S.

This effort is not the first for the U.S. There have been two major birth cohort studies previously conducted in the U.S.: the National Collaborative Perinatal Project (Niswander and Gordon, 1972) and the Child Health and Development Studies (van den Berg *et al.*, 1988), both initiated in the 1950s and 60s. These large-scale longitudinal birth cohort studies in the U.S. focused on the etiology of neurologic diseases (e.g., cerebral palsy) using data collected primarily through questionnaires and medical records abstraction, with limited biological sampling and no environmental sampling.

The state of public health research has changed immensely since the time when the National Collaborative Perinatal Project and the Child Health and Development Studies first began. The fields of genetic and molecular biology have exploded, with many new and exciting advances in laboratory tools. A large number of exposure assessment tools have been developed for a variety of toxicants. Nutritional assessment methods have matured. New geographic mapping techniques are available. Computer technology has increased our capacity to collect, store, and analyze data. All of this has resulted in greatly expanded methods for research design and data collection and analysis. In some cases, these methodological advances have resulted in more complex ethical and logistical issues.

NIEHS and US EPA funded eight Centers for Excellence in Children's Environmental Health Research in 1998, and funded four more in 2001. The Centers were required to conduct community based research on the causes and mechanisms of children's disorders having an environmental etiology. A number of these Centers (the Center for Children's Environmental Health at Columbia University, the Center for Children's Environmental Health and Disease Prevention Research at the Mount Sinai School of Medicine, the Cincinnati Children's Environmental Health Center at Children's Hospital Medical Center, the Friend's Children's Environmental Health Center at the University of Illinois, Urbana-Champaign, and the Center for Children's Environmental Health Research at the University of California, Berkeley) are currently conducting longitudinal birth cohort studies and examining the influence of various environmental exposures on the health of children in both urban and rural environments.

The Center at the University of California, Berkeley, known as CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas) is a community-university partnership studying pesticide and allergen exposures to pregnant women and their children and the potential effects of these exposures on growth, neurodevelopment, and respiratory disease. This paper describes the research activities of CHAMACOS and how lessons learned can provide some insight into how research on minority children living in rural communities can best be included in the upcoming National Children's Study.

Children and Pesticides

In the U.S., there are approximately 1.2 billion pounds of pesticides used every year (United States Environmental Protection Agency, 1999). Of this, approximately 950 million pounds are used in agriculture (United States Environmental Protection Agency 1999). In California, close to 150 million pounds of pesticide active ingredient are used annually for agricultural purposes (California Department of Pesticide Regulation, 2002). Recent studies have shown that pesticides are commonly detected in rural, suburban, and urban households in the U.S. (Berkowitz *et al.*, 2003, Bradman *et al.*, 1997, Buckley *et al.*, 1997, Camann *et al.*, 1997, Lewis *et al.*, 1994, Simcox *et al.*, 1995, Starr *et al.*, 1974, Steen *et al.*, 1997, Whitmore *et al.*, 1994), and that exposures to children, pregnant women, and other adults are widespread (Centers for Disease Control and Prevention, 2001).

In developing rodents, chronic exposures to low levels of organophosphate pesticides (OPs) have been shown to affect growth, neurologic functioning, and neurodevelopment (Eskenazi *et al.*, 1999). In children, acute OP exposures due to poisonings have impacted the parasympathetic, sympathetic, and central nervous systems (Eskenazi *et al.*, 1999). Children are particularly vulnerable to exposures to environmental agents, including pesticides, because of their unique activity patterns: increased hand-to-mouth behaviors and more time spent on potentially contaminated floors. As well documented, children also take in more air, food, and water per unit of body weight than adults (National Research Council, 1993).

Building Community Infrastructure

The CHAMACOS research is based in the Salinas Valley of Monterey County, California, a region often referred to as the “nation’s salad bowl” and home to an estimated 38,000 farmworkers and their families (California Employment Development Department, 2002). The Salinas Valley is located southeast of San Francisco and San Jose, and runs approximately 60 miles from North to South. The largest city in Monterey County is Salinas, with a population of approximately 156,000 (California Department of Finance, 2002), comprising over one-third (38%) of the county’s population (California Department of Finance, 2002). Of the entire county, 15% of the population lives in rural areas (United States Census Bureau, 2000). Monterey County has communities living at both ends of the economic spectrum, with wealthy families in the coastal towns of Carmel and Pebble Beach, and the more impoverished living 30 miles inland in the Salinas Valley. Almost a quarter of the county population is under the age of 14 (United States Census Bureau, 2000), and an estimated 17% of those under 18 years live in poverty (United States Census Bureau, 2000). The county is 47% Hispanic (United States Census Bureau, 2000); some 22% of residents were born outside of the U.S. (United States Census Bureau, 2000).

The structure of CHAMACOS emphasizes community involvement in all aspects of the research. Our community partners include local community clinics, hospitals, health departments, advocacy groups serving farmworkers, and agricultural industry groups. CHAMACOS has two external advisory committees: a Community Advisory Board and a Scientific Advisory Board. Our Community Advisory Board reflects many constituent groups, from farmworkers and health care providers to county officials and growers. The community partners and the Community Advisory Board help to shape and guide the research and to disseminate results.

The Salinas Valley community, like many communities, had never before been the site of such a large and detailed public health research project when CHAMACOS began in 1998. Working with our community partners, CHAMACOS scientific investigators have spent many hours in meetings, focus groups, and informal discussions with representatives from the larger community working to forge a common understanding of the aims and goals of the research project. We have aimed to build trust by bringing different constituent groups (e.g., farmworkers and growers) together for honest and open conversations about the research. It has been important to emphasize what the research can accomplish, and a realistic time-line. CHAMACOS researchers repeatedly emphasize that their role, as scientists, is to provide information for policy makers but not to make policy changes, and to provide unbiased answers to research questions. We have had many discussions about protecting the confidentiality of individuals and about the process and timing of providing results to the community.

This lack of previous community experience with research has made finding qualified staff members, who have the necessary language skills and cultural understanding, a challenge. All members of the field staff have been recruited from the local Salinas Valley community, are bilingual and bicultural, and many have a personal and/or family background in farmwork. Few have any previous experience

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working in the research field, and few have completed a 4-year college degree. In employing members of the community, CHAMACOS has provided advanced training to many Salinas Valley residents in home inspection, phlebotomy, interviewing, laboratory processing and management, and the neuropsychological assessment of children. Many Center staff members in Salinas have become certified as US EPA Worker Protection Standard educators. These skills will enhance their future employability and the ability to provide services to the community, which is chronically short of well-trained bilingual employees.

CHAMACOS has worked to raise community awareness of the research project. The CHAMACOS acronym was carefully chosen; the word “chamacos” means small children in Mexican Spanish, which has helped to raise name recognition, and we worked with a local Mexican artist to design a logo that reflects the culture. CHAMACOS has been featured repeatedly in articles in local newspapers and on local Spanish-language radio and television shows. We have also distributed, to participants and to the wider community, calendars and newsletters that provide education on child nutrition, safety, and lead poisoning prevention, as well as updates on the research.

CHAMACOS is a member of a coalition group of social service agencies (South County Outreach Effort), and our staff members regularly attend these meetings as well as community events, such as health fairs and the annual Dia del Trabajador Agrícola (Farmworker Day), a local festival attended by approximately 4000 people. CHAMACOS staff gives presentations on the research study, as well as hands-on demonstrations of sample collection techniques. For example, we use a doll that urinates to demonstrate how child urine samples are collected. CHAMACOS researchers give presentations to medical residents, community doctors, and county health departments on the potential hazards to children of lead, pesticide, and respiratory irritant exposures. In collaboration with the Salinas Allergy Clinic and the American Academy for Asthma, Allergy, and Immune Disease (AAAAI), we are monitoring area pollen and mold levels, which are published regularly in the local newspaper and are made available on the internet. These data provide a valuable service to area physicians and residents and also increase CHAMACOS visibility.

All of these efforts have helped CHAMACOS build trust across the Salinas Valley community, and, over the 5 years that CHAMACOS has existed as a research center, a strong sense of commitment and pride in being part of the study has developed among the partners, participants, and staff of CHAMACOS. This infrastructure has been instrumental in the success of this project.

CHAMACOS RESEARCH METHODS

The aims of the CHAMACOS study are threefold: (1) to estimate sources, pathways, and levels of in utero and postnatal pesticide exposures of children; (2) to determine the relationship of pesticide exposure and growth, neurodevelopment, and respiratory disease; to determine the association of environmental allergens

and respiratory symptoms and illness, and to determine whether pesticide exposure modifies this relationship; and (3) to develop home- and field-based interventions to reduce pesticide exposures to children.

Study Design

The current primary research activity of CHAMACOS is a longitudinal birth cohort study of pregnant women and their children. Pregnant women are enrolled through two community clinics. Questionnaires are completed at enrollment (M= 14 weeks gestation), 27 weeks gestation, and at delivery. Child neurodevelopment and growth assessments are completed during the neonatal period and when the child is 6, 12, and 24 months of age. At each postnatal visit, mothers are interviewed about their child's health and exposures. Medical records are abstracted from pregnancy until delivery for the mother and up to age 30 months for the child. Biologic specimens are collected throughout pregnancy and from the child at each postnatal visit. Home assessments are conducted to determine pesticide exposure and triggers for respiratory disease; environmental specimens are collected at these home visits.

Study Population

The CHAMACOS study population is a marginalized, low-income, mostly Spanish-speaking population of pregnant women and their infants. As shown in Table 1, about 75% of participants have at least one household member who works in agriculture. CHAMACOS participants have minimal education (44% completed no more than sixth grade) and many may not have legal residency status in the U.S. Thus, this population is not easily contacted, enrolled, or followed in a research study. However, an efficacious time to access this population is during pregnancy, a time when the women are eligible to receive free prenatal care through California's Comprehensive Perinatal Services Program (CPSP). CPSP, overseen by county Health Departments, provides prenatal care, including health education, nutrition services, and screening for social services, to women within 200% of the federal poverty line regardless of their immigration status.

Because of the need to protect the privacy of their patients, CHAMACOS partners, Clínica de Salud del Valle de Salinas (CSVS) and Natividad Medical Center (NMC), were not able to provide the study staff direct access to the pregnant patients. The clinics were only able to serve as a means of introducing CHAMACOS to their patients; if the patients were interested in participating in the study, the clinic could then provide the woman's name and contact information to CHAMACOS.

All women who enrolled in CPSP at NMC or CSVS during a 1-year period (between October 1999 and October 2000) were screened for eligibility by a CPSP worker using a standardized single-page form in English and Spanish. The CPSP worker obtained contact information for a woman if she met the following criteria: Spanish or English speaking; less than 20 weeks pregnant; 18 years of age or older; MediCal eligible; receiving prenatal care at NMC or CSVS; and planning to deliver

Table 1. Sociodemographic characteristics of CHAMACOS study population (n=601).

	n (%)
Age (years)	
18-24	290 (48)
25-29	179 (30)
30-34	88 (15)
35-44	43 (7)
Marital Status	
Married/Living as Married	481 (80)
Not Married	118 (20)
Education Completed	
≤ 6th Grade	261 (44)
7-12th Grade	219 (37)
HS Graduate+	120 (20)
Parity	
0 births	211 (35)
≥1 births	388 (65)
Country of Birth	
United States	77 (13)
Mexico	509 (85)
Other	14 (2)
Years in US	
≤ 1 year	166 (28)
2-5 years	153 (26)
6-10 years	125 (21)
11+ years	91 (15)
Entire life	65 (11)
Language Spoken at Home	
Mostly/Only Spanish	527 (88)
Both equally	26 (4)
Mostly/Only English	37 (6)
Other	9 (2)
Monthly Income	
At or below Poverty*	350 (62)
Poverty – 200% Poverty	196 (35)
>200% Poverty	22 (4)
Housing Density (people/room)	
≤ 0.5	9 (2)
0.51-1.00	116 (23)
1.01-1.50	188 (38)
≥ 1.51	184 (37)

* U.S. Bureau of the Census, Current Population Survey

at NMC. So that we could describe the differences between those who agreed to participate and those who did not, we also obtained information on parity, country of birth, and farmworker status of the woman and her family members. In order to be sure that potential participants were given adequate information on the CHAMACOS project at the time of screening and to avoid overburdening the clinic staff, potential participants were shown a video (in English or Spanish) describing the CHAMACOS project and what participation in the project would entail. This medium worked very well for this low-literacy population.

A total of 1800 women were screened for enrollment and 1130 were determined to be eligible. Of these, 601 women enrolled in the CHAMACOS study (53% participation rate), which exceeded the targeted enrollment of 550 women. After screening, many eligible women felt it was necessary to obtain their husband or partner's approval before commencing participation in the study. Women who lived in shared housing situations were also reluctant to enroll without the consent of housemates or landlords. Those who agreed to participate were more likely than women who refused to participate to be Spanish speaking and born in Mexico. Participants were also more likely to have field workers living in their home. No differences in maternal age or parity were seen between women who agreed to participate and those who refused.

Table 1 presents demographic characteristics for the 601 women who enrolled in CHAMACOS. The mean age of participants at enrollment was 25.6 years (SD 5.3); 20% of the women were not married. Approximately 85% of the women were born in Mexico, over a quarter had been in the U.S. for 1 year or less, and over 90% spoke Spanish at home. Almost all women had a monthly household income that was within 200% of the federal poverty line. On average, 6.5 (SD 2.9) people lived in the woman's household, with 1.5 (SD 0.7) occupants per room. These are very crowded housing conditions when compared to both the Monterey County and U.S. averages (United States Census Bureau, 2000).

Participant Tracking

Conducting a longitudinal cohort study with a migrant farmworker population is a daunting task. However, because the growing season in the Salinas Valley extends from March to November, and there is additional work in the fields for most of the remaining months, much of this particular farmworker population does not leave the area. In addition, many pregnant women and children stay at home for the few months that the men may migrate to find work elsewhere on the migrant stream, usually in Yuma, Arizona. Nevertheless, this is a poor population that moves frequently within the area and leaves little forwarding information. This means that keeping track of study participants is often difficult.

CHAMACOS infants were born between February 2000 and June 2001. As of October 2002, 436 women and their children remained in the study (a 25% loss to follow-up rate of those children still living). Most of the loss occurred in the initial phases of the follow-up and has lessened after the initial postnatal visit at 6 months.

Specifically, of the 601 pregnant women who enrolled, 18 miscarried and 5 had stillbirths or neonatal deaths. Of the remaining 578 women, 8.6% were lost before delivery, 9.5% were lost between delivery and the child's 6-month visit, and 6.5% were lost between the child's 6- and 12-month visits. Only 2.5% have been lost so far between the child's 12- and 24-month visits. Over half of the 25% lost to follow-up have moved and cannot be located; therefore, only about 10% have been lost due to lack of interest in continuing participation.

The CHAMACOS study has developed and instituted a number of strategies for maintaining contact with study participants. There are multiple visit points for participants in the study, and study staff make regularly scheduled phone calls between the child assessments so that participants never go more than 6 months without speaking with a CHAMACOS staff member. At each visit we ask for contact information for at least four people who are likely to know the participant's whereabouts, including the participant's mother (in Mexico in many cases). At every contact point, participants are reminded and encouraged to contact CHAMACOS with any change in telephone number or address. Participants are also given a key chain with the CHAMACOS logo and a reminder to call with phone number or address changes on one side. On the other side, to personalize the key chain and increase its value, we insert an up-to-date picture of their child at each visit. Participants who have moved out of the area or who no longer wish to fully participate in the study are offered two additional enrollment options: (1) telephone enrollment, in which they complete brief questionnaires over the telephone and give access to medical records; and (2) medical records enrollment, in which they provide access to medical records, but do not complete any questionnaires. In the summer and fall of 2002, for the portion of telephone-enrolled participants who had moved outside of the area, CHAMACOS study staff traveled to their homes in the Central Valley (150 miles away) and Southern California (400 miles away) in the CHAMACOS RV to complete child assessments and abbreviated home visits.

Interviews and Assessments

In order to accomplish the study aims, at multiple time points highly trained bilingual/bicultural staff interview the mothers and their partners, visit their homes, and conduct assessments of the children. As much as possible the same study interviewer and environmental assessment team completes questionnaires and home visits with a family over the 3 years of their participation in the study. The stable and trusting relationships that are built between study staff and participants have greatly helped to keep participants enrolled in the study.

Table 2 shows the schedule of questionnaire and home environmental assessments. Questionnaires obtain information on socio-demographic characteristics, socio-economic status, medical history, lifestyle exposures (tobacco smoke, alcohol, drugs), occupational and residential exposures to pesticides, exposures to respiratory irritants and allergens, housing characteristics, diet and breastfeeding prac-

Table 2. CHAMACOS questionnaire and home visit contact points.

	2 nd Trimester	3 rd Trimester	Delivery	6 Months	12 Months	24 Months
Maternal Questionnaire	✓	✓	✓	✓	✓	✓
Paternal Questionnaire			✓			
Home Visit	✓			✓	✓	✓

tices, household members, and social support and depression (maternal). Home visits obtain information through questionnaire and direct inspection on home pesticide use and storage, presence of respiratory irritants and allergens, distance to nearby fields, housing characteristics and quality, and presence of environmental hazards. At the time of the home visit, exact latitude and longitude coordinates for the home are measured using a global positioning system (GPS) device.

Table 3 shows the neurodevelopment test battery and the age at which tests are administered. At each visit, a growth assessment of the child is completed using standardized methods to determine length/height, weight, and head circumference. We attempt to assess a number of different neurodevelopmental domains that are as specific as possible to those affected by OPs in animal models (Eskenazi *et al.*, 1999). In addition to these preidentified domains, the selection of the test battery has been based on several criteria: age appropriateness; availability and preferably standardization in both English and Spanish (or easily translatable into Spanish); and reasonable administration time.

To date, CHAMACOS has completed nearly 3000 questionnaires, over 1400 visits to participants' homes, and almost 1500 child neurodevelopment and growth assessments. We are currently conducting the 24-month child assessments, questionnaires, and home visits.

Scheduling Appointments

Appointments with participants are scheduled at their convenience, including evenings and weekends. Many of the women worked during their pregnancy, especially in the first two trimesters, and it was important to conduct their first interview while they were still working in order to measure occupational exposures to pesticides. During pregnancy, appointments were scheduled to coincide with the woman's medical visits. This helped to assure adequate prenatal care (since we pay for transportation) and collection of important prenatal data.

In the hospital after the delivery, we aimed to interview mother and father, and to perform the Brazelton exam on the neonate. However, when the women presented at the hospital for delivery, many did not identify themselves to hospital staff as a CHAMACOS study subject. Also, despite having a list of study participants who were approaching their due date, hospital staff frequently did not have the time to notify the CHAMACOS field office of a participant's delivery. This meant that

Table 3. CHAMACOS child neurdevelopmental test battery.

	Neonatal	Six Months	12 Months	24 Months
Brazelton Neonatal Behavioral Assessment Scale	✓			
Bayley Scales of Infant Development (Motor and Mental Scales)		✓	✓	✓
Preschool Language Scale (PLS)		✓	✓	
Autonomic Nervous System Assessment (for 1/2 of cohort)		✓	✓	
MacArthur Inventory of Communicative Development (maternal report)			✓	✓
Visual memory (Piaget)			✓	✓
Home Observation for Measurement of the Environment		✓	✓	✓
Child Behavior Checklist (maternal report)				✓
Developmental Milestones		✓	✓	✓

CHAMACOS staff had to constantly survey the delivery room admissions, including on weekends. At the time of delivery, many women left the hospital much sooner than the permissible 48-h stay. The little time that they did spend in the hospital after delivery was often filled with hospital education about breastfeeding and other infant care issues, as well as a lengthy discharge process. This made it difficult for CHAMACOS to complete the entire neonatal study protocol while the woman and infant were in the hospital, as had been planned. For example, less than half of the Brazelton Neonatal Behavioral Assessments could be done in the hospital and CHAMACOS staff had to arrange for an additional visit.

The father was interviewed at delivery because it was the most likely time that he would be available, given that many of the fathers were farmworkers who worked long hours in the fields, or who were not living full-time in the Salinas Valley. Nevertheless, the fathers were less likely than the mothers to participate in the study. Of the 497 women who were enrolled at delivery and had a husband or partner, only 54% (n=268) of the partners completed questionnaires, 16% refused to participate, and a large proportion of the remaining 30% were unable to take time off from work to complete the interview.

Transportation Challenges

Many CHAMACOS participants do not own or have access to a car, and because of their immigration status do not have driver's licenses. In the northern portion of the valley, and in the city of Salinas, there are bus and taxi services available, but in the southern portion of the valley the limited public transportation runs infrequently. Many people utilize informal transportation services provided by private car owners, who charge exorbitant fares; this makes it difficult for many participants to travel to the Salinas office for their appointments with CHAMACOS or to receive medical care. For participants living in Salinas, CHAMACOS contracts with a local

taxi company for participant transportation and pays the taxi company directly so that participants do not incur any cost. For participants living outside of Salinas, or who are unable to come to the office, CHAMACOS travels to them in a specially outfitted recreational vehicle (RV) that serves as an assessment room on wheels. This has greatly increased our ability to complete visits, not just with participants who live far from the office but also with participants who have difficulty leaving the house, often because they cannot afford childcare, or are childcare providers themselves.

Consent and Ethical Issues

The CHAMACOS project has presented a number of difficult ethical issues and human subjects protection challenges. In the course of designing and planning the study, special care was taken to protect the privacy of participants and to ensure that participation in the research did not adversely effect their immigration status, housing, employment, child custody, or any other aspect of their lives.

Because of the complex nature of the study, participant consent is divided into two documents covering two different time points. The first consent, signed at enrollment, covers the mother's participation from enrollment through delivery. The second consent, signed at delivery, covers the mother and child's participation from delivery until the child turns 30 months old. This allows us to ensure in this low literacy population that participants understand all aspects of the study and fully consent. In addition, with the mother's agreement, the father was approached at delivery and asked to sign a written consent. There was some question as to whom would need to give consent for the home visit portions of the study, primarily because participants often live in the same household with multiple roommates or other families. We have not sought consent from other household members; however, in cases of shared housing, we limit our home inspection to the portion of the home in which the participant lives (e.g., the communal living spaces and her family's bedroom(s)). Only a small number of participants have not agreed to the home inspections.

CHAMACOS has obtained a Certificate of Confidentiality from the National Institutes of Health so that CHAMACOS researchers cannot be compelled to reveal to anyone outside of the study the identity of study participants or information about them. Because in many cases CHAMACOS staff members live in the same communities as participants, special emphasis has been placed during staff training on participant privacy, and, at times, staff members remove themselves from visits with participants with whom they have some outside connection. In a number of cases, husbands have requested to be present when their wives are being interviewed, but in order to protect the woman's privacy and to ensure that we collect accurate information this is not permitted.

The frequent and intimate contact between CHAMACOS and participants raises the possibility of finding something illegal or dangerous in the home or family situation. A number of husbands, landlords, and household members have ex-

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pressed concern about the home visits because they do not want strangers in the home, and, for some property owners, because they do not want housing violations to be uncovered and reported. After consulting with a local legal advocacy group (a member of the Community Advisory Board) and the Monterey County Health Department, we decided not to report housing violations to the authorities; because of the tenuous housing and immigration situation of many participants, wherein they have few rights or legal recourses, there were concerns that participants could be evicted, or even deported. However, there is a clause in the consent forms that states that if CHAMACOS sees something that is immediately endangering the mother or the child we may discuss it with the mother, or, potentially, seek outside help.

In the consents signed by both the mother and the father, our IRB approved a clause that stipulates agreement to future testing of banked specimens, including genetic testing. While there is no direct link between uniquely coded banked samples and participant identifiers, this information can be decoded by the Principal Investigator should the need to contact participants arise. However, it may be difficult to find the participants in future years given the mobility of this community. Thus, it is particularly important to obtain consent that will allow for future analyses. Participants are specifically asked if it is acceptable for their banked specimens to be used for tests in the future. Because many of the markers of gene environment interaction are still in development, we broadly worded this portion of the consent, as shown below:

Some of the urine, blood, breastmilk, and dust samples we have already collected and those we plan to collect will be frozen and stored. Some samples may be used in the future for genetic testing which may help us learn how you and your baby react to chemicals in the environment. We may also want to use these samples to test for you and your child's health and exposure to some chemicals. Some of these tests are not available now, and we are not sure what the best tests are now.

Can we use samples we have already collected and those we plan to collect for possible future testing?

☐ Yes

☐ No

Thus far, we have received IRB approval on a case-by-case basis to analyze specimens linked to the individual's data (e.g., health outcomes and covariates) for lead, PON1 polymorphism, and endocrine-disrupting chemicals. However, it may be necessary to remove all identifiers if future analyses are not considered to be directly covered by the consent form. In every situation, special care will be taken to protect the identity and rights of participants. In all cases, results will be presented to the scientific and local communities in an anonymous summarized format.

One of the major discussion issues is the appropriateness of reporting individual level pesticide levels, or other laboratory results, to participants when the clinical significance of these results remains unclear, or the methods used to obtain the results have not been clinically validated but designed for more experimental purposes. At the project inception we decided not to provide individual pesticide metabolite results to participants because there were no previous benchmark studies of pregnant women to provide an appropriate clinical reference. We are currently revisiting this policy because of recently released data on non-pregnant populations (Centers for Disease Control and Prevention, 2001).

Language and Translation

Because over 90% of the CHAMACOS study population is Spanish speaking, all study instruments and assessment tools have to be available in both English and Spanish. In addition, the Spanish language versions must be appropriate not just for speakers of the Spanish language, but speakers of the Spanish language that is unique to the regions of Mexico where participants come from, as well as unique to the California region where they now live. This has meant that all materials must be translated and back translated, and then reviewed by study staff, who are bilingual members of the local community. It has also meant that the published Spanish translations of some assessment tools (such as the Child Behavior Check List) have had to be altered slightly to use the words and phrases that study participants use and understand.

The CHAMACOS study population is of low literacy, with almost half of the participants (44%) never having continued school past the sixth grade. Therefore, the words and phrasing used for all study materials have to be simple and easy to follow. Consent forms are always administered orally, regardless of the participant's reading ability, to ensure that true informed consent is given. All questionnaires are administered orally, even those designed to be self-administered (such as the MacArthur Communicative Development Index). Because of the low level of education and limited exposure to standardized testing even so-called "non-verbal" self-administered tests (e.g., Raven Standard Progressive Matrices) have to be administered by a trained interviewer.

Complex Lives

CHAMACOS participants face multiple social, psychological, and economic challenges that may affect their health and their ability to access health care and make healthy lifestyle choices. Thus, in addition to the wealth of information collected about participant's health and exposures, participants are also asked social support and depression scales, and a modified version of the Home Observation for Measurement of the Environment is completed. These scales allow us to assess some of the social factors that may impact health outcomes.

Since asking about sensitive issues may increase our awareness of family's difficulties, our staff members have been trained to provide information about community

resources, which participants can access when they need help with food, clothing, shelter, and mental health. Because this population may be intimidated by the complexities of the medical care and social services infrastructure, on a number of occasions, study staff members have made the initial contacts for the participant to help ease their entrance into appropriate services. This has necessitated regular discussions with staff members to clarify that their role is that of a researcher and not a social worker or advocate. Because our staff members are not trained mental health professionals, we have also enlisted the help of a professional social worker specializing in the mental health of Latinos to provide guidance and a sounding board for our staff.

Distractions

In order to minimize bias and to ensure that the child's concentration and attention are being fully maximized, it is crucial to conduct child neurodevelopmental and growth assessments in standardized environments that are quiet and free of distractions. As shown in Table 1, many participants live in extremely crowded housing with multiple families and many children. For these reasons, and because of the difficulty of transporting equipment, child assessments are completed either in the CHAMACOS office or in the CHAMACOS RV, and not in the participants' homes.

Many of the women enrolled in the CHAMACOS study have other children not enrolled in the study. They often bring these children to the CHAMACOS visit, because they do not have money to pay for childcare or knowledge of potential childcare providers outside of family members. Few women are comfortable leaving their children at the daycare facility provided free of charge that is a 5-min walk away on the hospital campus. CHAMACOS has occasionally provided a mother with grocery gift certificates to reimburse her for childcare. More often than not CHAMACOS staff ends up tending to the children that are brought to the office, and entertaining them with children's videos, games, toys, books, and art supplies kept on hand for this purpose. Although costly, ideally a research facility would provide onsite childcare, with dedicated personnel for participants during their visits.

Participant Remuneration

Researchers need to find a balance between giving participants so much that it is coercive, and so little that it is difficult to enroll and retain participants. Giving cash or checks to CHAMACOS participants is not an option; check disbursement requires a valid social security number, which many participants do not have due to their immigration status, and keeping adequate cash reserves in the office raises security concerns.

We have tried to provide incentives that are beneficial to the family's health and well-being. CHAMACOS gives gift certificates to local grocery stores at the completion of each visit. At the time of delivery, each family was given their choice of a car

seat, stroller, or, if they already had these items, a gift certificate for Toys'R'Us. Telephone and medical records-only enrolled participants receive phone cards. At various time points, participants are also given small gifts with the CHAMACOS logo on them. Mothers were given a T-shirt to wear to the hospital for delivery so that hospital staff knew to collect additional blood samples and to contact the CHAMACOS office. The children receive T-shirts and sun hats. Although we have applied to get direct donations from local retailers and national chains, we have only been successful in receiving discounts. As an incentive to remain in the study, at the end of the 24-month visits (in the summer of 2003) a raffle will be held with prizes to include a computer, a bicycle, and a camera; only participants who complete their 24-month child assessment will be entered. We are currently attempting to obtain donations for the raffle prizes.

We have entertained other incentive ideas, but rejected them for numerous reasons, including financial. One idea was to increase the incentive amount based on the participant's length of enrollment, another was to provide an incentive for when participants informed us of change of address or phone number. These ideas may be utilized in future follow-up of the population.

Biological and Environmental Samples

In this section we highlight challenges to the successful collection of biologic and environmental samples in a birth cohort study and their potential solutions. These challenges include: the collection and processing of biologic samples from infants; cultural barriers to successful collection; and logistical issues in collecting samples over a wide geographic area that is also rural.

To date, CHAMACOS has collected over 56,000 biological and environmental specimens, which has created a large biorepository for future research on pregnant women and children. Table 4 shows the types of samples that are obtained, and the time-points of collection. The samples are being collected for the analysis of exposures to organophosphate pesticides and potentially endocrine-disrupting chemicals (including organophosphate pesticides, organochlorine pesticides, PCBs, pyrethroids, chlorinated phenols, and ethylene thiourea (ETU)), allergens, and endotoxin; various biomarkers of effect, including immunological biomarkers and cholinesterase; and biomarkers of susceptibility, including paraoxonase polymorphism. Table 5 shows the analyses completed or underway.

Biological and environmental samples have been collected, processed, and stored to maximize the potential for further analysis in the future, and to reduce additional research costs. CHAMACOS quality control and assurance procedures involve several components. Every biological sample that is collected is separated into several aliquots that are labeled with a unique bar coded identifier that does not contain personal identifying information about the participant. This identifier is a 14-digit number comprised of codes for clinic, family, family member (e.g., child), contact point (e.g., six-month child assessment), sample type (e.g., urine), and aliquot number. We extensively document collection, processing, storage, and shipping

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Table 4. Biological and environmental samples collected by CHAMACOS.

	2 nd trimester	3 rd trimester	Delivery	Six months	12 Months	24 Months
Maternal urine	✓	✓	✓	✓ (subset*)		
Maternal blood		✓	✓			
Cord blood			✓			
Breast milk			✓	✓ (subset*)		
Paternal urine			✓			
Infant urine				✓	✓	✓
Infant blood					✓	✓
House dust	✓			✓	✓	
Vehicle dust				✓ (subset)		
Burkard air sampling:						
Area sampling	Continual	→	→	→	→	→
House sampling	✓			✓	✓	
Pesticide Use Reporting Data	Continual	→	→	→	→	→

* Only for those women who are breastfeeding at the time of the six-month assessment.

Table 5. Planned analyses of collected CHAMACOS samples.

	Maternal Urine	Maternal Blood	Cord Blood	Breast Milk	Child Urine	Child Blood	House Dust	House Air	Area Air
Organophosphate (OP) Pesticides	✓			✓ (subset)	✓		✓		
Pollen and Mold Spores								✓	✓
Endotoxin							✓		
Endocrine Disruptors	✓	✓							
Lead		✓	✓			✓			
Paraoxanase Polymorphism and Cholinesterase		✓				✓			
IgE antibodies		✓ (subset)				✓ (subset)			
Th1/Th2 cytokines						✓ (subset)			

procedures, which includes the use of standard operating procedures, chain-of-custody forms, discrepancy reports, and tracking databases. CDC methods document that urinary pesticide metabolites are stable in samples stored under -20°C . CHAMACOS urine, blood, and dust aliquots are stored at -80°C . We are conducting storage-stability studies to verify that pesticides and endotoxin are stable under these conditions. For all analyses, we use validated assays and accredited analytical laboratories. Where appropriate (e.g. urine samples), field blanks, spikes, and duplicates are collected and shipped blind to analytical laboratories with each batch of samples.

Challenges in collecting samples

Home environmental samples: As shown in Table 4, house dust samples and Burkard slides (for mold and pollen counts) are collected at each home visit. Participants live throughout the Salinas Valley requiring extensive travel for the staff. The purchase of a study car has reduced wear and tear on staff members' personal automobiles and mileage reimbursement costs. To ensure the safety of study staff, all home visits are done in pairs. To ensure the comfort of the women and their partners, male staff members are always accompanied by female staff members. The equipment used for the home visits required careful cleaning after each use. Each team is supplied with two sets of sampling equipment so they can complete multiple visits without returning to the field office.

We were interested in collecting additional exposure information from some of our study participants, for example, time-activity analyses, dust wipes, food, clothing, etc. However, due to concerns that involvement in extensive substudies might overburden CHAMACOS participants, we have enrolled additional participants with similar demographic characteristics for small pilot investigations. We learned from these pilot studies that children's activity diaries were difficult for parents to complete and required multiple follow-up contacts. We also found that participants were reluctant to collect duplicate diet samples because they were uncomfortable with edible food being discarded. These pilot studies will help to shape future CHAMACOS data collection and will be used to develop multimedia multipathway models of pesticide exposure.

Urine: Pediatric urine bags are used to collect spot urine samples from 6, 12, and 24-month-old children. The urine bag is attached to the child with tape and then pulled through a slit cut in a diaper so that the bag is visible. Once the child voids, the bag is removed. Because the children are stronger and more mobile at 24 months, the diaper is covered with a pair of plastic underpants. Children often do not produce a urine sample in the time it takes to complete the visit, and this has become more of a problem as the children get older. In situations where there is no urine production, the staff provides the mother with materials and instructions on how to reapply the urine bag at home. Once the mother has collected the sample, CHAMACOS staff picks up the urine from the participant's home. Using this protocol, we have missed less than 2% of the child urine samples.

As is the case in many non-urban communities, the Salinas Valley lacks many of the resources and amenities that are available to researchers in large metropolitan areas that are needed to properly collect, store, and transport biological samples. For example, urine should optimally be frozen close to the time of collection in order to preserve the pesticide metabolites from degradation. This requires the availability of dry ice for both storage during transit from collection in the field (particularly in the southern portion of the county) and for shipping from the field office to the laboratory core in Oakland, CA (by overnight courier). However, there is only one supplier of dry ice in the Salinas valley, and this has resulted in insufficient supply at times. The shortage of dry ice has led to delays in sample handling at the field office, and shipment of samples to the core or analytical laboratories (Holland *et al.*, 2002).

Breastmilk: CHAMACOS collected samples from breastfeeding women at delivery and at 6 months. The delivery sample was usually collected in the first few days after delivery, a time when many women may not have had adequate milk production. In order to collect an adequate sample, we used a hospital-grade breast pump. In addition, many delivery breastmilk samples were picked up at the participant's home at a later time. We often had to assure many women that providing a breastmilk sample would not result in inadequate milk supply for their child.

Blood: Blood samples are collected at multiple time points: during pregnancy, at delivery, and from the children. Blood specimens have been processed and stored in various forms in order to be appropriate for many different assays (see Figures 1 and 2). In the southern portion of the county, there are no facilities for processing blood, such as a laboratory with a centrifuge adequate for separating whole blood into the products (buffy coats, plasma, serum and clots) required for the various planned assays (see Table 5). This means that samples collected have to be transported in a timely manner to the hospital laboratory for processing. There is only one courier service in the area, which operates only during normal weekday business hours. This has not been adequate, and in order to preserve our samples staff members frequently have to drive long distances to pick up samples.

The lack of facilities in the area contributed to our inability to preserve RNA or viable cells from maternal and cord blood samples. In order to extract RNA from blood, it is necessary to stabilize specimens in an appropriate buffer soon after collection and to freeze them promptly (Holland *et al.*, 2002). To overcome this obstacle, we changed the blood processing protocol for the latter samples of the 12-month and all of the 24-month children's bloods. These bloods are collected into both a red-top and green-top vacutainer (again after an aliquot is removed for lead testing), and now are transported within 48 h by FedEx® to the University of California, Berkeley, where there are state-of-the-art facilities with sterile biological hoods, nitrogen tanks, refrigerated centrifuges, flow cytometry facilities, and efficient CO2 incubators. Before changing the protocol, we determined that the conditions and timing of shipping from Salinas to Berkeley did not compromise the quality of the biomarkers analyzed. The samples are processed according to a

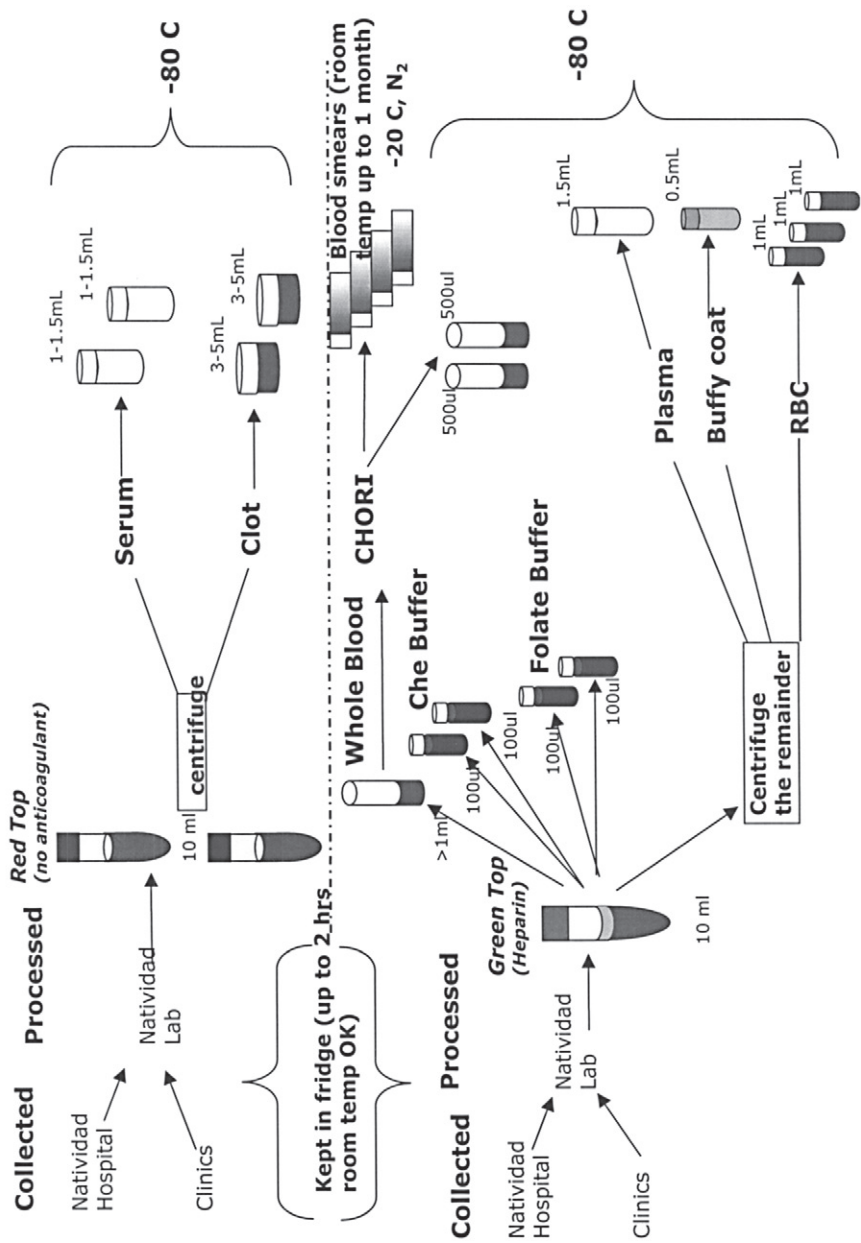


Figure 1. Prenatal blood processing protocol for CHAMACOS.

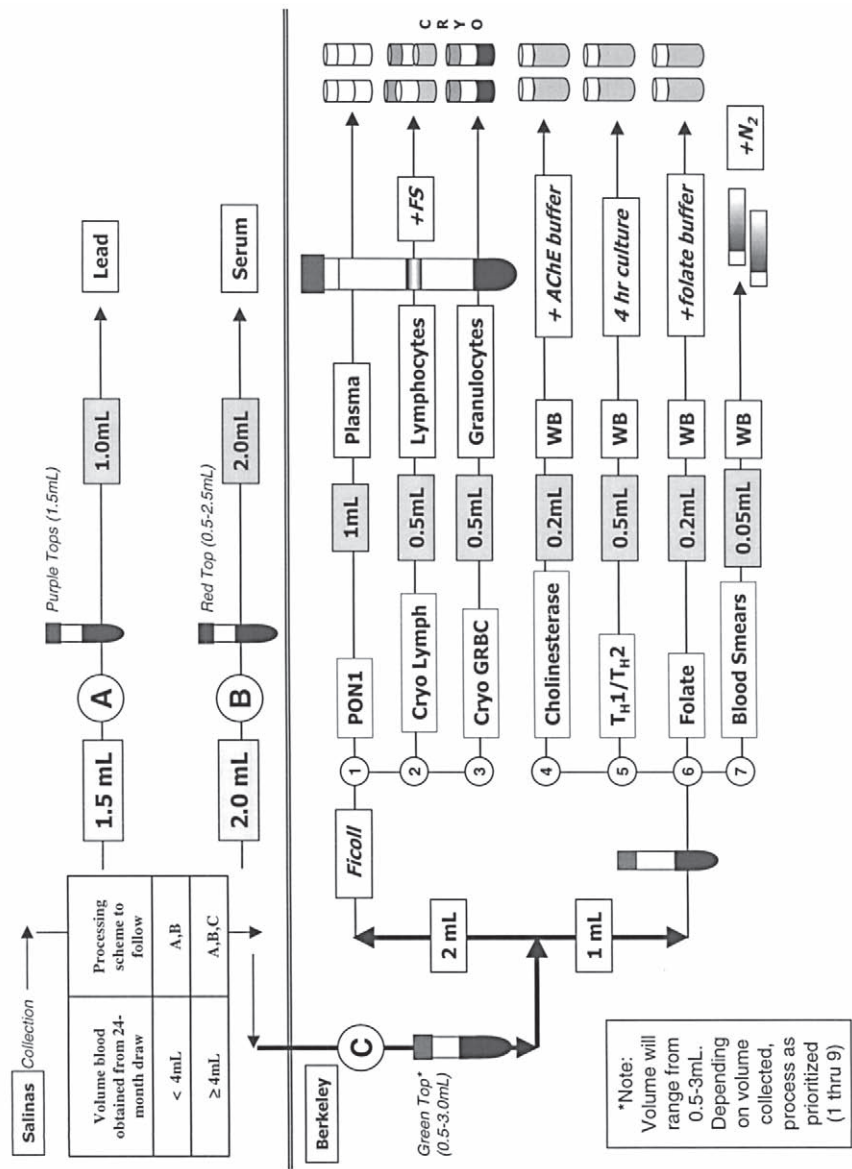


Figure 2. 24-month child blood processing protocol for CHAMACOS.

complex protocol (see Figure 2) that includes provisions to test for IgE, intracellular cytokines, immunological profile, cholinesterase, paraoxonase polymorphism, and other tests (see Table 5). We are also able to cryopreserve cells from pediatric blood samples, assuring the possibility of future reculturing, molecular cytogenetic analysis, and RNA isolation. According to our pilot data, RNA yield from proliferating cells is enhanced due to the increased cell number as well as an increased RNA yield per cell (Holland *et al.*, 2002). Thus, although the processing, storage, and shipping of such a large number of samples is labor-intensive, provisions have been made for a myriad of assays, including high-throughput methods of biomarker analyses based on PCR, chip, and microarray technologies currently in development.

Many challenges have arisen specifically relating to blood collection. Many CHAMACOS participants had been wary of providing blood samples, because of concerns about potential health effects of blood being drawn from pregnant women or young children. We have taken these concerns into consideration in a number of ways: (1) all blood is drawn for CHAMACOS as a part of regular medical care (e.g., 26-week gestation glucose tolerance test, child lead screenings at 12 and 24 months), and if this opportunity is missed, we do not draw an additional sample; (2) we have consulted with gynecologists and pediatricians to determine the maximum acceptable amount of blood that can be taken safely and in a manner that would reduce pain and perceived risk (e.g., it is less distressing for the mother for us to collect their child's blood in a few larger tubes than in multiple smaller tubes); (3) there are never more than two attempts to draw blood from a child. CHAMACOS staff work diligently to educate participants about the safety of blood draws and the very small volume of blood that is collected from participant children (approximately 5 mL). We have also ensured the successful collection and processing of blood samples by supplying collection kits to health care providers and aliquoting kits to laboratories.

Originally, CHAMACOS proposed that NMC begin using a venipuncture method for umbilical cord blood collection, but this was unacceptable to NMC clinical staff due to concerns about accidental needlesticks. Subsequently, CHAMACOS and NMC staff devised a collection method that minimized contamination and was amenable to the NMC staff, and CHAMACOS trained Natividad nursing staff, residents, and physicians in this method. After the placenta was delivered the cord was clamped, cleaned, and sterilized by the nursing staff. The blood was then drained into a sterile collection cup and aliquoted into red- and green-top vacutainers from kits supplied by CHAMACOS. Random samples of cord blood specimens have been tested for the presence of maternal cells, and none were found, indicating the success of this method.

We have described many of the difficulties encountered in the handling of biological samples in the field, and in shipping to the biorepository. It should be emphasized, however, that despite the obstacles encountered along the way we were able to maintain the integrity of the samples due to several factors, such as: (1) diligent adherence to standard operating procedures for all aspects of sample

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handling, processing, storage, and shipping; (2) constant oversight by supervisors and retraining of staff; and (3) an extensive quality control/quality assurance plan. In addition, because of the changes we have made to the general operating procedures of the clinics and hospitals, CHAMACOS has served to improve compliance with the state-mandated test for lead levels in the pediatric population of Monterey County. By hiring and training a pediatric phlebotomist, we helped to increase lead screenings for 12- and 24-month old children from an estimated 20% of children to 60% at 12 months, and, thus far, 78% at 24 months.

CONCLUSION: LESSONS LEARNED

Longitudinal birth cohort studies provide a rich source of information about pregnancy, childhood, and early etiology for late onset disease. Longitudinal cohort studies are more complex logistically and ethically today than in the past in part due to the advances in molecular and cell biology and in computer sciences. In anticipation of the National Birth Cohort study, lessons can be learned from other current smaller birth cohort studies such as CHAMACOS, which have also focused on environmental causes of developmental disease. Such lessons include:

1. Building community infrastructure and trust is essential, especially in difficult to reach populations.
2. All study instruments must not only be translated into the appropriate languages but must reflect the specific culture of the population. It is important to understand the cultural barriers to research. Focus groups with community members and staff are instrumental in understanding these barriers.
3. The medical care system is overburdened, particularly the facilities that provide care to low-income and rural populations. Therefore, a research study must rely minimally on clinical staff; researchers can make a study even more feasible by reducing their clinical burden, for example, by providing sample collection kits, screening/enrollment packets, lists of term women to the delivery ward.
4. In some populations, obtaining informed consent may require additional steps. This is especially true for low-literacy immigrant populations. Consents and study protocols must protect individual participants, and staff must receive formal training in these procedures. Ideally, consent forms should be written in a way that would allow materials and specimens to be used for additional studies.
5. Participant tracking is essential in longitudinal cohort studies and may be quite difficult, especially in a mobile population. Efforts should be made to keep frequent contact with participants, and to obtain names of a number of people who may know participants' whereabouts. We found that getting the

contact information of the women's mother was very useful. In addition, useful incentives can help to keep a participant interested in the study.

6. Research staff has to be flexible in their approach and be willing to work nights and weekends. Tagging a lengthy research protocol onto a clinical visit may not always be feasible, especially at demanding times (for example, post-delivery). Conducting an evaluation in the home with other children present may be disruptive, creating the need for other provisions, such as a mobile testing site.
7. There are many issues to be taken into consideration with regard to laboratory specimens, especially in rural environments. As far as possible, the specimens should be processed in a way that is optimal for many future uses. Special attention should be paid to the importance of careful handling and storage of these precious biological samples, with the goal of obtaining a large amount of information from often limited specimens (especially of pediatric origin), and minimizing future research costs by use of banked samples.

The challenges of collecting longitudinal birth cohort data are in part unique to the particular community. These challenges may be greatest in those communities with the most complex lives, such as those of other cultures or those who are impoverished. Nevertheless, it is essential to include these populations in the National Children's Study, since it is exactly these communities that are most at risk for exposure to environmental hazards.

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