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The Rationale for a National Prospective Cohort Study of Environmental Exposure and Childhood Development

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Evidence is growing that pre- and perinatal exposures and factors play a role in not only childhood but adulthood disorders. Therefore, there is a compelling need to undertake a national cohort study to evaluate the effects of such factors, ideally through adult life. Several recent developments, including advancements in computer technology, the management, storage, and analysis of biological specimens, and the rapid growth of genetic markers, facilitate the evaluation of the influence of environmental exposures on the subsequent risk of developmental abnormalities and disease. The rationale behind the establishment of such a cohort is discussed.

Key Words: **environment; prenatal; perinatal; neurodevelopment; cohort.**

INTRODUCTION

Human development is the consequence of a complex interplay between genetic and environmental influences. Genomic information directs the overall direction and timing of development and defines the range of developmental trajectories. Environmental factors—defined broadly as the entire gamut of extragenetic influences that operate from before conception through adult life—can modify development and in turn can either increase or decrease the risk of developmental anomaly and of disease.

Epidemiologic and clinical research has identified a series of extragenetic factors that can modify human development. Maternal cigarette smoking is known to lower birth weight (Berkowitz, 1988). Heavy alcohol consumption during pregnancy has clearly been implicated as a cause of morphologic anomalies and intellectual impairment in the offspring (Streissguth *et al*., 1983). *In utero* exposure to diethylstilbestrol (DES) can cause vaginal carcinoma in young women (Herbst and Bern, 1981) and reproductive tract abnormalities in males (Giusti *et al*., 1995). Exposure to lead can lead to decreased intelligence and shortening of attention spain, with subsequent increased risk of dyslexia, school failure, and possibly delinquent behavior (Bellinger *et al*., 1987; Dietrich *et al*., 1981; McMichael *et al*., 1988; Needleman *et al*., 1996; Wasserman *et al*., 1992). Intrauterine exposure to ionizing radiation has been linked to childhood cancer (Wakeford, 1995). Polychlorinated biphenyls (PCBs) have been found to increase the risk of neurodevelopmental impairment, even at low exposures (Jacobson and Jacobson, 1996; Jacobson *et al*., 1990; Koopman-Esseboom *et al*., 1996; Patandin *et al*., 1999; Rogan and Gladen, 1992). Neurological damage may also result from methylmercury (Grandjean *et al*., 1997) and pesticide exposures (Eskenazi *et al*., 1999).

Evidence is growing that perinatal factors play a role in the development of disease in later life. Low birth weight has been linked both to cardiovascular disease (Barker *et al*., 1989) and diabetes (Hales and Barker, 1992). The effect of perinatal factors on later health status is an exciting field for research, but is also a great challenge, as it will require long-term prospective cohorts to quantify environmental exposures and to evaluate perinatal factors that are not routinely recorded in medical charts or birth certificates. At the same time, technological advances in laboratory, techniques, data management, and analysis of biological specimens make possible assessment of the effects of early exposures on childhood and adulthood disorders.

In this paper, we discuss the rationale for a national, prospective study of the consequences of early life exposures on human health. Although the principal focus will be on the influence of environmental

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exposures upon childhood development, such a study needs also to incorporate a broad range of perinatal factors that may affect other diseases, in both childhood and adulthood.

MAGNITUDE OF POTENTIAL ENVIRONMENTAL EXPOSURES

Today there are over 80,000 commercial synthetic chemicals, most of which have been formulated since World War II. Pregnant women and their infants are particularly at risk for adverse effects of exposure. Specifically, there are 15,000 high-production-volume chemicals—pesticides, solvents, fuel additives, plasticizers, and cleaners. Many of these materials are contained in household products and are widely distributed in air, water, and food (US Environmental Protection Agency, 1998). Fewer than half of these high-volume products have been tested for their potential human toxicity, and fewer still for their toxicity to the young (National Academy of Sciences, 1984). The potential impacts of these myriad chemicals on the health and development of the fetus and infant are only beginning to be understood. These impacts appear to be affected by the timing of exposure, the route of exposure, interactions among extragenetic factors, as well as interactions between environmental and genetic factors.

TIMING OF EXPOSURE

Little is known about the effects on child health of preconceptional exposures to the parents. Toxicants can damage oocytes, which could lead to abnormal childhood development, if the embryo survives (Basler *et al*., 1976). Chemicals could also cause germ cell mutations, although there is some evidence that gene mutations are more likely in sperm, while chromosomal anomalies are more frequent in oocytes (Hatch and Marcus, 1991; Martin *et al*., 1983). Chromosomal anomalies, such as Down's Syndrome, have in turn been associated with childhood leukemia (Mertens *et al*., 1998).

Prenatal exposure to environmental agents may give rise to a variety of adverse outcomes, depending on the stage of gestation when exposure occurs. An environmental insult during the implantation period, which begins at the end of the first week after fertilization (Pritchard *et al*., 1985), traditionally was thought to result in either embryonic loss or cell damage that could be repaired during subsequent normal development (Wilson and Fraser, 1997). Experimental evidence, however, suggests that such insults may cause developmental abnormalities (Iannoconne *et al.*, 1987). The embryonic stage, which corresponds to the interval from implantation until the eighth week (Pritchard *et al*., 1985), is the period of major organogenesis and is thus the critical period for teratogenic effects (Wilson and Fraser, 1997). The vulnerable period for individual organ systems is generally narrow. For example, thalidomide exposure has been associated with phocomelia and other anatomic abnormalities during the period 34 to 50 days after the last menstrual period (Lenz, 1963). However, recent evidence also suggests that thalidomide exposure between 20 and 24 days of gestation (or approximately 34 to 38 days after the last menstrual period) can cause autism (Miller and Stromland, 1993), presumably through an injury at the time of closure of the neural tube (Rodier *et al*., 1996). Thus, exposure during the embryonic stage has also been linked to developmental impairment in childhood.

The fetal period, beginning 8 weeks after fertilization (Pritchard *et al*., 1985), is associated with cell differentiation, for example, of the sex organs and the central nervous system, and with the growth and functional maturation of organ systems. As a result, insults during this period are most commonly associated with deviation from normal growth and development, such as intrauterine growth retardation and subsequent developmental disabilities (Tuchmann-Duplessis, 1983; Wilson and Fraser, 1997).

While the influence of environmental exposures as a function of gestational age is a generally accepted principle, it should be emphasized that these observations are largely based on animal experiments and may not be generalizable to humans, as evidenced by the thalidomide experience, where initial animal studies found no teratogenic effects (Somers, 1962). Experimental data also suggest that the type of adverse outcome is dependent on the magnitude of the exposure dose. It has been hypothesized, for example, that the outcome may follow a gradient comprising functional deficits, growth retardation, congenital malformations, and fetal death as the level of exposure increases (Barr *et al*., 1979).

Growth during the prenatal period results primarily from an increase in the number of cells in the body (hyperplasia), but the major part of postnatal growth occurs from an increase in cell size (hypertrophy). Much of the functional maturation of organs also occurs during various postnatal age periods. As an example, the growth of neuronal cells is relatively complete by age 2 years, but full myelinization of neuronal tissue is not achieved until approximately 18 years of age (National Research Council, 1993).

Postnatal exposure to environmental toxins can occur either directly (e.g., by inhalation, dermal, or hand-to-mouth contact) or via breast milk. Occupational exposures as well as environmentally derived heavy metals and chemicals have been detected in breast milk. The most commonly found heavy metals in breast milk have been lead, mercury, and cadmium (Wolff, 1993). A number of organochlorines, most prominently DDT and PCB, have also been detected in breast milk (Wolff, 1983).

SUSCEPTIBILITY TO TOXICANTS IN THE ENVIRONMENT

Because the tissues and organs of fetuses and infants are rapidly growing and developing at various stages, these growth processes create windows of great vulnerability to environmental toxicants. Children's patterns of exposure also differ from those of adults.

The National Academy of Sciences' report, "Pesticides in the Diets of Infants and Children" (1993), identified several factors that may increase the susceptibility of children to toxicants. Some of these factors also apply to the fetus. First, children tend to be more heavily exposed than adults. Based on body weight, children drink more water, eat more food, and breathe more air than adults. Increased dermal contact and oral ingestion of toxicants, such as pesticides, among infants and toddlers are also attributed to their hand-to-mouth activity and to the fact that they play close to the ground. In addition, children tend to be more exposed to such potential toxicants as indoor pesticides because they spend more of their time at home than adults.

Second, the metabolic pathways of fetuses and infants are immature compared to those of adults. Differences exist in the absorption, metabolism, detoxification, and excretion of xenobiotic compounds (Spielberg, 1992; Warner, 1986). While fetuses and infants are able to metabolize certain toxicants to their active form and thus are relatively resistant to their effects, they are less able to detoxify chemicals such as organophosphate pesticides. Thus, children may be more or less sensitive than adults, depending on the particular toxicant exposure, but in most cases are more vulnerable.

As noted above, cellular and tissue growth is very rapid during the prenatal period and during the first months of life. Growth and development, particularly of the nervous, endocrine, and reproductive systems, also continue during childhood. As a result, levels believed to be safe for adults may result in permanent brain damage if neurotoxic exposure occurred during the prenatal and early childhood period (National Research Council, 1993).

Finally, because children are exposed to toxicants at an earlier age and can be expected to have more future years of life than adults, they have a longer time period to develop diseases and disorders that may be linked to early exposures. Since we still know relatively little about the causes of diseases with long latency periods, such as cancers, it is possible that early environmental exposures play a role in the development of such diseases. Also, the potential impact of multiple and cumulative exposures need to be taken into account.

EARLY EXPOSURES AND PEDIATRIC DISORDERS

With the retreat of infectious diseases, childhood diseases today predominantly consist of a series of chronically disabling conditions that have been termed the "new pediatric morbidity." These diseases include neurodevelopmental impairments, disorders of endocrine and reproductive development, asthma, and childhood cancer. Because many of these disorders appear to be increasing, environmental factors are suspected of playing a role in their etiology. There are no United States registres of neurodevelopmental disorders, which include learning disabilities, intellectual retardation, attention deficit hyperactivity disorder, autism, and juvenile delinquency. However, a 1988 survey of United States households estimated that approximately 17% of children under the age of 18 had ever had at least one developmental disability (Boyle, 1994). Many of these deficits are permanent. Among the disorders that may be affected by so-called endocrine disrupters, it is noteworthy that the incidence of hypospadias has doubled (Paulozzi *et al*., 1997). Testicular cancer in young men aged 15 to 29 years has risen by 68% (De Vesa *et al*., 1995), although it is not clear whether there has been a concomitant increase in cryptochidism (Berkowitz *et al*., 1993), which is the major risk factor for testicular cancer in young men (Pottern *et al*., 1985). Nevertheless, since this increase in testicular cancer appears to represent a birth cohort effect, the influence of environmental factors, whether *in utero* or postnatally, needs to be explored. The mortality for asthma has doubled and this condition has become the leading cause of hospital admissions among urban children (Centers for Disease Control and Prevention, 1996). Asthma currently affects more than 4.8 million children and adolescents under the age of 18 in the United States (Adams and Marano, 1995).

Childhood cancer has also increased: brain tumors have risen by 30% and acute lymphocytic leukemias are up 10% over the past 15 years (De Vesa *et al*., 1995). Although the causes of these disorders are not known, certain environmental links, such as between nitrites from cured meats and childhood brain tumors (Preston-Martin *et al*., 1996), have been suggested. Thus, an important environmental influence on these disease cannot be ruled out.

EARLY EXPOSURES AND ADULT DISORDERS

Much of the social and geographic variation in chronic disease risk is currently unexplained by risk factors present in adults. Some investigators have looked earlier in the lifecourse of individuals, even into the prenatal period, for evidence of additional risk factors. Ecologic studies linking infant mortality and other measures of early life deprivation at the community level to adult mortality led to the hypothesis that undernutrition early in life could cause an increased susceptibility to increased cholesterol level with a plentiful diet in adult life (Barker and Osmond, 1986; Forsdahl, 1977). A large number of individual level studies followed of potential early antecedents of adult health, many of them by investigators at the Environmental Epidemiology Unit at the University of Southampton, England (Barker, 1994). This group has focused on fetal and infant growth as proxies for early nutrition and has found these measures to inversely correlate with cardiovascular mortality and morbidity (Barker, 1994; Barker *et al*., 1989). Later studies have suggested links between fetal growth and potential cardiovascular disease antecedents, such as high blood pressure, adverse serum lipid profiles, elevated plasma fibrinogen, impaired glucose tolerance, and decreased arterial compliance (Fall *et al*., 1995; Leon *et al*., 1996). Together, these findings, supported by some animal studies (Dahri *et al*., 1991; Persson and Jansson, 1992), have been interpreted by some as indicating that an environment which impairs growth *in utero*, or early childhood, can influence an individual's metabolism and/or physiology in a way that increases the risk of cardiovascular disease in adult life.

However, studies to examine this "fetal programming" hypothesis have not yielded entirely consistent results. Positive studies have been subject to criticism, with the limitations cited including loss to follow-up, crudely measured early life factors, and inadequate control for confounding, especially by familial factors (Joseph and Kramer, 1996; Paneth *et al*., 1996; Paneth and Susser, 1995). Moreover, while adult morbidity may be linked with prenatal events, associations with specific environmental exposures have not been adequately investigated. While some of these limitations can be addressed in longitudinal studies of existing cohorts, such as the Collaborative Perinatal Project (Broman, 1984) and the California Health and Development Studies (Van den Berg *et al*., 1988), others will require careful design and long-term follow-up of new cohorts using state-of-the-art methods.

ESTABLISHED OR SUSPECTED NEURODEVELOMENTAL TOXICANTS

A number of environmental exposures are established or suspected risk factors for neurodevelopmental impairment. Initial concern regarding these neurodevelopmental toxicants generally arose from accidental or high-level exposures, but data are now available linking low-level exposures to such deficits.

Lead is a well-established neurotoxin in both children and adults. In children, lead is recognized to produce a range of toxic effects that extend from acute, clinically obvious symptomatic poisoning to subclinical neurobiological and developmental impairment. Increased body lead burdens in school children have been associated with juvenile delinquency (Needleman *et al*., 1996). Several recent prospective studies of newborn children have found evidence for the neuropsychologic toxicity of lead at low doses (Bellinger *et al*., 1987; Dietrich *et al*., 1981; McMichael *et al*., 1988; Wasserman *et al*., 1992). In each of these investigations, correlations have been sought between developmental decrements and umbilical cord blood lead levels at birth. All of the studies have found slowing of development and subclinical, but apparently irreversible, dysfunction in clinical neurologic function at cord blood lead levels above 10 μg/dl. Despite the dramatic decline in blood lead levels that followed the removal of most lead from gasoline in the 1970s, the Centers for Disease Control and Prevention estimated that 930,000 children aged 1–5 years had blood lead levels of 10 μg/dl or higher between 1991 and 1994 (Centers for Disease Control and Prevention, 1997). Inner city, indigent children living in deteriorated housing are particularly susceptible to lead poisoning and the prevalence of elevated blood lead levels is especially high in this population.

PCBs, which are chlorinated aromatic hydrocarbons, provide another developmental insult. Their production was halted in 1976. However, PCBs are

biologically persistent in sediments, fish, and shellfish in many waters in North America and are widespread via the food chain. Maternal consumption of rice oil contaminated with PCBs in Japan (Kuratsune *et al*., 1997) and Taiwan (Chen *et al*., 1992) demonstrated that such high-level exposure can lead to delayed development and lower IQ scores among offspring. Five cohort studies in North America and Europe indicate that levels in the general population range have subtle adverse effects on neurodevelopment, including slower psychomotor and mental development (Jacobson and Jacobson, 1996; Jacobson *et al*., 1990; Koopman-Esseboom *et al*., 1996; Patandin *et al*., 1999; Rogan and Gladen, 1992). The data also suggest that prenatal exposure has a greater effect than postnatal exposure (Longnecker *et al*., 1997). However, the evidence is not entirely consistent, perhaps due to differences in exposure assessment, study populations, and measures of neurodevelopmental status (Schwartz, 1996). Further limitations of the existing literature are the lack of studies on minority and low socioeconomic status subjects and on the influence of the individual congeners of PCBs.

Another dangerous toxin is methylmercury. Microorganisms in rivers, lakes, and streams convert organic and inorganic mercury to methylmercury. Accidental *in utero* exposure to high levels of methylmercury has produced devastating neurological damage, including cerebral palsy, deafness, and mental retardation (Cox *et al*., 1995; Matsumoto *et al*., 1965). However, the effects of low exposures are less clear. A cohort study from the Faroe Islands reported that consumption of primarily pilot whales was associated with mild cognitive deficits at age 7 (Grandjean *et al*., 1997). In contrast, in a study from the Republic of Seychelles, maternal and child hair methylmercury levels were not associated with any adverse effects on neurodevelopment at 66 months (Davidson *et al*., 1998). The reason for the discrepant findings may lie in differences in timing of exposure, measures of cognitive testing, and the timing of the follow-up testing.

Increasing attention is also being paid to the potentially adverse health effects of both indoor and outdoor pesticide exposures. More than 1 billion pounds of pesticide are purchased each year in the United States (Schierow, 1996). Indoor pesticide exposure is of particular concern for infants and young children, as they spend most of their time within the home (Gurunathan *et al*., 1998; Silvers *et al*., 1994). Chlorpyrifos, which is an organophosphate, is one of the most widely detectable pesticides in American homes and is the most commonly used indoor pesticide in New York (Their *et al*., 1998). Acute high-dose exposure to chlorpyrifos produces classic inhibition of the enzyme acetylcholinesterase in the nervous system, leading to a spectrum of cholinergic symptoms (Richard, 1995). Although there are no human data on the effects of low-dose exposure, substantial animal data indicate that low-level exposure may affect neurodevelopment and growth in developing animals (Ekenazi *et al*., 1999). Other commonly used indoor pesticides include chlordane, heptachlor, dieldrin, malathion, parathion, diazinon, and dichlorvos (Whitmore *et al*., 1994).

Finally, alcohol is also a known neurotoxicant. Fetal alcohol syndrome, resulting from maternal ingestion of alcohol during gestation, includes characteristic morphological abnormalities, with intellectual impairment to various degrees as a prominent feature as well (Abel and Sokol, 1987). The mechanism by which alcohol can cause fetal damage may also be relevant to the potential effects of metabolites of toxicants.

EXISTING CHILDHOOD COHORTS

Cohorts that began either perinatally or at birth are in existence. For instance, the Collaborative Perinatal Project (CPP) was established by the National Institute of Neurological and Communicative Disorders and Stroke as a prospective epidemiologic study of the relationship between factors related to pregnancy, labor, and delivery and subsequent health and neurodevelopment in infants and children (Broman, 1984). Fourteen medical centers within 12 universities collected data on over 58,000 pregnancies and the health of surviving children through age 7 or 8. Because the CPP collaborating centers largely enrolled pregnant women from urban hospital prenatal clinics, the CPP cohorts were of lower average socioeconomic status than were children born in the United States during the same time period. Similarly, the California Health and Development Studies (CHDS) examined approximately 20,000 pregnancies, birth outcomes, and health in surviving children (Van den Berg *et al*., 1988). Families enrolled in CHDS resided in the Oakland area and were members of the Kaiser Foundation health plan and were, therefore, of higher average socioeconomic status than CPP enrollees. While differing from CPP in some methodologic details, CHDS spanned the same enrollment period as CPP (1959–1966) and included data on most of the same key pre- and perinatal factors, and in both studies, maternal serum was obtained and stored at -20°C. Together, these cohorts have

a unique combination of attributes: large sample size; geographic and demographic diversity; and detailed, prospective, standardized prenatal and perinatal measures of clinical, social, and environmental factors.

The 1946 birth cohort, which included a sample of all births in England, Scotland, and Wales, is the only cohort that has provided long-term follow-up of a subsample (Wadsworth *et al*., 1992). However, since the cohort was formed at birth, no data on prenatal exposure or events are available. More recent cohorts with continued follow-up have also been established in Europe, including the Avon Longitudinal Study of Pregnancy and Childhood in England (Golding, 1996) and the Danish Perinatal Study (Baker and Mednick, 1984). While follow-up of birth cohorts into adult life is a challenge, this is particularly true among relatively mobile United States populations. Nevertheless, investigators at some sites have proven it to be feasible. For example, a subset of the Providence CPP cohort was recontacted at ages 18 to 27 years to examine the relationship between prenatal and delivery complications and psychiatric disorders in adult (Buka *et al*., 1993). The 1758 members of the Baltimore CPP cohort were followed up at ages 27 to 33 years to study early life factors that predict educational attainment (Hardy *et al*., 1997). The CHDS cohorts are currently being followed for a nested case-control study of prenatal determinants of schizophrenia (Susser, 1999).

However, none of these cohorts has specifically focused on environmental exposures. Yet, there is growing evidence that the developing fetus and infant are particularly vulnerable to such exposures. Animal studies link prenatal chemical exposure to "latent" health effects that emerge in later life. Human studies link prenatal growth and development to adult health and researchers have found that prenatal environmental exposures can affect fetal growth. Therefore, a pregnancy cohort study that focuses on such exposures is greatly needed: one that is designed to disentangle the relationships among environmental exposures, perinatal factors, and long-term outcomes. The purpose of this paper is to outline the rationale behind and some of the methodological difficulties related to such a cohort.

ESTABLISHMENT OF A CHILDREN'S COHORT STUDY

In setting up a cohort, it is important to use comparable approaches to prior cohorts, especially those with stored biological specimens and good neurodevelopmental assessments. Obviously, this does not mean that only previously used protocols should be included. However, if the basic measures used in previous studies are included, this would permit comparisons of outcomes as well as exposure levels.

The rationale behind a childhood or lifelong cohort is to examine the influence of early life exposures. Therefore, women should be recruited during early pregnancy, and ideally before conception. Although this approach excludes women with late or no prenatal care, this design would enable optimal exposure assessment.

The nature and composition of the study population is an important issue to be considered in setting up a cohort study. Ideally, the cohort should be population-based, but logistically that may be very difficult. A multicenter hospital and/or HMO-based study would appear more feasible. The Collaborative Perinatal Project, for example, comprised 14 medical centers. A heterogeneous population with respect to race/ethnicity and socioeconomic status is important. This may require oversampling of certain population groups. In our Children's Environmental Health Cohort Study at Mount Sinai Hospital, we are recruiting equal proportions of African American, Hispanic, and Caucasian prenatal patients to ensure such heterogeneity. Because of the racial/and ethnic composition of our patient population, Asian patients could not be recruited, but obviously should be included in a larger cohort study.

RECRUITMENT

Informed consent to participate in the proposed cohort study must be obtained according to federal regulations and those of local institutional review boards. The consent process must include measures for ensuring protection of confidentiality for the study subjects. Consent is to be obtained in a setting where the patient can clearly understand the purpose of the study, what will be required of her, and what risks and benefits may be related to the investigation. According to the Office for Protection from Research Risks (OPRR), a consent form should be written in language understandable to a person with a sixth grade education. In addition, many patients are reluctant to have additional needle sticks for blood sampling. As a result, it is expedient to request additional blood when venipuncture is being performed for routine, clinical studies. In general, invasive procedures should be minimized, as the subjects in a prospective children's or life-long cohort

are essentially healthy volunteers who may not directly benefit from the research. Further, retention and follow-up can be undermined by repeating invasive procedures.

Particular attention should be paid to consent procedures for patients who do not speak English. For example, in areas where there is a high proportion of Hispanic patients, all study instruments should be available in Spanish. The same is true for Asian patients. Also, all instruments need to be piloted, particularly because of the diversity of dialects among non-English-speaking patients.

SAMPLING OF SPECIMENS

A variety of tissues and fluids for laboratory assessments of environmental exposure are available both prenatally and postnatally. Prenatally, the most common maternal samples are blood, urine, and hair. Amniotic fluid may also be available if an amniocentesis has been performed for clinical purposes. However, such fluid would be restricted to a subsample that would not be representative of the overall cohort. Paternal samples are also of importance particularly if genetic studies are to be undertaken. Postnatally, placental tissue and cord blood are commonly sampled for research purposes as these involve noninvasive procedures. Breast milk could also be obtained if the mother is lactating. Again, such sampling may not be representative of the overall cohort, as the frequency of lactation varies by race/ethnicity and socioeconomic status. Maternal and infant urine could be obtained, although infant urine is not always easy to collect. Infant blood is even more difficult to obtain because of the infant's relatively limited blood supply. Other potential biological specimens include nails, saliva, and buccal swabs, but their applicability to exposure measures relevant to neurodevelopment, for example, is unclear.

The timing of biological samples for exposure biomarkers should correspond to the critical period of development with respect to the study outcome. For example, in our study, which is focused on neurodevelopment, maternal blood and urine are obtained around 26 to 28 weeks of gestation; cord blood is collected at delivery as a measure of late fetal exposure; and infant urine is obtained at 1 and 2 years of age for evaluation of postnatal exposures.

SPECIMEN BANKING

Biological specimens that are to be archived for future analysis must be collected, processed, stored, and maintained in a proper fashion if they are to be usable in later laboratory studies. Records of sample deposit, retrieval, thawing, etc. must be maintained in sample logs. These records would be part of the chain-of-custody of the biological specimens.

In part, the processing and storage mechanisms depend upon what will be measured later. For example, if certain enzymes are to be determined, it would be essential to process blood quickly (to limit enzyme degradation) and to store blood at low temperatures (to prevent further decomposition over time). In general, these procedures must be validated before a study is undertaken.

In the case of environmental epidemiology, biomarkers of exposure are often of primary interest. During sample collection, contamination and degradation must be avoided. Using lead as an example, contamination of samples from lead in the air, on the skin, or in the collection tubes are potential problems to avoid. Similarly, cigarette smoke contains high levels of cadmium, so that samples to be analyzed for this metal must be processed in appropriately clean rooms. Some environmental agents, for example, polycyclic aromatic hydrocarbons, are light-sensitive and must be stored in amber containers. Other collection problems to consider are heat sensitivity, volatility, adherence to glass, elution of plasticizers, pH, and chemical instability.

Urine samples are usually stored with preservatives to prevent bacterial and chemical decomposition. Blood samples require collection in chemical-specific tubes, for the purposes of preserving whole blood with the proper anti-coagulant or its components (e.g., leukocytes for DNA) or compartments (e.g., serum). If the environmental agent resides in the red cell, then the sample may need to be analyzed immediately (i.e., zinc protoporphyrin) or it may be possible to store frozen, anti-coagulated specimens indefinitely (i.e., lead).

Storage itself is an important consideration (Donnelly *et al*., 1995; Komaromy-Hiller *et al*., 1997; Landi and Caporaso, 1997; Pero *et al*., 1998; Subramanian, 1995). Samples may need to be frozen at a very low temperature to maintain the integrity of all of the enzymes, but for organochlorines, higher temperatures (e.g., $-20^{\circ}C$) may be sufficient. Freezers with frost-free capability may lead to desiccation of the specimens, rendering them useless for most assays in which weight or volume is the denominator of the measurement. Freezers and refrigerators which contain specimens must be maintained in a facility with oversight and with personnel available to remove samples in case of power failure or mechanical breakdown. Resources for these

procedures and for storage facilities must be built into the research plan.

FOLLOW-UP PROCEDURES

Follow-up, especially if it is long-term, is undoubtedly the most difficult component of a prospective study. The subjects must clearly understand the nature and time frame of their commitment. Procedures for tracking patients need to be in place at the beginning of the study. Follow-up of inner city, indigent populations is particularly difficult because of frequent moves, telephone disconnections, and generally unstable living conditions. We recommend that information regarding other contact persons be obtained at the time of recruitment and that frequent contact be maintained either by telephone or mail during the follow-up period. Community participation through advisory boards is another component that may facilitate both recruitment and retention of subjects. In addition to follow-up visits or questionnaires, tumor registries and vital statistics can be used to ascertain disease outcomes and mortality. A follow-up study that spans decades is extremely complex, as it would entail changes in investigators and research staff, new and more sensitive laboratory assays, and possibly different diagnostic criteria.

CONCLUSION

Despite the difficulties of conducting an environmental childhood or life-long cohort study, the needs for such a study are compelling. Although knowledge has been accumulating regarding the underlying mechanisms of many childhood and adulthood disorders, identified risk factors generally explain only a fraction of cases. Furthermore, little is known about the effects of environmental exposures on these disorders. Given the escalating growth of synthetic chemicals in our environment, prospective cohort studies that begin with assessments of *in utero* exposures and that follow offspring through childhood and adulthood are needed to obviate preventable disease burdens.

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