Breast Cancer & the Environment Research Centers

NIEHS NCI
Early Environmental Exposures

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Progress Report

Department of Health and Human Services
National Institutes of Health

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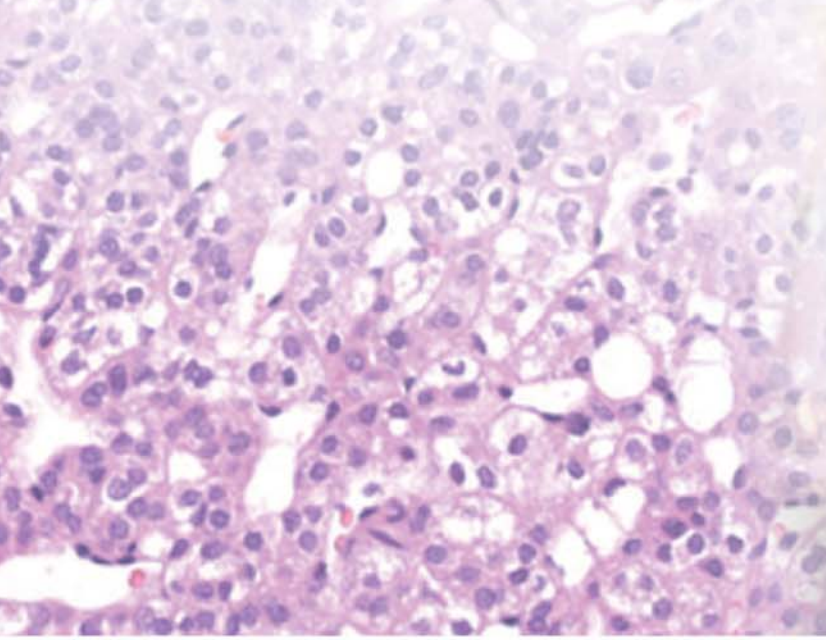
March 2009



The Breast Cancer and the Environment Research Centers

Fox Chase Cancer Center

Center Director

Jose Russo, M.D.

Collaborators

Mount Sinai School of Medicine
University of Alabama at Birmingham

Community Partners

The Renaissance University for Community Education of the Harlem Children's Zone Project: Girls, Inc.
New York City Parks Foundation
Community Science Specialists
Share
Avon Foundation
American Cancer Society
Huntington Breast Cancer Action Coalition, New York
Linda Creed Breast Cancer Foundation, Philadelphia, PA
Helen's Hope Organization, Philadelphia, PA
Great Neck Breast Cancer Coalition, New York
Rose of Hope Scholarship Fund from League of Women Against Cancer, Rydal, PA

Michigan State University

Center Director

Sandra Z. Haslam, Ph.D.

Community Partners

Faith Access to Community Economic Development
Susan G. Komen Breast Cancer Foundation, Lansing, MI Chapter
Michigan Breast Cancer Coalition
Michigan Environmental Council
American Cancer Society, Great Lakes Division

Bay Area – University of California, San Francisco

Center Director

Robert A. Hiatt, M.D., Ph.D.

Collaborators

California Department of Public Health
Kaiser Permanente of Northern California
Lawrence Berkeley National Laboratory
Marin County Department of Health and Human Services
New York University
Roswell Park Cancer Center
San Francisco Department of Public Health
University of Michigan
Zero Breast Cancer

Community Partners

Alameda County Department of Public Health
Bay Area Breast Cancer SPORE Advocacy Group
Bayview Hunters Point Health and Environmental Assessment Task Force
Breast Cancer Fund

University of Cincinnati

Center Director

Robert L. Bornschein, Ph.D.

Collaborator

Cincinnati Children's Hospital Medical Center

Community Partners

Breast Cancer Alliance of Greater Cincinnati
Breast Cancer Registry of Greater Cincinnati
Patterns, Inc.
Pink Ribbon Girls
Susan G. Komen Breast Cancer Foundation, Greater Cincinnati Affiliate
American Cancer Society, Cincinnati Area Office
Breast and Cervical Cancer Screening Project
National Breast Cancer Coalition
Sisters Network Cincinnati
The Wellness Community
YWCA Breast and Cervical Health Network

Acknowledgments

National Institute of Environmental Health Sciences

Gwen W. Collman, Ph.D.
Interim Director, Division of Extramural Research and Training

Caroline Dilworth, Ph.D.
Division of Extramural Research and Training

Elizabeth Maull, Ph.D.
Division of Extramural Research and Training

Leslie Reinlib, Ph.D.
Division of Extramural Research and Training

National Cancer Institute

Robert T. Croyle, Ph.D.
Director, Division of Cancer Control and Population Sciences

Deborah M. Winn, Ph.D.
Deputy Director, Division of Cancer Control and Population Sciences
Acting Associate Director, Epidemiology and Genetics Research Program
Division of Cancer Control and Population Sciences

Denise Buckley, E.L.S.
Writer-Editor, Division of Cancer Control and Population Sciences

Christie Kaefer, M.B.A., R.D.
Communications Coordinator, Epidemiology and Genetics Research Program
Division of Cancer Control and Population Sciences

Shannon Lynch, M.P.H.
Division of Cancer Control and Population Sciences

Gary L. Ellison, Ph.D., M.P.H.
Division of Cancer Control and Population Sciences

Contents

Foreword

Environmental exposures and personal susceptibility factors have been shown to be important in breast cancer development. Although genome-wide association studies have rapidly accelerated the identification of inherited susceptibility factors, there is great public concern regarding the possible environmental factors. Recognizing that the origins of breast cancer likely occur early in life and during times of rapid breast development, researchers need to learn more about exposures that are most influential during critical windows of susceptibility, such as puberty.

To address this complex problem in innovative ways, the National Institute of Environmental Health Sciences (NIEHS) and the National Cancer Institute (NCI) cofunded four Breast Cancer and the Environment Research Centers (BCERCs) to study environmental exposures that may predispose a woman to breast cancer throughout her life. Begun in 2003, the research initiative is a 7-year, $35 million public-private endeavor.

Two coordinated efforts are under way to examine the fundamental hypothesis that increased exposure to estrogens and estrogen-like compounds during a woman's lifetime increases her risk of breast cancer. The first is an epidemiologic study involving ethnically diverse cohorts of young girls that has the goal of understanding determinants of pubertal timing. This investigation aims to determine the onset of breast development, age at menarche, and the pubertal time course; as well as factors affecting these transitions, such as exposures to chemical agents, diet, exercise, obesity, family medical history, psychosocial stressors, and markers of genetic susceptibility. In parallel, the Centers are conducting

animal studies to characterize the molecular features of the mammary gland and determine how exposure to potential carcinogens during critical times in the life cycle influences cancer risk.

The Centers interact as a single program, with some specialization at each BCERC. The BCERCs also incorporate a transdisciplinary approach to improve their effectiveness. This vertical and horizontal integration of science is a central strategy across the National Institutes of Health (NIH). The success of the Centers in integrating various types of basic and epidemiologic science could provide a useful prototype in many contexts and diseases.

All of the BCERCs work with advocacy groups to add insight and experience to the research effort, leverage their expertise in outreach activities, and translate research results into improved understanding and prevention of breast cancer.

As the initiative moves into its next phase, goals include completing the epidemiologic study by following all of the girls to completion of pubertal development, studying the impact of other environmental agents early in life at the molecular level, and assessing gene-environment interactions that can modulate breast cancer risk. The ultimate goal is to discover possible environmental causes of breast cancer in order to protect future generations from this disease.

Robert T. Croyle, Ph.D.
Division of Cancer Control and Population Sciences
National Cancer Institute

Gwen W. Collman, Ph.D.
Division of Extramural Research and Training
National Institute of Environmental Health Sciences

SECTION

I

Introduction

The BCERCs are uniquely positioned to identify determinants of pubertal and developmental milestones associated with long-term risk of breast cancer.

In 2008, more than 182,000 women will be diagnosed with breast cancer, and more than 40,000 women will die of breast cancer. "Breast cancer" encompasses a group of diseases, each with a different epidemiologic profile, that differ in timing (pre- vs. postmenopausal) and in molecular characteristics that influence hormone responsiveness, as well as having different contributions from sporadic, polygenic (familial), and defined genetic syndromes. In addition, evidence increasingly indicates that breast cancer is a complex disease caused by multiple environmental and lifestyle factors interacting with genetic susceptibility across the life span. In examining etiologic factors along a continuum from *in utero* through the postmenopausal years (Figure 1a), possible influences involved in breast carcinogenesis exist at multiple levels, from genes and gene expression to neighborhood and societal influences (Figure 1b).

Little true primary prevention exists for breast cancer except for chemoprevention with agents such as tamoxifen. Preventive measures additionally could address known risk factors that in theory are modifiable, such as radiation exposure, use of hormone replacement therapy, control of body size or alcohol intake, physical activity, age at first full-term pregnancy, parity, or breastfeeding. In reality, however, decisions related to these factors often involve little consideration of breast cancer risk. Even less is known about possible environmental causes of breast cancer, another potential area for preventive actions.

Environmental factors are of intense interest to both researchers and community members, including women with breast cancer, but well-conducted studies of adult women have revealed little regarding possible environmental causes of breast cancer. The study of "windows of susceptibility" in the etiology of breast cancer is of increasing interest. The term "windows of susceptibility" refers to specific time periods when exposures to environmental factors may directly or indirectly affect the risk of developing breast cancer, although exposure to the same factors at other time periods may have no effect. Specific windows exist when physiologic changes occur in the mammary gland—including gestation, puberty, pregnancy, and lactation—and these windows may represent time periods of particular susceptibility to environmental factors that may influence breast cancer risk.[1,2] Thus, research focused on these critical periods of development may improve our understanding of the roles of environmental factors and their interplay with genetic susceptibility.

To address the gaps in current knowledge, the National Institute of Environmental Health Sciences (NIEHS), in collaboration with the

Women from several breast cancer organizations form the National Breast Cancer Coalition (NBCC), with a mission to eradicate breast cancer through increased funding and new strategies for breast cancer research and improved access to health care for all women; potential links between environmental exposures and breast cancer became an early priority.

1991

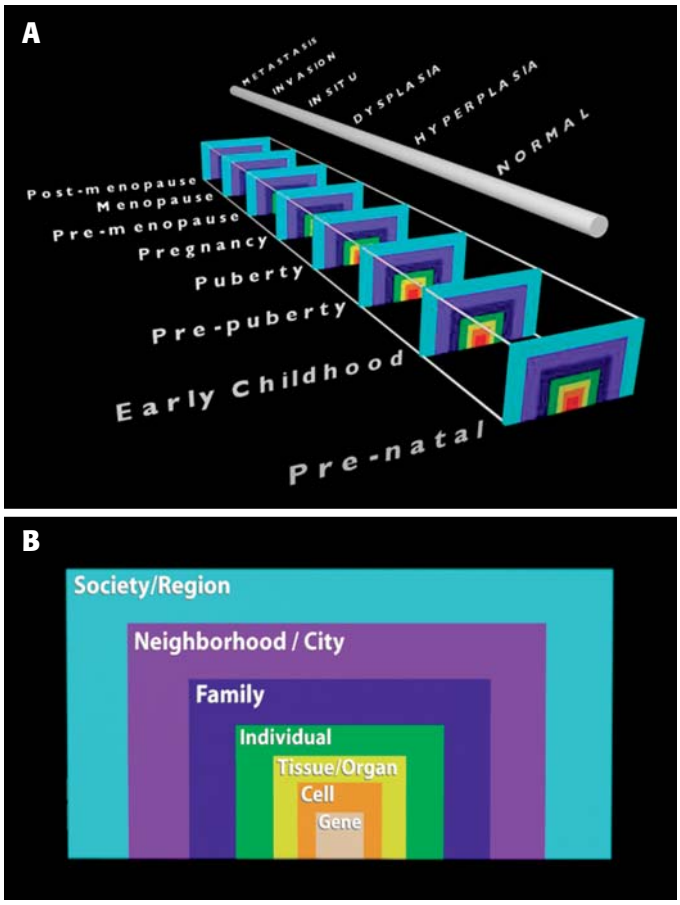


Figure 1. (a) This conceptual framework of the BCERC project illustrates the major phases of life course development and maturity for women, along with the parallel course of carcinogenesis. (b) At each phase, etiologic factors may come into play at multiple levels of biologic, behavioral, and social organization.

National Cancer Institute (NCI), issued Request for Applications (RFA) #ES-03-001 in October 2003 for Breast Cancer and the Environment Research Centers (BCERCs). Awards were made to four institutions: the University of California, San Francisco (the "Bay Area BCERC"); the University of Cincinnati; Fox Chase Cancer Center in Philadelphia; and Michigan State University. The goals of this program are threefold: the first goal is to integrate scientific information on histologic, pathologic, cellular, and subcellular changes that occur in normal mammary gland tissue across the life span and to compare this with exposure-induced changes. The second goal is to conduct a focused and coordinated epidemiologic study of determinants of puberty in girls, with attention to be paid to the timing of breast development and other endpoints. The overall goal of this program is to integrate basic biological, toxicologic, and epidemiologic data on the development and life span of the mammary gland in order to design public health messages to educate young girls and women who are at high risk of breast cancer. The investigators developed proposals based on their research interests and expertise that fit within these broad goals. The BCERCs are studying the role of environmental factors in female pubertal development as a potential window of susceptibility for breast cancer risk, arguing for the importance of puberty as a window of susceptibility. The rapid growth and development of

Table 1. Environmental Agents Studied and the Major Sources of Exposure

Class of Environmental Agent	Major Sources of Exposure
Phthalates	Plastics, personal care products, fragrances
Polychlorinated biphenyl (PCB) congeners	Contaminated food (e.g., fish, high fat foods) and water
Phenols (e.g., bisphenol A: BPA)	Drinking bottles, food can liners, water pipes, dental sealants
Perfluorinated compounds (e.g., perfluorooctanoic acid: PFOA)	Contaminated air and water, industrial sources
Phytoestrogens (e.g., enterolactone-ENL; genistein)	Diet: lignans, soy products
Cotinine	Tobacco smoke
Polybrominated diphenyl ether (PBDE) congeners	Brominated flame retardants, furniture foam, mattresses, carpet padding, hard plastic used in electronics; contaminated air, water, and food
Organochlorine pesticides	Contaminated food and water; persistent in the environment, now in diet and breast milk

The Department of Defense (DOD) initiates the Breast Cancer Research Program, which mandates bringing scientists and the public together for priority-setting and funding decisions; research proposals are solicited and initially reviewed for scientific quality and then for programmatic relevance.

1992

mammary tissue makes it a target for the carcinogenic action of estrogens and other hormones and exogenous chemicals that act like hormones. Increasing the length of time that the mammary gland is susceptible to these insults, for example by starting breast development earlier, may increase a woman's risk of breast cancer later in life.

Earlier age of menarche is an established breast cancer risk factor. The environment can affect breast cancer risk during this window of susceptibility; for example, it is known that radiation exposure during puberty confers a substantially greater risk of subsequent breast cancer than equivalent exposures later in life. Also, some studies of nutrition and breast cancer risk suggest that phytoestrogen intake during adolescence carries a greater influence on breast cancer risk than phytoestrogen intake in adulthood. (Phytoestrogens are substances structurally similar to estrogens that are found in foods of plant origin, such as soy products.)

In the BCERCs funded under this RFA, a major area of study is the role of chemicals in the environment, with a primary focus on hormonally active agents (endocrine disruptors) and the use of personal care or household products that are sources of these chemicals (Table 1; a more detailed version of this table is included in Appendix D). The BCERCs, in addition, have taken a broader perspective on what constitutes environmental factors by including lifestyle factors such as food intake and physical activity. The BCERCs' epidemiologic studies also examine other aspects of the environment, such as the psychological, family, and social environment, as well as factors such as the built environment that may alter physical activity or food availability patterns. The collection of residential and school address histories in these studies also will facilitate future linkage to databases focusing on area-level environmental exposures.

The BCERCs broadly address the multiple factors that may influence pubertal onset and long-term risk of breast cancer, with laboratory studies aimed at understanding biological mechanisms in rodents and tissue culture models, and with epidemiologic studies of pubertal development in young girls.

The historical context of the BCERCs includes the efforts of numerous individuals and organizations over the past 25 years. Selected highlights that resulted in this research program are shown in the timeline below.

1. Research Aims and Strategic Approaches

The BCERC program was created to establish a national network to foster interaction and collaboration within and between Centers. Partnerships were mandated among scientists from various disciplines, breast cancer advocates, and community members. The BCERCs rapidly developed a transdisciplinary approach to integrate the diverse scientific and community perspectives, as shown in Figure 2.

Transdisciplinary science is best described as the interactive work of scientists from multiple disciplines on a common problem with a common conceptual framework, resulting in novel insights and approaches.[3] In relation to the BCERCs, the "problem" is uncovering the impact of a broad array of genetic and environmental factors on the etiology of breast cancer, in particular during early development and puberty. The team science approach within the BCERC program incorporates basic science researchers from multiple fields, epidemiologists and clinicians, and the breast cancer advocacy community, with all investigators in the network using the skills of their own disciplinary training to address this common question. Integration of the science comes from the interaction of discovery from studies of animal and tissue culture models with that of the epidemiologic studies. Integration of community and advocacy perspectives in this research adds another dimension in which ideas and concerns from the public are incorporated into the science in an ongoing man-

Congress mandates the Long Island Breast Cancer Study Project in response to community concerns about high breast cancer rates in that area, with identification of lifestyle and demographic factors; the project receives joint funding through the National Cancer Institute (NCI) and National Institute of Environmental Health Sciences (NIEHS).

1993

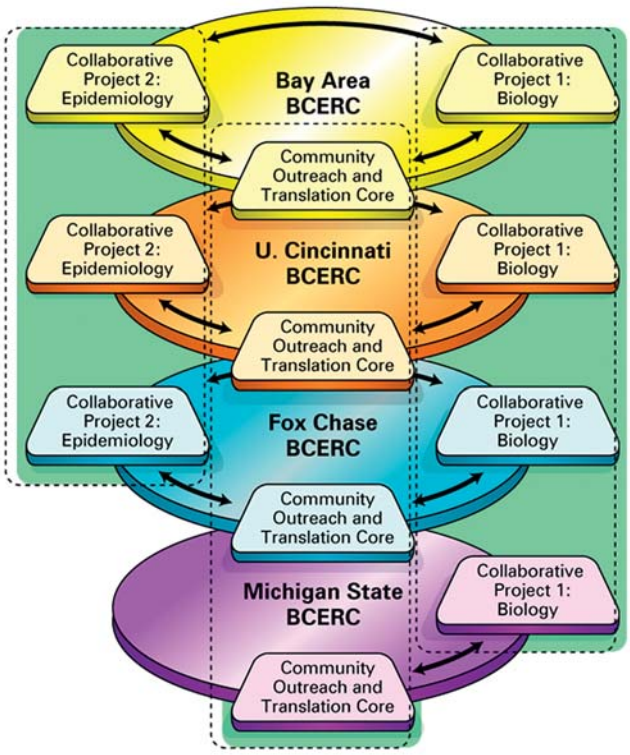


Figure 2. The transdisciplinary structure of the BCERC project allows for the integration of diverse scientific and community perspectives at the four closely collaborating Centers.

ner. Findings from the research are disseminated back to the community periodically during the course of the research projects.

Although the individual Centers have Center-specific aims, the BCERC program has overarching aims centered around the premise that periods of susceptibility exist in the development of the mammary gland when exposures to environmental agents may impact the breast and endocrine systems that can influence breast cancer risk in adulthood.

The Centers are working in close collaboration to pursue three specific approaches to this underlying premise:

1. The conduct of basic science studies in laboratory animals and cell culture systems;
2. The establishment of multidisciplinary epidemiologic studies in human populations; and
3. The creation of Community Outreach and Translation Cores (COTCs) to inform the research projects of concerns and suggestions raised by the advocacy community, and to develop and implement strategies to translate/communicate findings from the laboratory and human studies to the public.

The goals of these three approaches are described below, followed by sections that provide the rationale for the windows of susceptibility approach, the research designs for the Biology and Epidemiology Projects and the Community Outreach and Translation Cores and collaborations, and the governance structure for the joint projects. The projects focus on two major hypotheses: first, that puberty is a window of biological susceptibility due to specific processes; second, that the advent of puberty initiates a window of hormonal stimulation ending in menopause. These are not mutually exclusive, and some environmental agents may perturb both.

Project 1: Basic Science Research (The Biology Project)

Goal: To conduct collaborative experiments using rodent models and cell culture models to characterize molecular and morphologic changes in the mammary gland over the life span and to determine how environmental exposures affect mammary gland development and susceptibility to mammary carcinogenesis.

The RFA designated the title for Project 1 as "Environmental Effects on the Molecular Architecture and Function of the Mammary Gland across the Lifespan." The aim is to integrate gene expression, proteomics, and metabolomics to understand the effects of selected chemicals or other environmental exposures

Members of the Massachusetts Breast Cancer Coalition help to create the Silent Spring Institute to study links between the environment and women's health and breast cancer. The Silent Spring Institute subsequently takes a lead role with academic research institutions in studying environmental links to high breast cancer rates in Cape Cod.

1993

on mammary gland pathology during specific windows of time.

Basic scientists are investigating the mechanisms of exposure-induced changes that impact mammary gland development and susceptibility to mammary gland carcinogenesis by examining periods of susceptibility and the effects of relevant environmental exposures on the mammary gland at the molecular, cellular, and system levels. Interactions between environmental agents and genes that impact environmental susceptibility are being explored and will inform the BCERC program's epidemiologic research to help select relevant human genetic polymorphisms and biomarkers.

Project 2: Epidemiologic Research (The Epidemiology Project)

Goal: To examine the determinants of puberty in girls, integrating how environmental, genetic, biologic, lifestyle, and socioeconomic factors act together and independently.

The RFA-designated title of this project is "Environmental and Genetic Determinants of Puberty." The overarching aims are to: (1) determine the timing of the onset of puberty, with a focus on breast and pubic hair development and windows of susceptibility; and (2) establish the determinants of age at onset of puberty, including prepubertal adiposity, total energy intake and other dietary exposures, levels of physical activity, exposures to endocrine disruptors and other hormonally active agents, social environment factors, and genetic polymorphisms. The focus is on changes before and during puberty in girls, in recognition of epidemiologic factors in breast cancer linked to pubertal maturation. Three cohorts, totaling 1,270 young girls, are being followed.

Community Outreach and Translation Core (COTC)

Goal: To develop and implement strategies to translate/communicate findings from the laboratory and human studies.

The RFA designated the COTC in conjunction with breast cancer advocates to develop and implement protocols to translate/communicate and disseminate the findings from the laboratory and human studies. The overarching aims of the COTC are to build and promote partnerships and collaborations between the BCERC researchers and the breast cancer and environmental advocacy communities, policymakers, and public health professionals and to develop and implement protocols to translate/communicate the findings of the Centers into meaningful messages for the public and policy makers. In conjunction with epidemiology researchers, the COTC develops and implements protocols to report back individual results and translate/communicate study findings to the study families. The COTC also develops evaluation tools that assess the impact of community-university partnerships on the research, collaboration, and translation/communication aims of the BCERCs; evaluates the effectiveness of the translation/communication and dissemination protocols according to criteria standardized across the Centers; and serves as a structure for participation of breast cancer advocates to inform the research questions of the basic science and epidemiologic projects.

2. Rationale for Windows of Susceptibility Approach

It has long been known that risk factors for breast cancer develop and manifest over the course of a woman's lifetime. Widely recognized factors include age at menarche, age at first full-term pregnancy, and age at

1997

The California Breast Cancer Research Program provides funding for the "Adolescent Risk Factors Study" to measure exposures to risks during adolescence and adulthood, using the Community-Based Participatory Research process. This study focuses on breast cancer in Marin County, CA, another geographic area with relatively high breast cancer rates.

2001

The NIEHS organizes a "Brainstorming Workshop on Breast Cancer and the Environment" with the National Breast Cancer Coalition in Charlotte, NC.

menopause, all suggesting that the timing of hormonally related events over the course of the life span are critical to breast cancer risk. Although these risk factors are well established, the identification of modifiable breast cancer risk factors in epidemiologic studies has largely been a frustrating enterprise. Because breast cancer occurs most often in late adulthood, such studies have largely focused on women in that age range. Conversely, the focus in the BCERC epidemiologic studies is on the prepubertal and pubertal stages, in recognition of epidemiologic factors in breast cancer that have been linked to pubertal development.

Human puberty is characterized by a complex series of biologic events, including the development of secondary sex characteristics, changes in body composition, accelerated linear growth, and the achievement of reproductive capacity.[4] Although the onset of puberty has been classified traditionally by the first signs of breast development, the appearance of secondary sex characteristics in girls occurs after the pubertal acceleration in linear growth,[5] also known as the pubertal growth spurt. The rationale for the focus on puberty is derived from several epidemiologic observations that women with breast cancer experienced menarche at a younger age.[6–11] For example, a recent pooled analysis of 19 studies of premenopausal women and 18 studies of postmenopausal women revealed that the risk of breast cancer was decreased by 9 percent and 4 percent, respectively, for each year that menarche was delayed.[12]

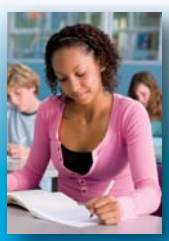
Later menarche may be associated with reduced risk of breast cancer for several possible reasons, including the relationship between menarche and onset of puberty and lifelong exposure to estrogen[13,14] and progesterone,[15] the number of proliferating cells in the intralobular terminal ducts,[16] the types of cells present in the pubertal breast, and susceptibility of rapidly developing breast tissue to environmental exposures.[2,17,18] Studies of mammary carcinogenesis in rodent models have also linked the pubertal period of mammary gland development to increased susceptibility to chemical carcinogens.[1,19] An important concept that has emerged from the study of breast development is that the terminal ductal lobular unit (lobule unit type 1) is the site of origin of the most common breast malignancy, ductal carcinoma.[20,21]

Evidence links prepubertal weight and adiposity with timing of puberty and age of menarche, although the precise mechanism is unclear.[22] The relationship between onset of puberty and menarche has changed over the past 50 years.[5,23,24] For example, in the United States, the correlation (measured by the Pearson correlation coefficient, where 1.0 indicates perfect correlation) between onset of puberty and age of menarche was greater than 0.9 for women born in the 1930s, 0.5–0.7 for those born in the 1950s, and 0.38–0.39 for those born in the 1970s, suggesting that factors that differentially impact menarche and onset of puberty have played an increasingly prominent role.[5] Although age at menarche is the established breast cancer risk factor in epidemiologic studies, it is unclear whether, from a biological perspective, this is the critical event during the pubertal transition. For example, in one analysis examining childhood growth records, age at menarche is associated with risk of breast cancer, but not when age at peak growth is included in the analysis; menarche may reflect age at peak growth, or earlier menarche, as well as age of peak growth, may reflect age at onset of puberty and breast development.[25]

Additional studies have demonstrated that other hallmarks of puberty and maturation also are associated with breast cancer risk. In a pooled analysis of several cohort studies, adult height emerged as a stronger risk factor for breast cancer than body mass index (BMI).[26] In other studies, women who reached maximum height early (age 12 years or younger) had a much greater risk of breast cancer.[27] Earlier age of peak growth is associated with greater growth velocity.[28] Additionally,

An "International Summit on Breast Cancer and the Environment Research Needs," funded by the Centers for Disease Control and Prevention (CDC) and the NIEHS core center at the University of California, Berkeley, is convened. Gwen Collman, Ph.D., of the NIEHS gives a presentation summarizing the 2001 workshop and introducing the idea of a structure for interdisciplinary research to address these issues.

2002



obese and tall children have greater levels of insulin-like growth factor 1 (IGF-1) in response to growth hormone.[29] The age of onset of puberty in girls and age of menarche have declined over the past century,[30–32] suggesting that these secular trends may be influenced by several potential environmental factors, including the rising prevalence of obesity,[33–35] exposure to endocrine disruptors,[36–39] and the interplay of these factors on genetic susceptibility.[32,40] For example, higher urinary concentrations of phytoestrogens were associated with later breast development, especially in girls with lower BMI.[41]

As girls and young women exposed to radiation before age 20 are at higher risk of breast cancer than women exposed to radiation at older ages,[42] the biological basis for this window of susceptibility is a major research question. An important characteristic of the mammary gland is the cyclic ability to proliferate, differentiate, and regress during estrus/menstrual cycles, pregnancy, lactation, and lactational involution (the process by which the mammary gland regresses to its quiescent stage, in which milk is not produced). Biologically, age is a surrogate of various stages in breast tissue development as well as a marker of cumulative endogenous hormone exposures, both of which vary considerably across a woman's life span.[41] The pubertal breast contains the highest number and greatest proliferative activity of terminal duct lobular units,[43] which may account for the observed susceptibility of the pubertal mammary gland of humans and rodents to carcinogens.[1,44–46]

A number of environmental agents have been proposed as possible risk factors for breast cancer, but only radiation,[47,48] alcohol consumption,[49–53] and hormone replacement therapy[54] have shown consistent associations.[55] Ionizing radiation is the best documented exogenous exposure known to increase breast cancer risk.[42] Epidemiological studies repeatedly have found the second decade of life to represent the most sensitive window for susceptibility to radiation-associated breast cancer. Women younger than age 20 years at exposure are at higher risk of radiation-associated breast cancer than those exposed at older ages, while women more than 50 years of age at exposure have no measurably increased risk of breast cancer. Girls exposed to ionizing radiation at Nagasaki-Hiroshima who were in the age range when puberty occurs (approximately aged 10–14 years) were much more likely to develop breast cancer than older girls or adult women who were exposed to comparable radiation doses.[56] Land and colleagues reported that dose-specific excess relative risk (ERR)/Sievert, by age of exposure, was 3.94 at 0–4 years, 2.77 at 5–14 years, 2.65 at 15–19 years, and 1.33 at 20–39 years.[57] Similar effects of age at exposure were found for high-dose radiation exposures to the breast from fluoroscopy for tuberculosis and radiation therapy for Hodgkin's disease.

One of the great challenges in breast cancer research is that we do not know what women were exposed to when the breast was developing and vulnerable. The BCERC program begins to fill in the potential links from early life exposures to breast cancers diagnosed later.

The impact of endocrine disruptors on breast development and pubertal maturation has been reviewed recently.[36–39] A study of 316 girls conducted by NIEHS researchers found that higher transplacental exposure to PCBs (polychlorinated biphenyls) and the DDT (dichlorodiphenyltrichloroethane) metabolite DDE (dichlorodiphenyldichloroethylene) was associated with earlier pubic hair appearance but not age of menarche.[58] Other examples include PBB (polybrominated biphenyl) exposure associated with early menarche and pubic hair development,[59] higher phthalate levels among girls with precocious thelarche (breast budding),[60] smoke exposure and early menarche,[61] and delayed menarche

In October, Kenneth Olden, Ph.D., Sc.D., L.H.D., discusses the Request for Applications (RFA) for "Breast Cancer and the Environment Research Centers" at a Town Meeting in Marin County, CA.

2002

Thirteen grant applications are submitted in response to the RFA; four groups are selected for funding in October, 2003. These groups are:

2003

and pubertal development with lead exposure.[62] The literature also points to the impact of certain environmental exposures on body composition. These include phthalates and obesity[63]; phytoestrogens and reduced adiposity[64]; and prenatal smoke exposure and obesity.[65] The timing of these exposures during the postnatal period through the time of sexual maturation may be a critical factor in the health outcomes observed. In general, children have a greater potential than adults for adverse outcomes related to environmental agents; because of differences in metabolism or behavior, children may reach higher internal dose levels than adults, and they have immature mechanisms for detoxification.

Many classes of chemicals and sources of exposure may be important in altering the pubertal transition and breast cancer risk through an effect on the endocrine system. Phthalates, parabens, and organic solvents are found, among other sources, in personal care products such as cosmetics.[66] Bisphenol A (BPA) may be leached from tin cans and polycarbonate containers.[67–69] Pesticides are found in household use and in residues or bioaccumulation from agricultural use and have been associated with earlier menarche.[32,58,70,71] A new class of brominated fire retardants (e.g., polybrominated diethyl ethers [PBDEs]) has been found at increasingly high levels in environmental and biologic samples,[72] likely from degradation of hard plastics and furniture that contain them. The potential interaction of such exposures with genetic factors has been suggested in studies such as those that indicate that elevated PCBs interact with polymorphisms in *CYP1A1* to influence breast cancer risk.[73]

The broader concept of "environment" includes the social environment and the built environment. Psychosocial factors may influence onset of puberty. For example, higher levels of stress and negative relationships are associated with earlier maturation in girls,[74–76] as is absence of a biologic father.[75–89] Those who live in socioeconomically deprived neighborhoods are more likely to be physically inactive,[80–82] to have less healthy dietary habits,[83] and to be obese.[80,81,83–85]

Genes, environment, and body composition interact with timing of puberty. Leptin, which is related to body composition, is necessary but not sufficient for initiation of puberty.[86] Polymorphisms in the promoter region of the leptin gene impact tissue leptin secretion, and can be impacted by BMI and diet.[87] Polymorphisms in genes controlling estrogen and androgen pathways may impact timing of puberty. High activity of *CYP3A4* alleles, more common in African-American than white or Hispanic girls, is associated with early puberty.[88]

The transdisciplinary framework for the BCERC program was created to promote interaction among the disciplines of basic science research, epidemiology, and the lay community represented by the Community Outreach and Translation Core.

Research using animal models helps to define risks for normal mammary gland development and exposures that impact cancer susceptibility that cannot be directly explored in human studies. The restricted window of carcinogen susceptibility evident during or around puberty in both rodents and humans has been attributed to the greater content of highly proliferative target cells in the developing breast. Tissue-specific stem cells or early progenitors are thought to be the critical cellular target in carcinogenesis, based on the idea that stem cell transformation can lead to unlimited progeny, as has been discussed from several perspectives.[89] Mammary tissue-specific stem cells play a key role in development and regeneration following lactation and involution. Two fundamental properties define these cells: the ability to self-renew, i.e., to maintain a constant pool in a certain tissue (through symmetric division); and their

The Fox Chase Cancer Center consortium, which includes research projects at University of Alabama at Birmingham and Mount Sinai School of Medicine in New York; Michigan State University; University of Cincinnati, with Cincinnati Children's Hospital Medical Center;

The University of California San Francisco (Bay Area) consortium, which includes research projects at Kaiser Permanente of Northern California and Lawrence Berkeley National Laboratory and a COTC chaired by Zero Breast Cancer

multipotency, which confers the potential to generate all of the differentiated cell types present in that tissue (through asymmetric division). It is generally accepted that "stemness" is not a single property but a number of properties that can be manifested under different conditions.[90] A stem cell must be undifferentiated (relative to other epithelial cell types, but not necessarily relative to embryonic cells) and capable of proliferation, self-maintenance, and regeneration of the tissue after injury.[91] It must be capable of producing many differentiated progeny and retain the ability to switch between these options when appropriate. Hence, the properties—and probably the number—of stem cells may change in response to circumstances, including environmental exposures.

The expansion of susceptible stem/progenitor cells during puberty to form the developing mammary gland is an evolving additional hypothesis that could explain the sensitivity of the pubertal mammary gland to environmental factors that impact breast cancer risk later in life. Emerging evidence indicates that the composition and maturation of the gland can be altered by diet, exercise, and environmental exposures, and that exposure during puberty is particularly relevant. Recent studies have shown that the regulation of cell type and fate is determined by developmentally regulated factors from within the tissue, such as transcription factors and hormone receptors, and from systemic signaling, including hormones and metabolic factors.

Because of the complexity of the associations described above, the transdisciplinary framework for the BCERC program was created to promote a high degree of interaction between the diverse scientific disciplines of the basic science research, the epidemiology studies, and the lay community represented by the COTC. The challenge is to integrate information across multiple scales of order and time that influence the lifelong susceptibility to breast cancer. The basic science research provides detailed mechanistic information derived from experimental models that motivate new conceptualization of mammary gland development and the carcinogenesis process. The epidemiology studies provide an epidemiologic resource for investigation of the relationships among various environmental and genetic factors and the biological markers of puberty and physical attributes, including consideration of higher order societal and community structure. The COTC integrates the perspectives of breast cancer advocates and community participants into the research agenda, and coordinates the translation of research findings to community participants and to the public. The objective of this framework is to maintain continuous information flow among the projects and between Centers, such that a more refined understanding of the relationship between environmental and genetic factors, onset of puberty, and breast cancer risk is delineated.

3. Research Design: Project 1 (Biology Project)

All four Centers focus on rodent models, and the Centers cover a range of exposures and research questions among them (Table 2). All Centers use multiple assays to evaluate the effect of exposure on the maturation of the mammary gland, as evidenced by molecular, cellular, and morphologic events. These include the following techniques:

- Genetic factors are studied using strain comparisons and genetically engineered mice. Molecular architecture is defined by gene expression profiling that involves a concerted effort to integrate information across species and platforms.
- Cellular composition and phenotype are measured by immunostaining and microscopy, quantitative image analysis, or flow cytometry.
- Morphological analysis of mammary development is conducted using tissue whole mounts.

The events are correlated with susceptibility to breast cancer induced by chemical carcinogens such as dimethylbenz[a]anthracene (DMBA) or ionizing radiation. Additionally, one Center uses cultured primary human epithelial cells to study the effects of ionizing radiation.

These projects are conducted at:

- The University of California, San Francisco (UCSF)/ Lawrence Berkeley National Laboratory (LBNL), part of the Bay Area BCERC;
- The University of Alabama at Birmingham (UAB), part of the Fox Chase Cancer Center (FCCC) BCERC;

Table 2. Environmental Exposures and Experimental Models by Study Site

Exposure	Model	Center	Research Question
Endogenous hormones	Mouse, rat	MSU	How do species differences and genetic background affect the regulation of mammary gland development by endogenous hormones? How is mammary development regulated during puberty?
	Mouse	Bay Area	
Dietary fat	Rat	University of Cincinnati	How does maternal and/or pubertal diet alter mammary development during puberty and susceptibility to carcinogens across species and in different genetic backgrounds?
	Mouse	MSU	
Endocrine disruptors, e.g., BPA, BBP, TCDD	Rat	FCCC	Are altered genomic and proteomic expressions associated with increased susceptibility for mammary cancer? What effect do these substances have on susceptibility to breast cancer in rodent models? What are their mechanisms of action (as determined via genomic and proteomic technology)?
	Mouse	MSU	
Genistein	Rat	FCCC	Does prenatal and/or prepubertal genistein alter gene and protein expression to account for breast cancer chemoprevention?
PFOA	Mouse	MSU	What effect does PFOA have on pubertal mammary gland development and susceptibility to carcinogenesis and in different genetic backgrounds?
Ionizing radiation	Mouse, human	Bay Area	In addition to its action as a mutagen, how does radiation alter susceptibility to cancer?

Key: MSU, Michigan State University; FCCC, Fox Chase Cancer Center; BPA, bisphenol A; BBP, butyl benzyl phthalate; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PFOA, perfluorooctanoic acid.

- The Michigan State University (MSU) BCERC; and
- The University of Cincinnati BCERC.

4. Research Design: Project 2 (Epidemiology Project)

The Epidemiology Project, which consists of an observational longitudinal study, is conducted at three sites (Table 3):

- Mount Sinai School of Medicine (MSSM), part of the Fox Chase Cancer Center (FCCC) BCERC;
- Cincinnati Children's Hospital (CCHMC)/University of Cincinnati (Cincinnati);
- Kaiser Permanente Northern California (KPNC), part of the Bay Area BCERC.

The baseline cohort consists of 1,270 girls. The racial and ethnic composition of the cohort varies by clinical center, reflecting the source populations. Overall, 34.4 percent of the girls were identified by parents/guardians as White, non-Hispanic; 25.3 percent as Black, non-Hispanic; 4.3 percent as Black, Hispanic; 29.9 percent as Hispanic; 4.5 percent as Asian; and 1.7 percent as "Other." Mean ages at baseline, by site, are 7.34 years (MSSM), 7.13 years (Cincinnati), and 7.38 years (KPNC).

Before recruitment began, the investigators and study coordinators from each Center met to prepare a common protocol, methodology, and training materials. The procedures used are summarized in Tables 3 and 4. Additional details about specific data collected at each of the three sites and about the timing of data collection are provided in Table D7 in the Appendix.

- Pubertal maturation has been assessed using the criteria established by Marshall and Tanner for breast maturation (Figure 3) and pubic hair stages,[92] with photographs that demonstrate the maturation stages, published by van Wieringen.[93] Clinical assessments are performed annually in the MSSM and KPNC cohorts, and semiannually in the Cincinnati cohort.
- A substantial biorepository has been established and continues to be expanded.
  - Urine specimens were obtained annually at all three sites. The laboratories of the Centers for Disease Control and Prevention (CDC) examined the specimens collected at the baseline examina-

Table 3. Study Participant Sources, Selection Criteria, and Other Key Characteristics of the Cohorts of Girls

	MSSM (FCCC)	KPNC (Bay Area)	Cincinnati
Study participant source	Community centers, schools, pediatric clinics in East Harlem, NY	KPNC membership	Schools in greater Cincinnati metropolitan area; subset recruited through Breast Cancer Registry of Greater Cincinnati
Recruitment strategy	Interviewer recruitment at pediatric clinics; presentations at community groups, interviewer recruitment at school events	Letters mailed to study-eligible families, followed by telephone call	Presentations to school administrators; half of schools with presentations to parents; half with mailed letters to parents
Ages of girls at baseline	6–8 years	6–8 years*	6–7 years
Other criteria for inclusion in study	Self-reported Black or Hispanic race/ethnicity; not diagnosed with select medical conditions known to impact growth and development; only one sibling per family enrolled	Mother/child were KPNC members when the child was born and at the time of invitation to the study; resident of selected Bay Area communities; not diagnosed with select medical conditions known to impact growth and development	Not diagnosed with select medical conditions known to impact growth and development
Number of girls enrolled in study	447	444	379
Data collection setting: clinic	Community centers; pediatric clinics	Three KPNC research or medical facilities	Schools; Cincinnati Children's Hospital
Data collection setting: questionnaire	Community centers; pediatric clinics	Three KPNC research or medical facilities	Questionnaire mailed to families
Mode of questionnaire administration	Interviewer administered in-person; select forms self administered	Interviewer administered in-person; select forms self administered	Self administered
Questionnaire respondent	Parent or legal guardian	Parent or legal guardian	Parent or legal guardian
Languages used	English, Spanish	English, Spanish	English
Dates of baseline exams	October 2004–October 2007	June 2005–August 2006	September 2004–January 2007
Current followup	In up to fifth year of data collection	In fourth year of data collection	In up to fifth year of data collection
Time interval between visits	Approximately 12 months	Approximately 12 months	Approximately 6 months

*Ages 6–7 years at time of recruitment.

tion, after a pilot of about 30 specimens from each Center was analyzed to determine the feasibility of measuring environmental chemicals of interest.[94] Classes of compounds analyzed include phthlates, phenolic compounds, phytoestrogens, and cotinine. Additional samples from both the baseline year and subsequent years are stored at each Center.

- The CDC analyzed baseline blood samples from the KPNC and CCHMC cohorts for organohalogen and related compounds including PBDEs, pesticides, and perfluorinated compounds. Additional samples are available at both sites for future analyses of environmental exposures of interest, such as heavy metals.
- DNA was extracted from specimens, and selected novel single nucleotide polymorphisms (SNPs) not previously studied in relation to puberty were examined.

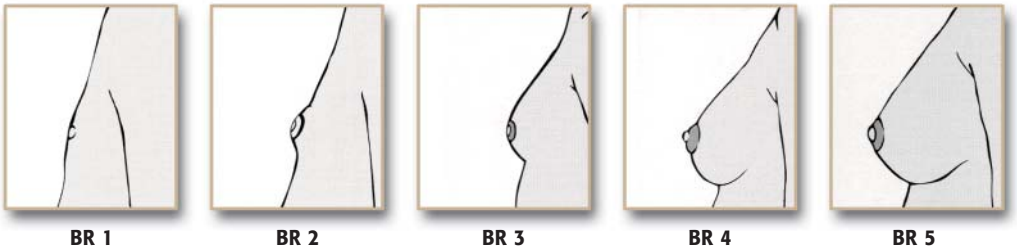


Figure 3. The Tanner staging system recognizes five stages of breast maturation, from prepubertal (BR 1) to the adult breast (BR 5).

Table 4. Questionnaire Content, Clinical Examination Content, and Other Special Procedures

Questionnaire Content
Demographic factors
Socioeconomic status
Physical activity
Personal care and household product use
Environmental exposures
Health history
Household characteristics
Residential and school histories
Psychosocial assessments
Family environment
Clinical Examination Components
Height, weight, BMI
Waist and hip circumference
Bioelectrical impedance analysis
Skinfolds
Tanner staging (a measure of breast and pubic hair development)
Blood pressure
Biospecimen Collection
Blood (for biomarkers and DNA)
Blood (for endogenous factors)
Urine (for biomarkers)
Urine (for endogenous factors)
Saliva (for DNA)
Buccal swabs (for DNA)
Other Special Procedures
Diet (24-hour recall)
Pedometer (mean steps per day)

- Parents or guardians completed detailed questionnaires annually to assess various factors that may be important in the onset of puberty and other hallmarks of sexual maturation. Topics are summarized in Table 4. Psychosocial assessments included the Behavioral Assessment System for Children (parental scale) (BASC-P), the Center for Epidemiologic Studies Depression scale (CES-D), and the Family Environment Scale (FES).

- A 24-hour dietary recall was conducted every 3 months in the first year of the study at all three sites. Additional assessments include second-year assessments at KPNC, repeat assessments at Breast Stage 2 at MSSM, and annual assessments at CCHMC. (See Table D7 in the Appendix.)

Table 5 shows breast maturation at age 7 years in study participants, demonstrating notable racial differences. The degree of agreement between the master trainers and the research staff conducting the examinations was measured with 127 dual examinations. The kappa statistic for agreement between examiners was 0.67, indicating that "substantial" agreement[95] exists between the master examiners (who were trained by the master trainers) and the research staff.

5. Design of the Community Outreach and Translation Core (COTC)

The COTCs have implemented multiple novel strategies to accomplish their aims. The approaches include the following:

- Conduct large- and small-scale educational programs and develop materials targeted to the public, breast cancer advocates, and/or study participants and their families;

- Conduct training programs for breast cancer advocates that address biology and/or epidemiology research methods and protocols to enhance advocates' critical understanding of scientific studies;

- Create materials and conduct activities that support the recruitment and retention objectives of the epidemiology studies;

- Conduct formative research to identify effective dissemination strategies and priorities for translating/communicating study findings;

- Conduct pilot research to identify key issues to be addressed when assessing collaborations between researchers and advocates.

6. Collaborations

Collaborations within and between the projects (basic science, epidemiology, and COTC) and sites are strengthened through twice yearly meetings and a minimum of monthly project-specific calls among investigators, with representatives from the National Institutes of Health

Table 5. Breast Maturation Status at Age 7 Years, by Site and Race/Ethnicity, in the BCERC Cohort as of June 30, 2008

Study Site	Mount Sinai School of Medicine (MSSM)			Cincinnati			Kaiser Permanente Northern California (KPNC)			Overall
BCERC Center	Fox Chase Cancer Center			University of Cincinnati			University of California, San Francisco			
Tanner Stage	B1	B2+ (%)	Total	B1	B2+ (%)	Total	B1	B2+ (%)	Total	B2+ (%)
White, non-Hispanic				184	29 (13.6)	213	172	7 (3.9)	179	36/392 (9.2)
Black, non-Hispanic	52	8 (13.3)	60	62	29 (31.9)	91	69	16 (18.8)	85	53/236 (22.5)
Black, Hispanic	27	3 (10.0)	30				4	1 (20.0)	5	4/35 (11.4)
Hispanic	112	21 (15.8)	133	7	1 (12.5)	8	95	12 (11.2)	107	40/248 (16.1)
Asian				4	0 (0)	4	45	2 (4.3)	47	2/51 (3.9)
Other				13	3 (17.6)	16	3	0 (0)	3	3/19 (15.8)
Total	**191**	**32 (14.4)**	**223**	**270**	**62 (18.7)**	**332**	**388**	**38 (8.9)**	**426**	**132/981 (13.5)**

Note: This table includes only those participants who were age 7 years or younger at the time of recruitment or who had attained age 7 at the time of the analysis. B1, Tanner Breast Stage 1 (no evidence of breast maturation); B2+, Breast Stage 2 or 3.

(NIH) staff. Collaborative interactions between Centers include comparisons between species, shared infrastructure for expression profiling, and cross-calibration of endpoints. Discussions among projects have motivated examination of additional environmental exposures, as well as additional genes of interest for polymorphism analysis in the human study cohort.

The BCERC program is in a unique position to facilitate the exchange of emerging scientific information and technologies between basic scientists, clinicians, population and environmental scientists, and the advocacy community to test hypotheses expeditiously that emerge from coordinated studies of chemical exposures (such as BPA). Scientific discovery can be accelerated, and involvement of the advocates facilitates effective dissemination of findings to the public. This information should be useful in developing clinical and public health programs that target breast cancer prevention in girls and young women.

7. Governance

The BCERC program is managed by NIEHS staff in collaboration with NCI staff. A Steering Committee, comprised of the Principal Investigators and a COTC representative from each Center and representation from NIEHS and NCI, coordinates cross-center efforts and annual meetings. A Publications Committee with representation from all Centers and projects, along with NIH program staff and Working Group members (see Appendix E for members), has established guidelines and reviews and tracks proposals for cross-project analyses and publications as well as requests for ancillary studies.

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SECTION

II

Cross-Center and Transdisciplinary Accomplishments

A major accomplishment across Centers is the measurement of 51 environmental agents and their metabolites in biospecimens from approximately 1,190 girls.

The research and activities conducted by the BCERCs are highly integrated across the biologic and population sciences and incorporate participation by community members and advocates. A later section of this report describes the accomplishments of individual BCERCs. This section focuses on accomplishments that have resulted from the interaction of investigators at two or more Centers and illustrates the benefits of working in the cross-disciplinary environment made possible by this NIH Cooperative Agreement.

Environmental Chemical Exposures, Biomarkers, and Experimental Findings

A major accomplishment across Centers is the measurement of 51 environmental agents and their metabolites in biospecimens from approximately 1,190 girls (Table 6). The Epidemiology Project Biomarker Subcommittee, working with the COTCs and CDC collaborators, identified several families of chemicals based on biologic activity (relevance to pubertal development, e.g., hormonal activity) and feasibility (biomarker variability, validity, and reliability in the study context). The chemical families pursued include phenols and phthalates found in many personal care products and plastics; phytoestrogens found in foods; persistent pesticides (such as DDT); flame retardants used in hard plastic and foam furniture; PCBs; perfluorinated compounds (PFCs) used in a variety of materials, most notably Teflon; other pesticides; and cotinine, a tobacco smoke metabolite. The data include the first report in children of high levels of a broad range of hormonally active chemicals such as enterolactone, benzophenone-3, and mono-ethyl-phthalate. This investigation confirms that significant levels of the chemicals of interest are

Table 6. Baseline Biomarkers Measured in Girls in the BCERC Cohorts Through October 24, 2008

	MSSM	KPNC	Cincinnati
Urine			
Phthalates	10/07 (366); 2/08 (59)	1/08 (435)	3/08 (325)
Phenols	10/07 (366); 2/08 (59)	12/07 (435)	2/08 (325)
Creatinine	6/08 (366); 6/08 (59)	8/08 (435)	8/08 (326)
Cotinine	5/08 (429)	6/08 (435)	6/08 (326)
Phytoestrogens	4/08 (425)	4/08 (436)	4/08 (326)
Blood			
PFCs	NA	5/08 (384)	5/08 (229)
PBDEs/OCs/PCBs	NA	10/08 (393)	10/08 (270)
Lipids	NA	10/08 (393)	10/08 (270)

NOTE: Dates are given as MM/YY, with number of samples in parentheses. The numbers include approximately 10% quality control samples. Pilot samples (30 per site) are not included.

KEY: PFCs, perfluorinated compounds; PBDEs, polybrominated diethyl ethers; OCs, organochlorine pesticides; PCBs, polychlorinated biphenyls. KPNC, Kaiser Permanente Northern California; MSSM, Mount Sinai School of Medicine.

found in the girls. The BCERC data will be an important contribution because they confirm and extend findings from the National Health and Nutrition Examination Survey (NHANES).

In a BCERC pilot study, investigators reported a wide range of concentrations in phthalate, phenol, and phytoestrogen urinary metabolite levels,[1] as well as levels of PBDE, organochlorine pesticides, PFCs, PCBs, and cotinine metabolites.[2] Differences were found by site, race, and BMI for some biomarkers. At the MSSM site, a 6-month study of variability of these same biomarkers demonstrated reasonable reproducibility in individual levels over time.[3]

The chemicals being studied in the Biology Project were selected to accommodate the structure of the experimental models and to complement the studies in girls. A major focus of the Biology Project is to examine environmental exposures at levels that approximate the exposure of the general population. Several compounds are being explored in both the Biology and Epidemiology Projects. Featured below are examples that illustrate fruitful transdisciplinary interactions between the Biology Project, Epidemiology Project, and COTC.

High levels of a number of hormonally active chemicals, such as enterolactone, benzophenone-3, and mono-ethyl-phthalate, were common in the girls.

Phthalates and Phenols

The individual BCERC Epidemiology Projects identified phthalates and phenols *a priori* as high priority based on observed urinary levels in the micromolar range in the population and knowledge of their hormonal activity. Although the three-site analysis of epidemiologic findings has not begun, site-specific findings to date support few effects on pubertal development. The studies in animal models suggest, however, that only critically timed exposures may alter pubertal milestones. Therefore, sophisticated methods may be needed to detect such effects in the cohort of girls. To identify better ways of analyzing the data, BCERC investigators are re-examining their analytic models in the Epidemiology Project in light of the animal model findings. BBP (but not BPA) exposure in rats during puberty appears to affect lipid homeostasis, with an aggressive pattern of fat metabolism. In animals exposed prepubertally to BPA, oncogenes are highly expressed shortly after the pubertal period (50 days). This signature predicts a mammary response to DMBA carcinogenesis. In addition, other studies suggest that effects may be detectable in certain subsets of girls, such as leaner girls or those with certain genetic characteristics.

Phytoestrogens

These dietary agents are one of many high priority chemicals for the Epidemiology Project based on their levels and known effects on reproductive function. To complement the epidemiologic work, Biology Project investigators at MSU initiated animal studies on the effects of enterolactone in 2007 after the human biomarker study data[1] were made available. Pubertal enterolactone exposure in two strains of mice, C57BL/6 and Balb/c, revealed delayed onset of puberty as measured by later age at vaginal opening, with similar responses in the two mouse strains. No significant effect was observed, however, on pubertal mammary gland development at the doses studied. Studies are planned to measure enterolactone serum levels in mice to determine whether they are comparable to levels in girls. To date, the findings from the animal studies are consistent with delayed onset of puberty in girls, although not mammary development. The dose effects may vary by species, suggesting genetic variations in response that also can be investigated in the Epidemiology Project by examining polymorphisms. In this and other studies, an effect also has been suggested in relation to low or high BMI.

Other Environmental Exposure Analyses

In the initial pilot studies conducted by BCERC investigators, brominated fire retardants were found at higher levels in girls from California compared to girls in Ohio (see further discussion below), as were several PCB congeners and organochlorinated pesticides (e.g., DDE).[2] There was also considerable variation in cotinine levels across the three sites, as shown in Table 7, indicating that girls experience widely different exposure to environmental tobacco smoke, which has been implicated as a risk factor in breast cancer.

Table 7. Distribution of Cotinine in Urine (µg/L) in Girls at BCERC Epidemiology Project Sites

	N	N < LOD (%)	Median	Min	Max
KPNC	421	19 (4.5%)	0.2	<LOD	129.0
MSSM	409	0 (0%)	1.1	0.05	1586
Cincinnati	321	1 (0.3%)	0.6	<LOD	260.8
TOTAL	**1151**	**20 (1.7%)**	**0.5**	**<LOD**	**1586**

KEY: LOD, limit of detection

A secondary aim of the Epidemiology Project is to identify sources of environmental exposures whose biomarkers have been measured. The potential sources documented from interviews with parents or guardians are diet, personal and home products, home interior structural materials (e.g., floor and window coverings), and neighborhood characteristics. For at least some biomarkers, it may be possible to capture major inputs, such as fragranced products, which can be a source of MEP (the metabolite of DEP, diethylphthalate, used to sustain fragrances). Using the baseline questionnaire data from all BCERC girls in conjunction with biomarker data, the BCERC researchers are examining the reported use of 30 products to predict urinary endocrine disruptor biomarker concentrations. If recalled information can be used to rank participants by hormonally active exposures, it will be possible to implement this approach in a manner similar to food frequency questionnaires. A "product use questionnaire" potentially could be a powerful epidemiologic tool that could be used in larger studies at a lower cost than biomarker analyses. Additionally, this approach could be used as an exposure assessment tool in prevention studies. Preliminary data have shown few associations, but data analysis on the entire population is just now underway.

COTCs at two sites, in collaboration with researchers across Centers and members of the working group, developed fact sheets about some of the chemical compounds studied above (see Appendix C). These fact sheets provide BCERC researchers, COTC members, and community breast cancer and environmental advocates background information on these environmental exposures to assist in:

- Understanding the research being presented and published by BCERC investigators;
- Interpreting the literature as BCERC findings are disseminated; and
- Developing outreach materials for the lay public.

Special Cases of Environmental Exposure: The PFOA Investigations

Through BCERC collaborations with CDC, investigators were able to pursue unexpected findings in the pilot studies of biomarkers in the Epidemiology Project to ultimately measure perfluorooctanoic acid (PFOA) serum levels in 617 girls (Table 8). This opportunity has uncovered a potential new reproductive toxin and illustrates well the interaction of epidemiologists and basic scientists as well as the critical role played by one of the COTCs. (The role of the COTC is discussed in the section "Community Participation," below.)

Table 8. PFC Biomarkers for KPNC and Cincinnati Girls, Pilot and Main Study Combined, Compared With NHANES Data

Location	Total Number of Participants	PFOA Serum Concentration ≥ 8.6 ng/mL	
		N	Percentage
Cincinnati	266	115	43.40
KPNC	351	43	12.25

NOTE: A PFOA serum concentration of 8.6 ng/mL was the 95th percentile value for 12- to 19-year-olds in NHANES (2003–2004). PFC, perfluorinated compound; PFOA, perfluorooctanoic acid.

Data from a 2005 biomarker feasibility study among girls in Cincinnati unexpectedly showed that approximately 50 percent of the samples had PFOA concentrations that were above the 95th percentile of NHANES III participants, age 12–19 years, and a substantial variation in PFOA levels existed between the study participants in different communities. Results were confirmed by the CDC, and the elevation in this population was verified in additional analyses on blood collected a year later.

The BCERC findings could impact public health policy decisions regarding PFOA disposal sites in the United States and production activities in other countries.

After the Cincinnati PFOA exposure data were presented to all BCERC investigators, a new cross-center and transdisciplinary collaboration was formed with

BCERC investigators at MSU to study the effect of pubertal PFOA exposure in female mice. The effects of PFOA (doses 0.1–10 mg/kg BW) were examined in two strains of mice (Balb/c and C57BL/6) that are known to have genetically determined differences in mammary gland development and responses to hormones. PFOA treatment caused: (1) pathological changes in the liver, (2) delayed onset of puberty, (3) changes in uterine development, and (4) alterations in mammary gland development. Significant differences in effects between the two mouse strains existed, however. This finding underscores the need for caution when drawing conclusions about the effects of PFOA and possibly other environmental pollutants on the basis of studies in a single mouse strain and is particularly relevant for identifying the effects of PFOA in the genetically heterogeneous human population.

Studies are in progress to determine the effect of pubertal PFOA exposure on susceptibility to mammary cancer development in mice. Targeted real time quantitative reverse transcriptase polymerase chain reaction (QRT-PCR) array analysis of livers from control- and PFOA-treated C57BL/6 mice recently revealed a strong induction by PFOA of detoxification enzymes and repression of hormone-metabolizing enzymes. These results strongly suggest that the effects of PFOA on the mammary gland and uterus are mediated systemically through alteration of estrogen levels as a result of PFOA effects on the liver rather than through a direct effect of PFOA on the mammary gland and uterus. PFOA levels currently are being measured by the U.S. Environmental Protection Agency (EPA) to determine how the mouse serum concentrations at the different doses compare with the serum concentrations in the Cincinnati cohort of girls.

Pubertal Maturation Studies in Humans

The animal findings described above prompted the BCERC epidemiologists and the CDC to add PFCs to the exposure biomarkers to be measured in the Epidemiology Project for use in a multisite (Cincinnati and KPNC) analysis of effects of PFOA on growth and pubertal maturation.

These analyses first were conducted in June 2008 using Cincinnati cohort data only, as soon as the main study biomarker data were received from the CDC. Relationships between PFOA serum concentrations ≥ 11.6 ng/mL and earlier thelarche and adrenarche, lower BMI, and decreased low-density lipoprotein (LDL) cholesterol were reported. In analyses with an additional 4-month followup, these effect estimates held or became slightly stronger. The pubertal maturation findings are consistent with MSU's findings on mammary gland development in C57BL/6 mice at low doses. The decreased LDL cholesterol in exposed girls may be indicative of altered hepatic function due to PFOA exposure.

These epidemiologic findings will inform the direction of future studies in animal models, and animal findings will provide insight into the potential mechanisms by which PFOA might affect humans. Animal studies will be undertaken to explore the peroxisome proliferator-activated receptor alpha (PRAR-α) agonist activity of PFCs, especially PFOA. Most important, if the detected pubertal maturation effects persist in the larger multisite cohort and in other cohorts, the findings would be indicative of a potentially significant impact on future breast cancer rates. These findings could impact public health policy decisions regarding PFOA disposal sites in the United States and production activities in other countries.[4]

A major focus of the Biology Project is to examine environmental exposures at levels that approximate the exposure of the general population.

Brominated Fire Retardants

PBDEs are a newer class of chemicals to be studied for reproductive health effects, so relatively few data are available, despite the known increase in levels generally found in biospecimens.[2,5–8] This prompted additional attention by the COTCs, as described below in the community participation section.[5–8]

In the pilot study conducted on serum from the first 30 girls at the Cincinnati and KPNC sites, PBDE levels were higher in girls from California compared to those in Ohio, even after adjusting for race, age, and BMI. This pattern persisted in additional data received from CDC on 100 girls from each of the two sites.[5] Furthermore, the geometric mean levels in California girls

were double those of adolescents (12–19 years of age) in NHANES III.

These findings are consistent with recent reports showing higher levels of PBDEs in adults from California than from other states in NHANES data, and in dust samples taken in the San Francisco Bay Area compared to Massachusetts. This is a public health concern as levels of PBDEs have been rising in environmental and biological specimens, especially in the United States compared to European countries. California likely has particularly high levels because of a history of more stringent flammability standards that have been met by adding these chemicals to foam and furniture. Determining the health effects of these fire retardants will be quite important, as animal data suggest possible disruption of thyroid and reproductive function.

Data on all PBDEs now have been received from the CDC lab. Analyses of the California data only, examining levels in relation to pubertal outcomes in year 2, show somewhat higher levels for several PBDE congeners among girls who were pubertal compared to prepubertal girls. Adjusting for other variables such as the child's race, age, BMI, and household income altered these patterns. African Americans tended to have higher PBDE levels and also entered puberty earlier than other racial groups, which may have confounded the association. After adjustment, girls who were pubertal were less likely to have PBDE levels in the higher quartiles.

Lower levels among pubertal girls also were seen consistently for other organochlorinated compounds, such as PCBs, in crude data (adjustment not completed yet). The number of girls in the study who are pubertal still is somewhat small, so it will be important to add the Cincinnati data, as well as to examine associations with puberty at later followup visits to increase the power of the statistical analyses. Since higher levels of several chemicals were found in African-American girls, the researchers are interested in examining associations within racial groups when the Cincinnati data are added, to explain the reasons for the racial disparities.

Diet, Physical Activity, and Body Size

It is well known that body size (height and weight) is a determinant of onset of puberty and also affects risk of breast cancer. The BCERCs have focused on understanding the effects of adiposity, dietary factors, and physical activity on pubertal maturation and mammary gland development. A wealth of epidemiological data focusing on adiposity, food and nutrient intake, and physical activity has been gathered. At the basic science level, the findings have been extended to explore how they influence mammary gland carcinogenesis in rodent models. Studies of breast development and hormone responsiveness to high-fat diets in two mouse strains with varying susceptibility to diet-induced obesity have been a major focus of the MSU Biology Project. In addition, the Cincinnati Biology Project has investigated the effects of maternal fatty acid exposure on the development of offspring. (These projects are described in greater detail in the sections of this report describing findings from these two Centers.)

In the Epidemiology Project, cross-sectional associations of BMI with Tanner stage at baseline have demonstrated clearly that girls who have higher BMI are more likely to have evidence of pubertal development, after accounting for age. BMI also differs by race/ethnicity, with African-American girls across the three cohorts having higher BMI than other girls; African-American girls also are more likely to show evidence of onset of puberty at baseline.

Through genetic investigations, BCERC researchers will enhance the understanding of factors that determine timing and pathways through puberty.

The primary method of dietary assessment in the epidemiologic studies is 24-hour dietary recalls administered by telephone at all three sites during the baseline year of data collection. Dietary recalls were conducted approximately 3 months apart to account for seasonality of food intake, for a total of four dietary recalls within the first year. Data on consumption of organic food items also is being collected, under the assumption that organically grown and produced foods are less likely to be vehicles for hormonally active environmental chemicals. In addition, recent intake of selected food items that are rich sources of phytoestrogens has been queried independently. Data from the KPNC cohort have shown some associations between dietary data and onset of breast development, as described in the section

of this report that summarizes findings from the Bay Area BCERC.

In summary, the MSU Biology Project data demonstrate that a high-fat diet increases fat pad weights in the mammary gland and perturbs mammary gland development in mice, and findings from the Cincinnati Biology Project suggest that consuming a diet with elevated fat intake enhances the development of obesity and onset of puberty and alters mammary gland morphology in rats. Although preliminary analyses from the KPNC Epidemiology Project do not indicate that fat intake *per se* influences pubertal development, dietary patterns that are consistent with a more plant-based diet are associated with a lower likelihood of onset of breast development.

In another example of cross-project, cross-center transdisciplinary research, a group of BCERC investigators developed a study to explore genetic and proteomic markers of environmental exposures in the girls.

These observations are consistent with studies in adults indicating that dietary patterns characterized by lower fat intake and higher dietary fiber intake are associated with lower levels of circulating free estradiol, and with rodent studies indicating that high-fat diets increase mammary gland carcinogenesis. A major pathway by which diet may influence mammary gland or breast development is through increased adiposity; this may be accompanied by perturbations in inflammatory cytokine levels, sex hormone metabolism, and other effects. These mechanisms can best be examined in detailed rodent studies in the Biology Project that pursue leads from these observations, coupled with analyses of the interplay of growth, adiposity, dietary factors, and physical activity patterns in followup studies of the Epidemiology Project cohorts.

Genetic and Proteomic Studies

In the original grant applications for the Epidemiology Project, all sites stated that they planned to measure variation in candidate genes related to pubertal maturation, and particularly those genes involved in hormone synthesis and metabolism. Through such investigations, BCERC researchers will enhance the understanding of factors that determine timing and pathways through puberty.

The technology for investigating gene variants has advanced, including genome-wide or pathway-specific SNP typing and analyses. The Epidemiology Project is developing a strategy for these studies, including consideration of the best uses of the available DNA, the time and amount of DNA required for completion of genome-wide studies, and the potential need for reserving native DNA for future resequencing studies. Additional funding will be needed for any studies of genetic variants. DNA will be available from almost every girl but in varying quantities.

DNA from 806 girls at KPNC and MSSM has been analyzed for 128 novel SNPs in two pathways related to neuroendocrine development, with data analysis underway. Preliminary analysis of the incomplete dataset has revealed interesting associations with both BMI and Tanner Breast Stage, as well as corollary findings in gene expression in the rodent models (this cross-center project is described briefly in the FCCC section of this report). Interactions with the Biology Project have helped clarify the directions for future genotyping, which include:

- Genetic determinants of hormone levels and obesity in girls and in animals, and related gene pathway analyses;
- Genetic factors that regulate metabolism of xenobiotics;
- Neuroendocrine pathways (genotyping for girls and gene expression for laboratory studies; epigenetic profiles in girls and laboratory animals); and
- Inflammatory immune pathways.

In another example of cross-project, cross-center transdisciplinary research, a group of BCERC investigators developed an ancillary study and were awarded additional funding through the trans-NIH Genes, Environment, and Health Initiative to explore genetic and proteomic markers of environmental exposures in the girls. The team includes basic scientists, toxicologists, clinicians, and bioinformaticians from several of the

BCERCs. The goal of this new work is to develop sensitive and reproducible genomic and proteomic signatures in rats as a model system exposed to selected environmental chemicals that have been identified as (potential) endocrine disruptors, including BPA, phthalates (BBP and di-2-ethylhexyl phthalate [DEHP]), and genistein. Then investigators will use biospecimens from the cohort studies to see if they can replicate these signatures in highly exposed girls.

An earlier pilot study demonstrated that these compounds are measurable in the population and are suspected of altering susceptibility for biochemical insult. For this purpose, BCERC investigators will use both humans and animal models for sources of RNA, DNA, and serum proteins, which will be collected through blood (serum and buffy coat) and buccal swabs. In the human studies, scientists are targeting girls who have elevated levels of one of the selected environmental chemicals but normal levels of other chemicals, as determined by prospective measurement of urine concentrations in the prepubertal period. For the animal studies, rats are treated subchronically with the same compounds.

In both humans and rats, prepubertal genomic and proteomic biomarkers and chemical blood levels are being identified as a means of comparing patterns of response to environmental chemicals across species. Metabolites are being measured in the urine by BCERC collaborators at the CDC. Subsequently, samples will be collected at the time of puberty as defined by breast and pubic hair development in girls and vaginal opening in rats.

Inflammation and Immune Modulation

It is well established that immune cells such as monocytes, macrophages, and eosinophils play a role in mammary gland development.[9] The recruitment of tumor-associated macrophages also is implicated in tumor progression of many tissues including the mammary gland.[10,11] A common theme that emerged from the various BCERC studies in experimental animals that have utilized microarray approaches to investigate hormonal, toxicant, and dietary modulation of gene expression in the mammary gland is that the regulation of genes can influence the recruitment and activity of these cell types.

Investigating the effects of progestin/progesterone on mammary organoids that solely express the PRA isoform, the MSU BCERC found many genes associated with innate immunity upregulated by progestin treatment.[12] Prominent among these genes were those with chemotactic activity toward monocytes and macrophages, such as serum amyloid A, beta defensins, Receptor Activator of NF-κB Ligand (*RANKL*), and *CCL15*. The serum amyloid A proteins also increased the abundance of leukocytes in the mammary gland peri-epithelial stroma, *in vivo*, in progesterone-treated mice. These findings uncovered a previously unknown linkage of progesterone to the recruitment of leukocytes in mammary gland development and provided a possible mechanism for progesterone-driven tumor progression. The FCCC BCERC also found a number of genes associated with immune function upregulated after BPA[13] and BBP[14] treatments. Once again, genes encoding leukocyte chemoattractants were among those upregulated. These include *Ccl5* (*RANTES*), *CxCl10*, and *IL-16*. The theme of immune modulation and inflammation continues with the dietary fat studies conducted at the Cincinnati BCERC. A diet high in oleic acid showed elevation of two inflammatory markers, NF-κB and COX-2. This same diet caused prepubertal obesity and showed the highest incidence of tumors.[15]

Loss of GATA-3 in animal models has emerged as a predictor of tumor differentiation, estrogen-receptor status, and clinical outcome. This finding may have implications for early detection and treatment strategies for breast cancer.

Normal mammary gland development and tumor progression both require tissue remodeling and angiogenesis. In both instances, macrophages are important effectors. Once recruited to the mammary gland, they likely are polarized to the pro-angiogenic, tissue remodeling "M2" phenotype. Progesterone, BPA, BBP, and the high oleic acid diet may affect both normal development and tumorigenesis by modulating macrophage recruitment and activation. In light of the association of obesity with increased inflammation and breast cancer risk, particularly the association of high BMI with breast cancer risk in postmenopausal women, the Biology Project studies point to inflammation as a critical linkage between environmental influences and breast cancer. Further studies

in this area not only will elucidate mechanisms in mammary tumorigenesis, but also provide new biomarkers for Epidemiology Project investigations.

Normal Development and Windows of Susceptibility

As in humans, the mammary gland in rodents is unique in that the epithelial rudiment established during gestation remains largely quiescent until puberty, at which point epithelial morphogenesis generates the ductal tree that undergoes lactational differentiation during pregnancy. The implication that the biology specific to puberty creates a window of susceptibility is based on the epidemiology of radiation exposure and breast cancer risk in humans.[16]

BCERC Biology Project investigators, using molecular and cellular methods, extensively studied normal mammary gland development and tissue-based tools in rodent models. An early study from the Bay Area BCERC microdissected the specialized morphogeneic endbuds and mature duct regions from postpubertal mice and compared their expression profiles to epithelium-free distal stroma via a microarray that identified 1,681 genes expressed within the mammary epithelial microenvironment. The microarray data, 1,074 of which were enriched in endbud epithelium and stroma and 222 of which were enriched in mature ducts, were submitted to the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo).[17] GATA-3 was identified as the most highly expressed transcription factor in the mammary epithelium, within both the terminal end buds and the mature ducts. Subsequent studies showed that GATA-3 appears to play a fundamental role in maintaining the differentiated state of the luminal cell[18] and further that loss of GATA-3 has emerged as a strong predictor of tumor differentiation, estrogen-receptor status, and clinical outcome.[19] The latter finding may have important implications for future early detection and treatment strategies for breast cancer.

Puberty is well understood to be a time when normal breast development proceeds rapidly, and the expansion of stem/progenitor cells is required for this development to occur. Studies by FCCC BCERC investigators have proposed that immature breast cells respond to carcinogens more strongly than mature cells. Bay Area BCERC investigators hypothesized that puberty presents a special "window of exposure sensitivity" because breast stem cells are undergoing self-renewal during puberty and that a variety of environmental exposures could alter this key event. In support of these hypotheses, new techniques were developed to genetically mark stem cells,[20] map their distribution,[21] and define the hormonal hierarchy of mammary stem and progenitor cells.[22]

By defining the determinants of breast cancer development in rodent models, the scientists in the BCERC Biology Projects are providing scientific motivation for the examination of new biomarkers and potential genetic contributions in the epidemiologic studies.

Deregulation by environmental agents could establish the first steps in a carcinogenic process that manifests later in adult life. Consistent with this idea, new studies from the Bay Area BCERC suggest that the prototypic carcinogen increases stem cell number in the mammary gland of mice irradiated during puberty. The MSU BCERC focused on the proportion of a special subclass of hormone receptor-expressing cells. Estrogen receptor α and progesterone receptor A containing (ERα+ PRA+) cells decrease significantly during sexual maturation of the gland and are decreased permanently after pregnancy in both mice and rats.[23,24] In the rat mammary gland the greatest decrease occurs in a subpopulation of undifferentiated cells that are ER+ PRA+ and lack expression of STAT5A (STAT5A–). MSU analyzed the phenotype of mammary tumors induced by DMBA in pubertal rats and found a threefold enrichment in ERα+ PRA+ STAT5A– cells, which also represent the majority of proliferating cells within the tumors.

The BCERC projects have studied both known and suspected carcinogens and factors that modify their efficacy. The FCCC BCERC studied early exposure to endocrine disruptors and the response to chemical carcinogens.[13] These studies were expanded through expression profiling and proteomics that have revealed specific molecular alterations occurring prior to tumorigenesis. The Cincinnati BCERC examined the effects of dietary fat in rats on mammary cell differentiation. Tumorigenesis studies show that the greatest increase in susceptibility to mammary gland carcinogenesis occurs

with high-fat diets containing olive oil followed by butterfat compared to low-fat or control diets.

Prior to the initiation of the BCERCs, most studies examined psychosocial factors in relation to menarche, but little research investigated the impact on breast development.

New radiation studies suggest that underappreciated radiation contributes to carcinogenesis.[25] To this end, the Bay Area BCERC has completed studies in a mouse model showing that low radiation exposure to the host increases the frequency and decreases the latency of cancer in unirradiated p53 null mammary epithelium and that this action depends on transforming growth factor beta (TGFβ).[26]

By defining the determinants of breast cancer development in rodent models, the scientists in the BCERC Biology Projects are providing scientific motivation for the examination of new biomarkers and potential genetic contributions in the epidemiological studies. An underlying signature of inflammation that may be a biomarker of metabolic changes is emerging across the experimental studies as a biologically feasible means of increasing cancer risk.

Psychosocial Factors

As discussed in the Introduction to this report, psychosocial factors, particularly stressful family environments and the absence of a biological father, influence girls' timing of menarche.[27,28] Prior to the initiation of the BCERCs, most studies examined psychosocial factors in relation to menarche, but little research investigated the impact on breast development.

The BCERC Epidemiology Project permits a longitudinal assessment of multiple psychosocial risk factors, including family environment indicators (conflict, warmth), paternal absence, stepfather and other male caregiver presence, and childhood and maternal depressive symptoms, on breast and pubic hair development, while adjusting for a number of biological and physiological factors. Widely used standardized measures are being employed to assess psychological constructs in this study, including child psychopathology, maternal depression, family environment, and (in Cincinnati only) the child's self-reported depression. BCERC investigators also are gathering data on the social environment, including socioeconomic status (SES) and race/ethnicity.

The Bay Area and Cincinnati Epidemiology Projects currently are investigating the influence of family context and maternal and child psychopathology on girls' weight status, an established risk factor for early pubertal timing. Preliminary analyses by the Bay Area BCERC indicate that the absence of a girl's biologically related father is associated with overweight at baseline, which subsequently may influence puberty later in the cohort. In addition, through cross-project and cross-center communication with investigators and advisors from the Biology Project, several behavioral patterns have been observed in experimental animals that were exposed to PFOA and BPA, such as aggressive and "stressed" behavior patterns. As the girls in the study grow older, investigators will examine links to breast and pubic hair development. In addition, investigators from all three Epidemiology Projects will examine whether absence of the biological father in early life leads to accelerated breast development and whether family-level factors (SES indicators, family environment factors) either explain or augment this effect. In the epidemiologic studies, BCERC investigators will pursue questions related to PFOA, BPA, and other toxins and their effects on girls' early behavioral indices and subsequent risk for early breast and pubic hair development. They propose that early stressors may trigger the hypothalamic-pituitary-adrenal/gonadal (HPA-HPG) axes to initiate puberty early. In turn, epidemiologic studies have influenced Biology Project investigators' thinking about new animal experiments that incorporate stress.

The Built Environment

BCERC investigations into the impact of the built environment are being pursued at the Bay Area BCERC's KPNC site and the FCCC BCERC's New York (MSSM) site.

In the Bay Area BCERC, researchers are exploring how city planning policies and on-the-ground circumstances in the girls' neighborhoods are associated with their physical activity and BMI values. The results of preliminary analyses suggest that some city land use planning policy indices are associated with physical activity and

that neighborhood conditions are associated with girls' physical activity levels. Development density policy has not been associated with either physical activity or BMI, however.

The researchers in the two sites have access to preliminary data on both East Harlem BCERC girls (n = 234) and East Harlem boys (n = 104) from a parallel NIEHS/EPA Center for Children's Environmental Health and Disease Prevention Research cohort. East Harlem children have easy access to unhealthy foods: 45 percent live on a block with a bodega/convenience store, and 60 percent live on a block with fast food stores. The presence of bodegas on the same block as a child's home was associated with a higher BMI percentile.[29] Vending machine use has declined in schools in East Harlem, but stores that sell unhealthy foods abound just outside school doors, and East Harlem children make frequent use of them.

Going forward, the three BCERC sites participating in the Epidemiology Project are planning a joint analysis of the survey data that asks about perceived neighborhood environment for food and physical activity resources. Little is known about the contribution of factors within the neighborhood environment on dietary behaviors and physical activity behaviors and subsequent impacts on childhood growth and development, including BMI and pubertal development. A neighborhood questionnaire has been administered at all three Epidemiology Project sites, allowing for the collection of information on how children access neighborhood food stores and physical activity resources and how these relationships change as children grow older and gain increasing independence. In general, analyses will assess whether differences in behavior/environment interactions based on race/ethnicity, socioeconomic status, population density, and geographic area are present.

Community Participation

Each BCERC COTC has developed a specific plan and a set of integrated activities (highlighted in the site-specific sections of this report), but the COTCs also are working together and with scientists in both the Biology and Epidemiology Projects to optimize their effectiveness.

One example of the value of the COTCs is dramatically illustrated by the role they played in disseminating unanticipated findings about PFOA and PBDEs to participant families and the community.

After BCERC research results suggested that exposure to PFOA was a potential cause for concern, the Cincinnati BCERC team, including researchers and community advocates, strategized about sharing this information with study participants and the community. Advice was sought from colleagues in the other three BCERCs, program personnel from NIEHS and NCI, and an NIEHS advisory group. In the spring of 2007, data on PFOA levels and the most recent PFOA findings from the BCERC study were shared in three community meetings. In addition, fact sheets were prepared and distributed, an informational phone line was established, and a press release prepared. The process was designed to be transparent for the community, and evaluations indicated that the effort was successful in informing participants and the community to their satisfaction.

Similarly, when BCERC research produced novel findings on PBDEs, the Bay Area COTC took on PBDEs as a topic needing community input and translation. They developed a newsletter article and a white paper summarizing the history of PBDE use in California, as well as a fact sheet on the different commercial formulations of PBDEs in use, sources and pathways of exposure, basic science and epidemiologic studies related to PBDEs, and suggestions for reducing individual and population exposures to these compounds.

The COTCs are using a variety of methods and activities tailored to the circumstances of the respective communities served to engage community members, partners, and study participants in the Centers' Biology and Epidemiology studies. Among other activities, the COTCs are producing manuscripts that explore the history of breast cancer advocacy and describe the transdisciplinary process that has occurred as a result of the jointly funded NIEHS and NCI Cooperative Agreements. Other products have included published papers on the BCERCs and how they function in an integrated, transdisciplinary manner.[30–33]

The transdisciplinary nature of the BCERCs has been enhanced through collaborations between COTC members and both the Biology and Epidemiology Projects. The COTCs and Epidemiology Project researchers worked collaboratively to successfully recruit and retain

the large cohort of girls participating in the Epidemiology Project. In addition, researchers at three Centers, working with their respective COTCs and advocate partners, have opened their laboratories for tours, lectures, and hands-on exercises to enhance community awareness of how research is conducted. A fourth Center developed an educational tool kit for the public that describes why and how different types of mouse models are used to study various aspects of breast cancer biology. All of the COTCs, working together, have developed a system to report outreach events across the COTCs to facilitate sharing of materials and approaches used to reach various communities. It denotes the COTC that sponsored the event, the purpose of the event, the topics presented, estimated audience size and makeup, participation of other BCERCs, evaluation, cost, contact person, and availability of the information on each of the individual Centers' BCERC Web site.

Lessons Learned

Transdisciplinary research and research that involves community participation, such as the work of the BCERCs, are tremendously challenging.

Through working together successfully within and across Centers and with the community, the BCERC teams achieved the scientific synergism that was envisioned when the BCERC RFA was developed. As the BCERC project got underway, the Centers and federal partners learned several important lessons that can inform similar future initiatives. One is that it takes time for scientific and advocacy teams to build a common language, understanding, and trust. For example, the investigators working with animal models and the teams establishing the cohorts of young girls needed to sufficiently understand the details of normal human and animal development during puberty to be able to meld their hypotheses into a unifying paradigm of how environmental factors could impact this development. This is not a trivial issue when, for example, physiological processes in humans do not correspond exactly to those in animal models, and a clinical examination of the girls is the only option for assessing breast development. In addition to having regular teleconferences to discuss business, the BCERCs addressed this need by having an annual "Integration Meeting," in which all of the teams' key personnel participated. This was in addition to annual scientific meetings that were open to the public. The Integration Meeting was a scientific retreat that allowed participants considerable opportunities for discussion, learning, and planning of joint projects. Other challenges became apparent as team members worked toward efficient and fair conduct of the research. One such challenge has been the need to develop adequate ways to formally recognize the role of the breast cancer advocates in the research studies. Additionally, there have been prolonged discussions about formal processes and procedures across the four Centers, such as publications policies that helped recognize and value the intellectual and other contributions of team members, including COTC members.

The role of the advocates at the local level in assisting with recruitment for the epidemiologic study was apparent early on. It was recognized that it was more difficult to define the role of advocacy participation and input into animal toxicology and cancer biology studies. The BCERCs developed innovative and novel approaches to deal with this challenge. Community forums on cancer biology research relevant to the study led to the creation in the Bay Area BCERC of a 45-minute DVD entitled *Of Mice and Women: Modeling Breast Cancer and the Environment*, which helped to translate the principles of animal research to the community. Other centers opened their laboratories to groups of advocates to spend the day gaining hands-on experience in animal, biological, and genetic research. These were highly valuable activities.

Another challenge is that specific needs for data coordination and funding for various laboratory assays were difficult to anticipate in the RFA because the scale-up activities of the specific projects at each Center could not be anticipated until the grant awards were made. In addition to the environmental chemicals in their research plans, the BCERCs identified additional high priority chemicals that emerged during discussions with the general public and the advocacy community. Federal staff worked to identify resources to address these important needs. NIEHS also established a public-private partnership with the Avon Foundation which provided valuable support. The Avon Foundation helped enhance collaborations between the BCERCs and advocates through support such as meeting travel for advocates, data management, and other important activities across the program. ●

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SECTION
III

Individual Center Highlights

The BCERC model uses animal studies to understand biological processes that are difficult or impossible to study in humans.

Although the BCERC Centers work cooperatively on many projects, each Center has its own individual areas of interest and research goals. This section summarizes key findings from the research projects in progress at each of the four individual BCERCs and their collaborating institutions.

Fox Chase Cancer Center

How Environmental Agents Affect Phenotypic and Genotypic Manifestations of the Mammary Gland During Puberty in Humans and Animal Models

The main research question addressed by the FCCC BCERC is how environmental agents affect phenotypic and genotypic manifestations of the mammary gland during puberty in humans and animal models. An important corollary that crosses disciplines is what alters these effects, including timing of exposure, genetics, and obesity.

FCCC's collaboration with the University of Alabama at Birmingham Comprehensive Cancer Center focuses on the effect of environmental exposures to endocrine disruptors on the molecular architecture of the mammary gland during development in animal models and susceptibility to mammary cancer.[1] The specific aims of the FCCC BCERC's Biology Project are to: (1) determine the effects of prenatal and prepubertal exposures to hormonally active xenobiotics such as BPA, BBP, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the genomic and proteomic signatures of rat mammary glands during critical stages of development and differentiation; and (2) determine the effects of prenatal and prepubertal exposures to these substances on predisposition to chemically induced mammary cancer. Since timing of exposure is important to target organ toxicity and mechanism of action, the neonatal/prepubertal period was selected for the first studies.

The Epidemiology Project with the Mt. Sinai School of Medicine (MSSM) involves environmental and genetic determinants of puberty in a cohort of inner-city minority girls. The goals of this project are to: (1) establish and follow annually a cohort of girls with data collected on risk factors and biological specimens; (2) assess age at pubertal stages 2–5 as measured using the Tanner staging system (B2–B5 for breast, P2–P5 for pubic hair), age at menarche, and length of tempo; (3) measure environmental exposures known to influence the sex hormone milieu or cellular factors; (4) examine relationships of hormonal determinants with pubertal milestones; and (5) prospectively investigate the relationship between hormonally active exposures and pubertal milestones. Wide variability in pubertal development, individual susceptibility, obesity, physical activity, and environmental exposures has been observed in the cohort. The study has the potential to guide strategies for prevention and provide a greater understanding about risk for later disease. The project also has contributed to knowledge of hormonally active environmental exposures among girls that may be relevant to pubertal development as well as cancer risk.

The goal of the FCCC BCERC's COTC is to provide educational activities to participants in the Epidemiology Project to enable the community to receive direct benefits from the study. In addition, these activities also may enhance recruitment and retention of study participants. FCCC also has created programs to keep breast

cancer advocates current on scientific discoveries and include the advocates in research and policy decisions.

Major findings from the FCCC BCERC to date include the following:

1. **Effects of BPA in rats, including a dose-dependent increase in mammary tumor multiplicity and reduced tumor latency, have been observed at exceedingly low exposure levels that are well below most estimates of normal, environmentally achievable exposures in humans.**

   - *The experimental model.* Eight-week-old female Sprague Dawley CD rats were bred and maintained on the phytoestrogen-free AIN-93G diet. The rats were exposed to three different compounds (BPA, BBP, and TCDD), at different stages of development, either prenatally (Figure 4a) or prepubertally (Figure 4b).
   - *Choice of doses.* The doses took into consideration European estimates for BPA exposure in infants and preschool children[2–4] and that in the United States, an exposure of up to 50 µg BPA/kg body weight (BW)/day (50 ppb) is considered safe. Based on published pharmacodynamics and distribution data of radioactively labeled BPA, less than 0.01 percent of the dose administered to the lactating dam would be observed in the offspring carcasses.[5] Hence, the offspring are exposed to approximately 10,000-fold less BPA than the dose administered to the dam. Thus, each offspring is exposed to approximately 2.5 ng BPA/kg BW/day at the lower dose tested and 25 ng BPA/kg BW/day at the higher dose, both of which are well below human exposure levels.
   - *Experimental procedures.* Offspring were weaned at age 21 days, and females were euthanized at 21, 35 ± 1, 50 ± 1, and 100 ± 2 days. For the latter three ages, all females were sacrificed in the estrous phase of estrus. The fourth mammary

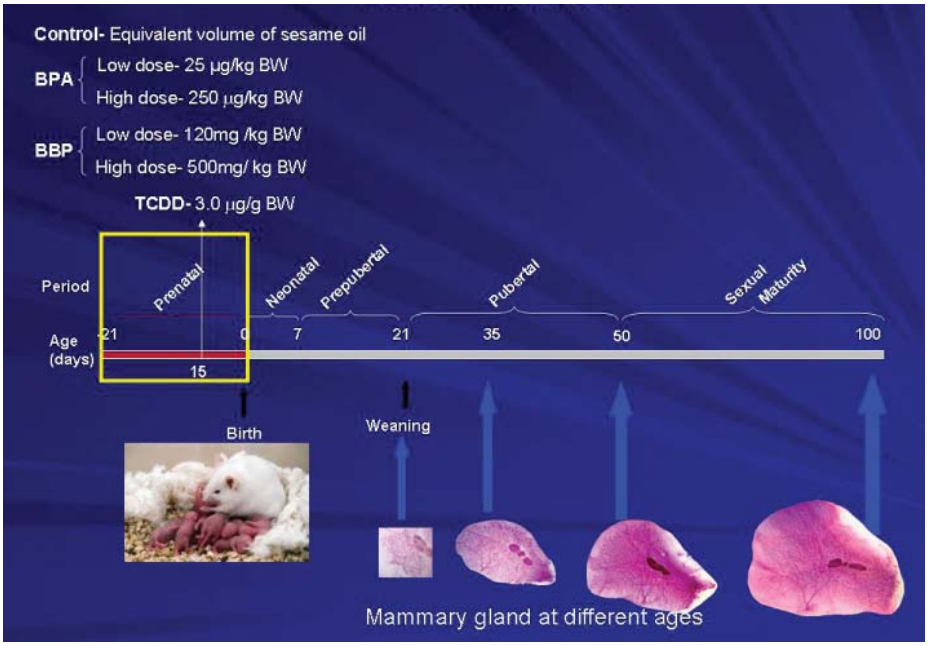


Figure 4a. Prenatal treatment.

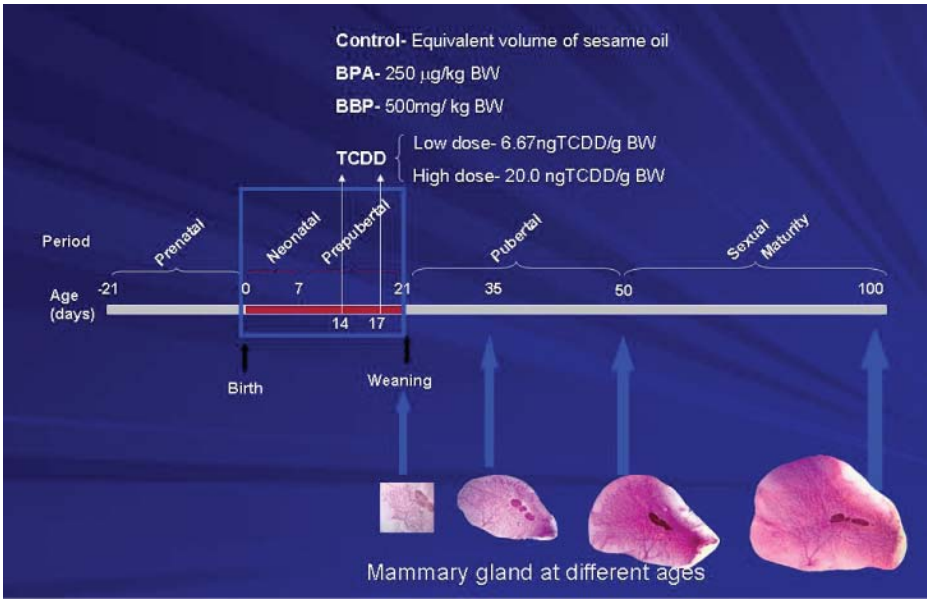


Figure 4b. Prepubertal or neonatal treatment.

glands were rapidly dissected, and the "mammary tree" was frozen for microarray analysis.

- *Tumorigenesis studies.* At age 50 days, female offspring from each treatment group were given a single gavage of 30 mg DMBA/kg BW. All animals were palpated twice weekly and weighed once weekly to monitor tumor development. Data were recorded on palpable tumor latency (time to first, second, third tumor), location, tumor burden, and multiplicity. Animals underwent necropsy at 12 months of age or when tumor burden exceeded 10 percent of body weight. All tumors and gross lesions were dissected out and paraffin-blocked for pathological evaluation. Coded slides were classified as to tumor type, tissue of origin, and degree of invasiveness.
- *Findings.* Rats exposed prepubertally to BPA and then at day 50 to the mammary carcinogen DMBA showed a dose-dependent increase in mammary tumor multiplicity and reduced tumor latency compared to controls[6] (Figure 5). No tumor response was observed after prenatal or prepubertal BBP or TCDD exposure.

2. Genomic analysis of mammary glands of rats exposed to BPA, BBP, or TCDD prenatally or prepubertally showed that each compound has a unique signature that affects multiple genes relevant to development and carcinogenesis.

- An important discovery from these studies is that BPA, BBP, and TCDD each have defined biological processes and cellular components, and the genomic signature in the mammary gland determined by each compound provides specific canonical pathways and networks of gene interaction, the most important of which appear to be transcription- and DNA-related genes, oncogenes, tumor suppressor genes, DNA damage response and repair genes, apoptosis genes, neurotransmitter genes, immunity and inflammation genes, fatty acid and lipid metabolism genes, and cell differentiation and development genes (Figure 6).
- Oncogenes are highly expressed at 50 days in animals exposed to BPA prepubertally but not prenatally.[7] Also, changes were observed at the proteome level. Protein expression in mammary glands of 50-day-old rats exposed prepubertally

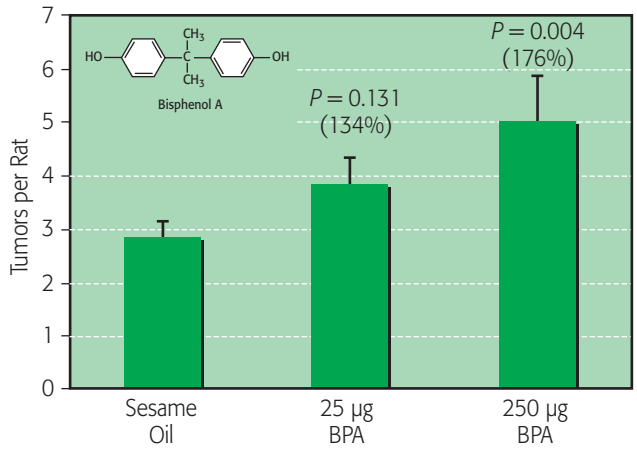


Figure 5. Oral exposure to the environmental chemical BPA only during the prepubertal period increases DMBA-induced mammary tumors in rats.

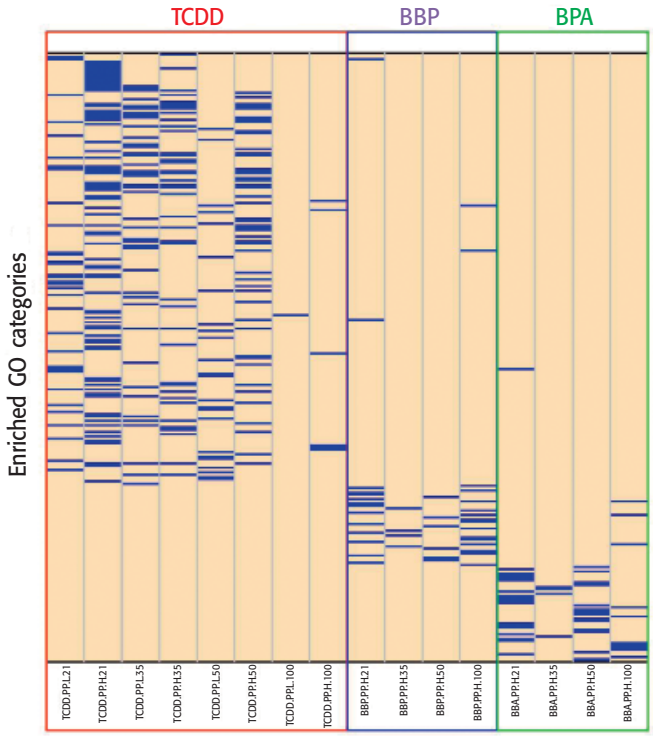


Figure 6. For each exposure in the prepubertal period, an image map was constructed with every available combination of compound (BPA, BBP, TCDD), dose level (low, high), and age (21, 35, 50, 100 days) on the x-axis and various enriched biological processes on the y-axis (selected as all terms with $P < 0.01$ from a conditional hypergeometric test). Analysis of the gene ontologies (GOs) demonstrates that each compound has distinct effects, as shown in the clustering of genes in blue. The effects observed are not only compound-dependent but also specific to the stage of life when the animal received the treatment, the dose administered, and the age when the animals were studied. Little overlap of GOs was observed for the three compounds, emphasizing that each compound induces a specific genomic signature.

to BPA was different than that of age-matched control rats.[6] In the absence of DMBA treatment, lactational BPA exposure resulted in increased cell proliferation and decreased apoptosis at 50, but not 21, days of age (shortly after the last BPA treatment). Using Western blot analysis, steroid receptor co-activators (SRCs) 1-3, Akt, phospho-Akt, progesterone receptor A (PRA), and erbB3 proteins were determined to be significantly upregulated at 50 days. The data clearly indicate that genomic and proteomic changes detected in the mammary glands of prepubertally exposed animals show signatures that predict the response to a carcinogen challenge.

- Prenatal or prepubertal low- or high-dose exposure to BPA or BBP in rats did not influence body weight, vaginal opening, uterine weight, or estrous cyclicity, but prepubertal TCDD treatment (6.67 and 20 ng/kg BW orally) resulted in a slight but significant decrease in body weight at days 21, 35, and 50, but not at day 100. Both TCDD doses resulted in significantly decreased uterine weights at day 35, delayed vaginal opening, and induced irregular estrous cycles. Dual-energy x-ray absorptiometry (DXA) and quantitative magnetic resonance were used to assess the body composition of 100-day-old rats exposed prepubertally and prenatally to BPA, and fat and lean content was found to be similar in exposed and control animals.
- TCDD appears to have a greater effect on tumor suppressor genes as compared to the other two compounds. The aggressive pattern of fat metabolism is more evident for TCDD and BBP (Figure 7, a and b)[8]; BPA does not seem to affect lipid homeostasis.

3. In the Epidemiology Project, biomarkers for several exposures, including phthalates, 2, 5-dichlorophenol, phytoestrogens, and certain phenols, have been found to be quite high, indicating exposure to high levels of endocrine disruptors in the cohort. These hormonally active exposures are derived from personal products and the indoor environment and thus are preventable.

- Metabolites of 1, 4-dichlorobenzene (from mothballs and air fresheners) in the study participants are more than 50 times higher than those report-

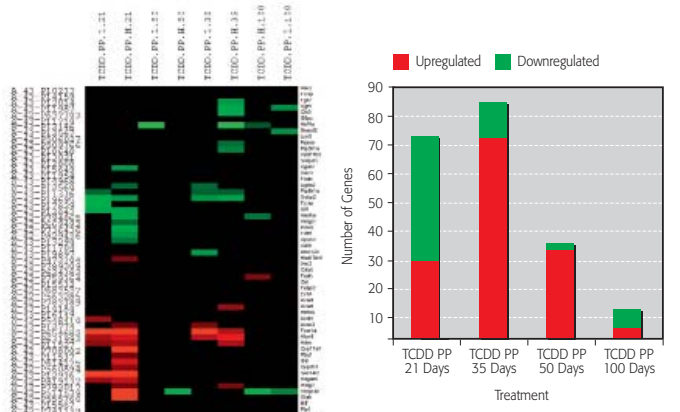


Figure 7a. (left) Heatmap of fatty acid- and lipid metabolism-related genes affected by prepubertal TCDD exposure. As the heatmap indicates, a high dose of TCDD received during the prepubertal period primarily affected animals at ages 21, 35, and 50 days, but no effect was observed at 100 days. This difference can be seen in the histogram (right), which indicates the number of upregulated (red) and downregulated (green) genes in the mammary glands of rats of specific ages that had been prepubertally exposed to TCDD.

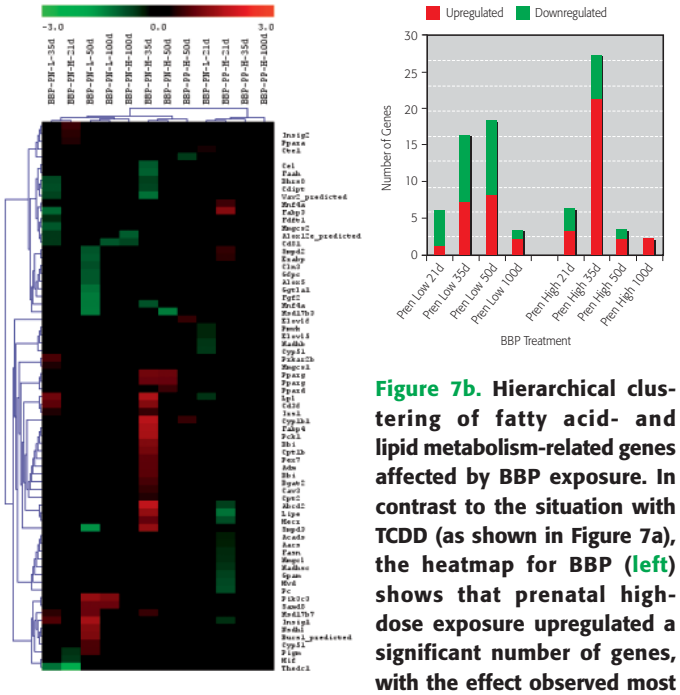


Figure 7b. Hierarchical clustering of fatty acid- and lipid metabolism-related genes affected by BBP exposure. In contrast to the situation with TCDD (as shown in Figure 7a), the heatmap for BBP (left) shows that prenatal high-dose exposure upregulated a significant number of genes, with the effect observed most prominently at age 35 days in the offspring. The histogram (right) shows the number of upregulated (red) and downregulated (green) genes in the mammary glands of rats of specific ages that had been prenatally exposed to BBP.

ed in children in a national survey.[9] Levels of some phthalate metabolites also are higher than national data. (Phthalates are anti-androgens and possibly obesogens.) Other environmental endocrine disruptors, including BPA, are ubiquitous in the girls in the Center's cohort.

- Obesity is common in the cohort, and associations between phthalate metabolites and obesity have been reported.[8]
- Sixty-nine percent of girls in the Center's cohort who were above Tanner breast development stage B2 at baseline were above the 85th percentile of BMI for age. Three completed panels of 20 biomarkers showed no significant effects on puberty among the MSSM girls, using the pubertal assessments available up to October 2008. This cohort, having a high prevalence of obesity, might not show effects of environmental agents that may be detected in underweight girls. Therefore, in the combined three-site BCERC cohort, where there is more variability in BMI scores, effects may be more evident. For example, the Center's cohort shows modest effects of exposure to triclosan and enterolactone in altering the timing of B2 in girls with lower BMI, based on baseline and one followup visit.
- A pilot study that demonstrated the reliability of biomarkers measured in a spot urine sample was completed.[10] Intra-individual variation shows quite remarkable stability in children's levels of endocrine disruptors, suggesting that one spot sample may be representative of a year's exposure for some agents.

4. **Several SNPs have been associated with either BMI or breast stage in the KPNC cohort (joint project with Roswell Park Cancer Center); similar associations involving SNPs of the same genes have been observed in rats.**

   - A preliminary analysis of selected genotypes among 397 girls from the KPNC cohort (from the Bay Area BCERC) was conducted by the FCCC BCERC. The analysis, a joint project with Roswell Park Cancer Center, found that several SNPs were associated with either BMI or breast stage at the $P < 0.15$ level. An analysis of the MSSM cohort is under way. SNPs of the same genes have been found in the dysregulated genes in the mammary glands of rats exposed to these compounds (Table 9 and Figure 7, a and b).[11] These data, although preliminary, establish a connection between the experimental data in animals and those in the girls under study.

5. **FCCC COTC activities, some conducted in cooperation with community-based organizations, have provided study participants and other community residents with environmental and community health information. The incentives provided by COTC events have helped to identify many study participants who had been thought to be lost to followup and made it possible to bring them back into the study. FCCC also has designed and implemented a workshop program for breast cancer advocates.**

   - A total of 427 people (participants and other community residents) attended COTC events in 2008. This effort was made more efficient by focusing on giveaway activities, including a back-to-school program in which a backpack with school supplies was given to study participants. These events brought many former study participants back into the program; for example, the back-to-school outreach event resulted in 54 completed visits.
   - The COTC has partnered with community-based organizations that have expertise in providing quality supplemental education for minority children and their families, including the New York City Parks Foundation and Little Sisters of the Assumption. The COTC also makes additional contacts with study participants using print materials created especially for this population. The communications include a biannual newsletter that informs participants of the progress of the Center studies and fact sheets that focus on one topic in pediatric environmental or community health and its relevance to the community. Two examples of the fact sheets that have been created and distributed are "Chemicals in Cosmetics" and "Your Child and Camphor."
   - In addition to study participants, advocates also are key stakeholders in research on breast cancer and should be included in research and policy decisions. The FCCC has done this by creating a successful program entitled "A Day in the Life of the Breast Cancer Research Laboratory…A Work-

Table 9. Comparison Between SNPs Found in Prepubertal Girls Exposed to BBP and the Gene Disruption Profile of Rats Exposed to BBP, BPA, or TCDD

SNPs Model	Gene	BMI for Age ≤ vs. > 85th Percentile[a]	Tanner-Breast Stage 2+ vs. 1[b]	Rat Exposed Prepubertally to		
				BPA	BBP	TCDD
rs1044498	*ENPP1* ectonucleotide pyrophosphatase/ phosphodiesterase 1 [*Homo sapiens*]		Yes		Disrupted	Disrupted
rs11542313	*GAD1* glutamate decarboxylase 1 (brain, 67 kDa) [*Homo sapiens*]	Yes		Disrupted	Disrupted	Disrupted
rs7566605	*INSIG2* insulin induced gene 2 [*Homo sapiens*]		Yes		Disrupted	Disrupted
rs2016520	*PPARD* peroxisome proliferator-activated receptor delta [*Homo sapiens*]	Yes		Disrupted	Disrupted	
rs1051424	*RPS6KB1* ribosomal protein S6 kinase, 70 kDa, polypeptide 1 [*Homo sapiens*]	Yes			Disrupted	Disrupted
rs180515	*RPS6KB1* ribosomal protein S6 kinase, 70 kDa, polypeptide 1 [*Homo sapiens*]	Yes			Disrupted	Disrupted
rs5888	*SCARB1* scavenger receptor class B, member 1 [*Homo sapiens*]	Yes			Disrupted	
rs4822063	*SREBF2* sterol regulatory element binding transcription factor 2 [*Homo sapiens*]	Yes	Yes		Disrupted	Disrupted

Key: SNPs, single nucleotide polymorphisms; BBP, butyl benzyl phthalate; BPA, bisphenol A; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; BMI, body mass index.
[a] BMI for age ≤ vs. > 85th percentile by 2- or 3-level SNP.
[b] Breast Stage 2+ vs. 1 by 2- or 3-level SNP.

shop for Breast Cancer Advocates." Four of these workshops have been conducted for a total of 64 participants.

Michigan State University

Interactions of Estrogen and Progesterone, Genetic Background, and Pubertal Environmental Exposures in Mammary Gland Development and Susceptibility to Mammary Cancer

The MSU BCERC's goal is to define endogenous hormonal regulation of mammary gland development across the life span and elucidate how hormones (estrogen, progesterone), environmental exposures (chemicals, phytoestrogens, diet), and genetic background (mice, rats) interact to increase or decrease susceptibility to mammary carcinogenesis. The MSU BCERC uses an interdisciplinary approach that includes endocrinology, immunology, molecular biology, toxicology, and bioinformatics. The MSU BCERC has hypothesized that the hormone-dependent expansion of mammary progenitor cells during puberty, which is necessary for formation of the mammary gland, is the basis for the pubertal window of susceptibility to environmental exposures that impacts breast cancer risk. Recent evidence indicates that progesterone, in addition to estrogen, contributes significantly to the etiology of breast cancer[12] (Figure 8). The mechanisms of progesterone's action in the mammary gland are not well understood, however. A major goal is to delineate how progesterone acts, along with mediation by two progesterone receptor isoforms (PRA and PRB), and how they impact breast cancer risk.

The following are among the MSU BCERC's key findings to date:

1. **Genetic background determines mammary gland responses to estrogen and progesterone. Two inbred mouse strains and two rodent species (rat and mouse) differ so greatly in estrogen and progesterone responses that caution is required in generalizing findings. These results imply that in genetically heterogeneous human populations, individuals may respond differently to the same**

hormone. Differentially expressed genes may affect hormonal regulation and have potential utility as biomarkers for both breast cancer susceptibility and the assessment of estrogen or progesterone sensitivity in the breast. The specific differentially regulated genes and pathways identified in these studies (i.e., *PRB, RANKL, cyclin D1, C/EBP*β*, Id2,* and *p21*) may provide new biomarkers for the assessment of breast cancer susceptibility.

- MSU BCERC researchers investigated estrogen receptor α (ERα), PRA, and PRB expression and function in wild-type mice and rats. Prior to these studies, receptor-specific functions were identified by examining the phenotype of transgenic or gene-deleted mice, whose mammary gland development often was abnormal.[13–17] Importantly, this analysis of receptor expression and function in wild-type mice and rats provides a more accurate picture of their functions in normal mammary gland development. Striking differences were identified between two strains of mice (BALB/c and C57BL/6) and between the mouse and rat in the patterns and regulation of ERα and PRA and PRB expression that determine the mechanisms by which hormones regulate mammary gland development.
- In the mouse, exclusive PRA expression prior to pregnancy limits mammary gland development to the formation of ducts.[18] In contrast, in the rat and human, expression of both PRA and PRB leads to the concomitant formation of ducts and lobules prior to pregnancy[12,19] (Figure 9).
- C57BL/6 mice are less responsive to progesterone than BALB/c mice[20] (Figure 10). This results in altered expression of progesterone-regulated genes that play important roles in mammary gland development [*PRB*, *RANKL*, *cyclin D1*, CCAAT/Enhancer Binding Protein β (*C/EBP*β)] and the downstream effectors of *RANKL*, nuclear *Id2* and *p21*. In contrast, estrogen responsiveness is greater in C57BL/6 than in BALB/c, suggesting that estrogen may play a compensatory role in C57BL/6 glandular development through its effect on the induction and activation of *STAT5A*, a known regulator of *RANKL*.
- MSU BCERC researchers determined that the rat pattern of ERα, PRA, and PRB expression is significantly different from that of the mouse and

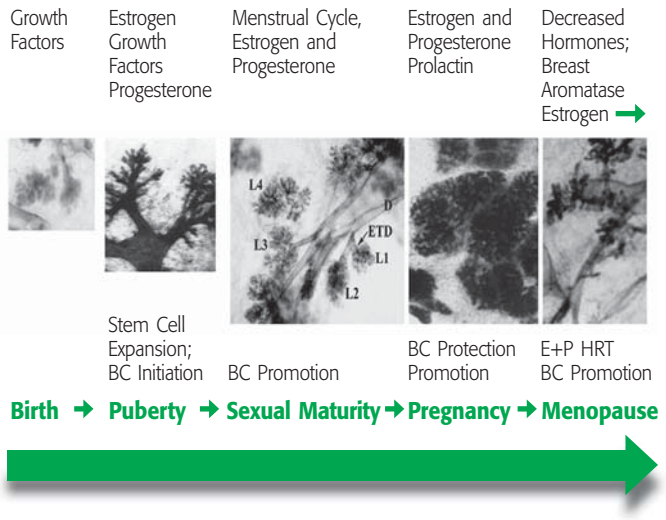


Figure 8. Breast development, hormones, and breast cancer risk. Schematic representation of known and hypothesized effects of hormones and growth factors at various stages of human breast development across the life span, and their impact on the development of breast cancer.

closely resembles the pattern in the human breast (Figure 8). In the adult rat mammary gland, an absolute requirement exists for *both* estrogen and progesterone to induce proliferation. Studies from this BCERC indicate that amphiregulin and not *RANKL* may be the critical progesterone-induced paracrine mediator in the rat. Clinical studies have suggested that amphiregulin also plays a role in human breast cancer progression, and its expression has been associated with aggressive disease.[21]

2. Patterns of receptor expression and function that are highly conserved across species are likely to be informative about their functions in the human breast and in the etiology of breast cancer.

- The MSU BCERC has determined that certain patterns of receptor expression and regulation are highly conserved across mouse strains and between the rat and the mouse. ERα+ PRA+ cells decrease during sexual maturation of the gland and are decreased permanently after pregnancy.[22] The greatest decrease occurs in a subpopulation of undifferentiated cells that are ER+ PRA+ STAT5A–. The MSU BCERC hypothesizes that these are progenitor cells whose prevalence at puberty and decreased abundance after pregnancy are linked to the pubertal window of suscepti-

bility and the protective effect of pregnancy, respectively.

- Analysis of the phenotype of mammary tumors induced by DMBA in pubertal rats shows a threefold enrichment in ERα+ PRA+ STAT5A– cells compared to the adjacent normal gland. Furthermore, 51 percent of the proliferating cells in tumors are ERα+ PRA+ STAT5A–. The possibility that these PRA+ STAT5A– cells represent a subpopulation of susceptible progenitor cells currently is under investigation.

3. **Inflammatory cells such as macrophages are known to play an essential role in normal mammary gland development and tumor progression. The MSU BCERC's unique findings of progestin induction of inflammatory genes and progesterone-induced recruitment of leukocytes to the mammary epithelial stroma indicate a novel role for progestins in mammary gland development and function, and present a heretofore unsuspected link between progestins and inflammation in the etiology of breast cancer. An inflammatory mechanism may underlie the increased breast cancer risk associated with endogenous and exogenous hormone exposures, such as oral contraceptives and postmenopausal hormone therapy, that include progestins. This also raises the possibility that other mechanisms (environmental chemicals, dietary factors) can induce an inflammatory state in the mammary gland that can impact normal development and/or cancer susceptibility and tumor progression.**

   - Researchers with the MSU BCERC identified PRA-regulated genes through microarray analysis of *in vitro* progestin-treated mouse mammary epithelial organoids obtained from pubertal or adult mice. Sixty-nine progestin-regulated genes were identified in both pubertal and adult epithelium, along with 38 genes regulated uniquely in adult epithelium and 96 genes regulated uniquely in pubertal epithelium.
   - Most striking is the novel observation that about 20 inflammation-related genes were upregulated by progestin, prominently in both pubertal and adult epithelium. Many of these genes were not known previously to be progestin-regulated. The most dramatically upregulated inflammatory genes were serum amyloid A1, A2, and A3 (*SAA-1*, *2*, and *3*).
   - The MSU BCERC confirmed progestin-induced SAA-1 protein expression *in vivo* in progesterone-treated mammary glands. SAA proteins are implicated in the induction of inflammation and increased expression of proinflammatory factors by leukocytes and lead to the recruitment and adhesion of these cells to sites of infection and

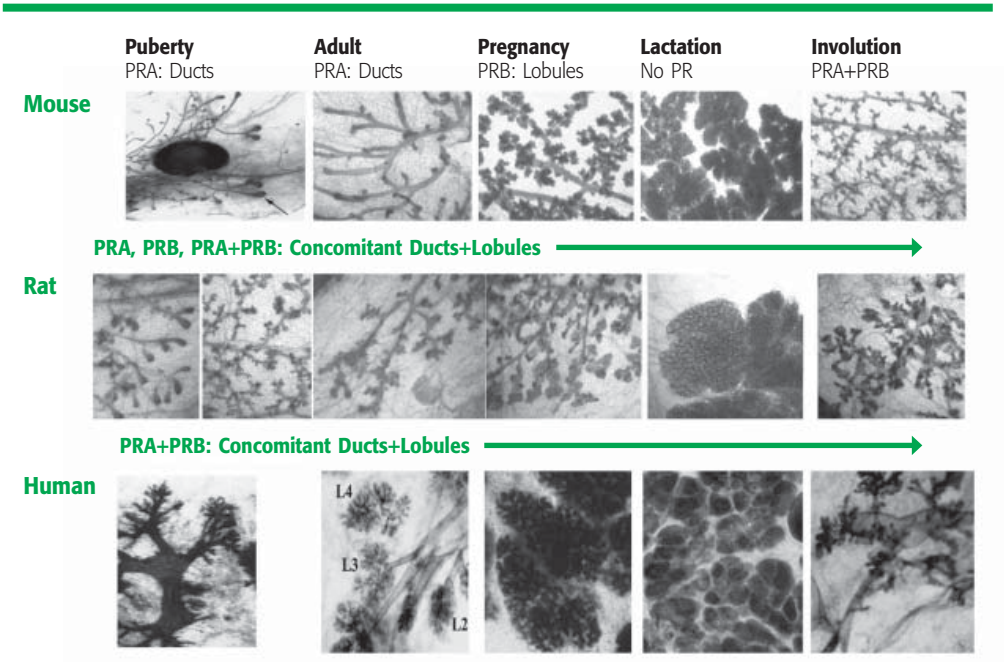


Figure 9. Species-specific function of progesterone receptor (PR) isoforms (PRA, PRB) during mammary gland development. Specific PR isoforms that are expressed at various stages of mammary gland development in the mouse, rat, and human from puberty through sexual maturity, pregnancy, lactation, and postlactational involution are shown in relation to differences in duct and lobule development. In the mouse, exclusive expression of PRA prior to pregnancy limits mammary gland development to ducts. Co-expression of PRA and PRB in rat and human mammary glands leads to concomitant duct and lobule development prior to pregnancy.

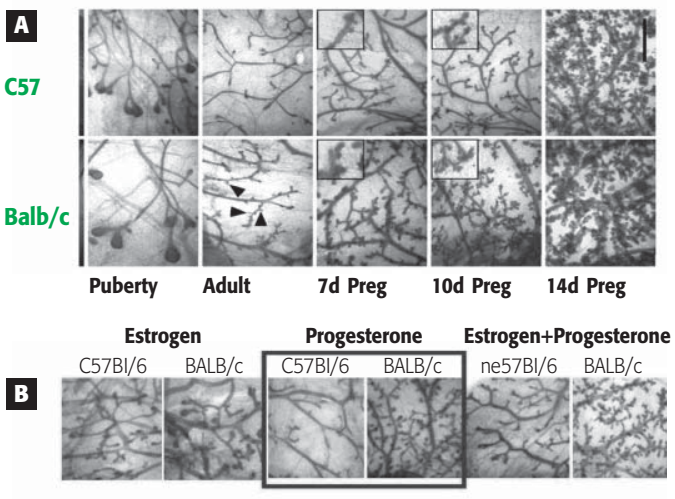


Figure 10. Genetic background determines mammary gland response to estrogen and/or progesterone. A. Prior to pregnancy, C57BL/6 mice exhibit an underdeveloped mammary gland with reduced secondary and tertiary ductal branching compared to Balb/c mice. C57BL/6 mice also exhibit delayed alveologenesis during pregnancy. B. Adult Balb/c and C57BL/6 ovariectomized mice were treated with exogenous estrogen, progesterone, or estrogen + progesterone. C57BL/6 mice are highly sensitive to stimulatory effects of estrogen but are less sensitive to the stimulatory effects of progesterone compared to Balb/c mice. The reduced responsiveness of C57BL/6 mice to progesterone causes delayed alveologenesis during pregnancy.

injury. In support of this, increased infiltration of leukocytes in progesterone-treated mammary glands was found (Figure 11).

4. **Genetic background impacts the effects of environmental exposures on pubertal mouse mammary gland development. It can alter the effect of diet on increased adiposity during adolescence, which can impact mammary gland development and hormone responsiveness, potentially influencing breast cancer susceptibility. Reversing adiposity restores the normal pattern of mammary gland development. This may shift, however, and extend the pubertal window of susceptibility to an older age.**

   - Increased body weight and adiposity have been associated with early onset of menarche in girls—a known risk factor for breast cancer. Elevated adiposity during puberty may alter breast cancer risk by perturbing breast development, which may have long-term consequences on the function and hormonal responsiveness of the adult gland. MSU BCERC researchers investigated the impact of pubertal obesity on breast development in two strains of mice (C57BL/6, BALB/c) fed a high-fat diet (HFD) or control diet (CD) from weaning through puberty. The HFD consisted of 60 percent of kilocalories (kcal) from fat, 20 percent of kcal from carbohydrate, and 20 percent of kcal from protein, whereas the CD had 12 percent of kcal from fat, 69 percent of kcal from carbohydrate, and 19 percent of kcal from protein. For both diets, 11 percent of kcal from fat came from corn oil, with the remainder of the fat (1% or 49% kcal) coming from lard.
   - The HFD diet produced a significant weight gain only in C57BL/6 mice. The weight gain was accompanied by elevated leptin levels, diminished glucose tolerance, and increased parametrial and mammary fat pad weights (Table 10).
   - The HFD diet caused inhibition of mammary ductal development in C57BL/6 mice that was associated with markedly reduced PRA expression, an indication of reduced estrogen levels and/or estrogen response (Figure 12). When C57BL/6 mice were switched from the HFD to the CD, they lost weight and resumed mammary gland development. In C57BL/6 mice, an HFD also resulted in refractoriness to exogenous estrogen- and/or progesterone-induced mammary gland development, indicating obesity-induced refractoriness to hormones at the target tissue level.
   - In BALB/c mice, an HFD caused only a modest increase in body weight and parametrial and mammary fat pad weights; no significant effect on mammary development or ERα or PRA expression was present.
   - Studies are in progress to determine the effect of pubertal exposure to an HFD, with or without resulting obesity, on susceptibility to mammary tumorigenesis.

5. **To facilitate the inclusion of community concerns in the research projects, the MSU COTC partnered with community advocates and other stakeholders concerned about breast cancer and environmental risks and systematically studied dissemination practices. The COTC is well positioned to provide the expertise necessary to communicate the findings of the epidemiology and biology studies to the concerned public.**

- To serve the advocate community, the MSU BCERC team organized special programs about community involvement in environmental decisions and media advocacy and public relations techniques. In addition, the MSU BCERC has instructed advocates about persuasive communication strategies for influencing policymakers. The MSU COTC, in collaboration with several advocates from other centers, is preparing an article addressing the evolution of the role of breast cancer activists in the scientific research process.[23]

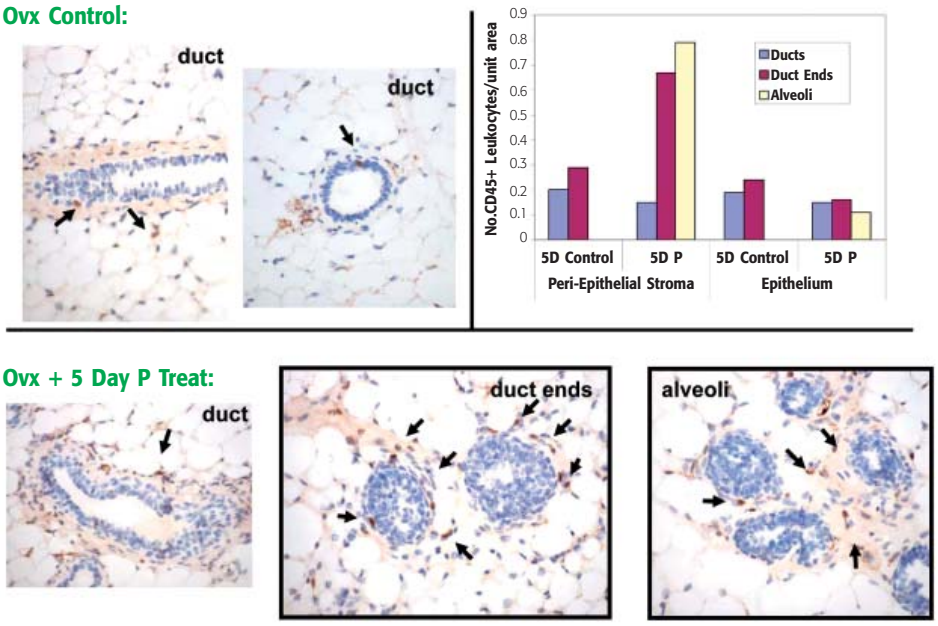


Figure 11. Progesterone-induced leukocyte infiltration in the mammary gland. Balb/c mice were ovariectomized (Ovx) and treated for 5 days with progesterone. Leukocyte infiltration was determined by immunostaining with leukocyte-specific CD45 antibody; leukocyte infiltration (indicated by arrows) was increased in the periductal stroma of stimulated duct ends and developing alveoli in progesterone-treated mammary glands. Graph: Quantitation of leukocyte infiltration.

- To optimize translation of new findings into the most effective risk minimization, MSU COTC faculty from the Department of Communication analyzed breast cancer information produced by the news media and breast cancer Web sites[24] and conducted focus groups with mothers and adolescent daughters.[25,26] Breast cancer Web sites were analyzed for their design tenets and theoretical information.[27] A Meaningful Message Study was conducted and analyzed for the types of women most likely to have meaningful messages about breast cancer[28] and the types and sources of these messages.[29]
- A Message Testing Study was conducted with more than 400 women to test potential design variations of messages for mothers of preadolescent girls, comparing the effectiveness of different message sources, levels of involvement, and messages that dealt with one of three topics:

Table 10. Impact of HFD on C57BL/6 and Balb/c Mice

Mouse Strain	Diet	Fasting Blood Glucose (mg/dL)	Fasting Plasma Insulin (ng/mL)	Leptin (ng/mL)	Mammary Gland 4 Weight (mg)	Mammary Gland 2 and 3 Weight (mg)	Parametrial Fat Pad Weight (mg)	Liver Weight (mg)
C57BL/6	CD	139.6 ± 5.6	0.48 ± 0.06	2.79 ± 0.58	113.6 ± 5.2	147.9 ± 6.2	89.3 ± 11.9	798.4 ± 14.8
C57BL/6	HFD	120.9 ± 4.5	0.47 ± 0.05	8.74 ± 1.99[a]	206.4 ± 18.6[a]	252.9 ± 2.1[a]	233.6 ± 30.6[a]	680.5 ± 18.0[a]
Balb/c	CD	102.9 ± 4.7	0.42 ± 0.06	1.96 ± 0.20	92.5 ± 5.0	124.2 ± 6.2	100.0 ± 11.3	736.5 ± 17.3
Balb/c	HFD	118.3 ± 4.9[c]	0.60 ± 0.04[c]	2.39 ± 0.16[b]	111.7 ± 7.6[b]	170.0 ± 8.6[b,c]	156.7 ± 22.1[c]	712.3 ± 12.3

KEY: CD, control diet; HFD, high-fat diet; ANOVA, analysis of variance.
[a] $P < 0.01$ when compared to C57 mice fed CD (n = 7 to 8, ANOVA).
[b] $P < 0.001$ when compared to C57 mice fed HFD (n = 6 to 7, ANOVA).
[c] $P < 0.05$ when compared to CD-fed Balb/c mice (n = 6, Student's t-test).

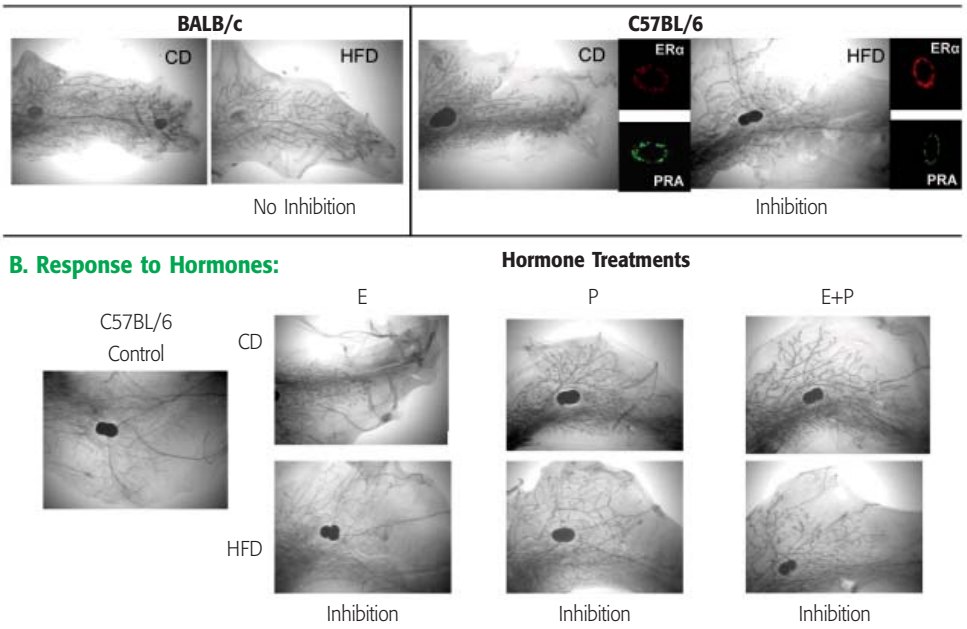


Figure 12. Genetic background determines the effect of a high-fat diet on pubertal mammary gland development and response to hormones. A. C57BL/6 and Balb/c mice were fed a control or high-fat diet from 3 to 7 weeks of age, during the pubertal period of mammary gland development. In C57BL/6 mice, the high-fat diet significantly inhibited ductal elongation and reduced PRA expression compared to Balb/c mice. B. After ovariectomy and exogenous hormone treatments with estrogen (E), progesterone (P), or estrogen + progesterone (E+P), C57BL/6 mice showed severely reduced morphological response to the hormones.

adoption of healthy lifestyle behaviors, limiting exposure to chemicals, and understanding normal mammary gland development.

Bay Area

Understanding Normal Breast Development in Animal and Human Models

The Bay Area BCERC has made significant contributions toward answering scientific questions surrounding environmental influences on the etiology of breast cancer. Its basic science studies, based at the University of California, San Francisco (UCSF) and the Lawrence Berkeley National Laboratory, have focused on the many unanswered questions related to normal breast development in the mouse model and human tissue cells. Based on the observation that many of the processes present in normal development (e.g., invasive growth) are parallel to processes in carcinogenesis, the underlying motivation has been to understand normal breast development as a lens for examining breast carcinogenesis. Investigators have explored the fascinating interactions and crosstalk of breast epithelial and stromal tissue as well as the nature of breast stem cells and their role in breast carcinogenesis. Ionizing radiation has been used as the prototype carcinogen because understanding the effects of this known carcinogen provides a pathway to understanding the effects of other, putative environmental carcinogens.

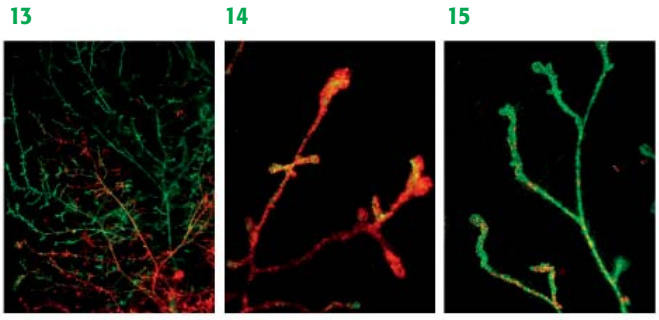
The Bay Area BCERC has collaborated with the epidemiologic studies in Cincinnati and at MSSM, part of the FCCC BCERC, to add to the number and diversity of girls contributing to the human longitudinal studies and to add particular expertise in methods from nutritional, environmental, physical activity, and social epidemiology, as well as psychology and pediatric endocrinology. In the Bay Area BCERC, 444 girls between the ages of 6 and 7 years were recruited from Kaiser Permanente Northern California (KPNC) to participate in the epidemiologic study, which is called CYGNET (for Cohort study of Young Girls' Nutrition, Environment, and Transitions). To facilitate clinical examinations and followup of the cohort, all study participants lived in defined geographic areas and received health care from KPNC, not just at the time of recruitment, but also at the time of birth in 1997–1998. This latter eligibility criterion makes it possible for the Bay Area BCERC to obtain data on the mothers' pregnancies and on the girls' early growth patterns and early medical histories. Furthermore, because the population targeted for recruitment all shared a common and familiar source of medical care and had unique medical records, potential participants were identified easily and were relatively willing to volunteer for the research project; the retention rate has been more than 90 percent during the first 3 years of followup.

The community outreach component of the Bay Area BCERC has been directed by Zero Breast Cancer, headquartered in Marin County, California. It was here that much of the original impetus for this research project was stimulated by very high rates of breast cancer incidence and mortality in Marin and other Bay Area counties compared to national norms. The advocates and activists brought their concerns to local, regional, and national attention, which resulted in the RFA that led to the BCERCs. Members of this COTC have been active co-investigators on the research teams for both the Biology and Epidemiology Projects and join in the regular scientific meetings, contribute community perspectives, and work interactively to improve bidirectional communication between scientists, advocates, study participants, and the community, while working to effectively disseminate the results of BCERC findings.

Major findings and accomplishments from the Bay Area BCERC to date include the following:

1. **Normal breast development during puberty is characterized by branching of breast ducts, where multiple layers of tissue and the expansion of the stem cell compartment are present. The critical regulators of differentiation and signals from growth factors have been identified using novel microscopy and image analysis.**

   - The new development and use of time-lapse video images that monitor epithelial branching initiation, elongation, and splitting in mammary organoids reveal the requirement for specific signals from the microenvironment (Figures 13 through 16). This new technical method will be valuable in the future in the monitoring of cellular growth and proliferation in the process of tumor formation stimulated by putative environmental carcinogens.[30]
   - Transcriptional profiling of morphogenic structures led to the discovery that levels of the protein GATA-3 are critical to the differentiation of luminal epithelial cells during puberty. Moreover, GATA-3 loss has been shown to be a more powerful predictor of breast cancer status than estrogen-receptor positivity.[31]



Figures 13 through 15. A whole-mount two-color image of mammary epithelial outgrowths derived from pooled mammary epithelial cells, separately transduced with either HIV-*H2BmRFP* or HIV-*Zsgreen*, that were transplanted into the cleared mammary fat pad at nonlimiting dilution and harvested from virgin recipient mice.[33] 13. A low magnification micrograph of outgrowth with ducts containing highly intermixed red and green fluorescent cells. 14. A high-magnification example of a predominantly red fluorescent duct with clusters of green fluorescent cells. 15. A high-magnification example of a predominantly green fluorescent duct with clusters of red fluorescent cells.

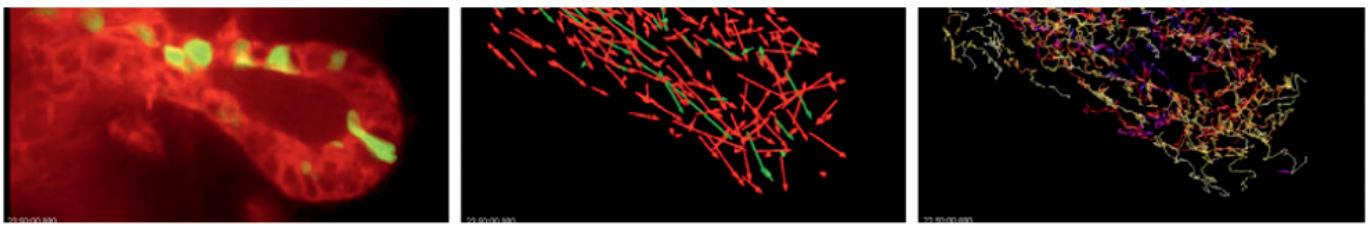


Figure 16. Mammary epithelial elongation in culture in a model of terminal end-bud invasion in puberty occurs through a multilayered active epithelial state.[34] Mammary epithelial fragments (organoids) were harvested from a mouse with EGFP knocked into the stem cell antigen 1 (Sca-1) locus. GFP positive cells were mosaically distributed throughout the epithelia. All cells were labeled with Cell Tracker Red. Left: During elongation, the invasion front remained multilayered, smooth, and free of labeled protrusions and was characterized by dynamic cell rearrangements within a partially depolarized epithelium. The epithelial nuclei were tracked during elongation using automated software (Bitplane's Imaris). Center: The red (Cell Tracker Red) and green (Sca-1 EGFP) channels were tracked separately, and the resulting displacements were color coded and displayed. Right: The trajectories also were calculated and are displayed using a color scale from dark blue (earliest) to white (most recent).

- Stem cells, which are the putative cellular target of carcinogens, have been studied using integrated microscopy, image analysis, and statistical analysis to map their distribution in the architecture of the mammary gland.[32] The specific signals that regulate these cells now can be more readily studied using these techniques combined with a new technique to mark the mammary gland.[33]
- The carcinogenic action of ionizing radiation, which is being studied as a prototype for other putative carcinogens, has been shown to alter the tissue microenvironment. This in turn has been shown to promote mammary carcinogenesis, perhaps through deregulation of stem cell number. This finding may represent a paradigm shift from the theory that radiation works directly to damage DNA and also may hold true for other putative carcinogens.

2. **The Bay Area BCERC has established a cohort of young girls, who were age 6–7 years at baseline exam, through annual clinic visits. This cohort includes substantial information from questionnaires; clinical examinations to assess anthropometry and breast and pubic hair development; and a biorepository including urine and blood, and saliva samples as a source of DNA. This accomplishment was undertaken in close collaboration with companion studies in Cincinnati and New York. Key observations from this study include the following:**

- Including the baseline exams that were conducted from June 2006 through August 2007, girls have had three annual visits; the study is about one-third of the way through the fourth annual visit.
- During annual visits, the girls' parents or guardians are asked their child's demographic characteristics and questions about other characteristics, such as their physical activity, medical history, household and personal product use, psychosocial environment, and neighborhood environment.
- Physical examinations were performed to assess height, weight, body composition, and Tanner stage of pubertal development.
- Retention rates are high; during the third full cycle of examinations, 404 girls (91%) were seen in the clinic.

3. **The Bay Area BCERC is actively analyzing baseline characteristics to examine associations with BMI and pubertal development at the time of the second annual visit; such analyses will be extended longitudinally as the girls continue to be followed through annual examinations. Preliminary analyses of this cohort suggest the following:**

- Girls in this study population in California are entering puberty in proportions similar to previous reports.[34] Substantial differences have been noted by race and ethnicity at each examination year, with 32 percent of African-American girls showing the first signs of either breast development or pubic hair at baseline (ages 6–7 yrs), compared to 15 percent of Hispanic girls, 10 percent of white girls, and 4 percent of Asian girls who had reached a similar stage of development.
- As expected, BMI at baseline is a major predictor of breast and pubic hair development at the second annual examination (first followup exam).
- Girls are exposed to many of the hormonally active chemicals of interest, and substantial variation in levels of exposure is present within the cohort.[35]
- Nutrient intake during the first year appears to be associated with breast development at the first followup exam (**Figure 17**). Girls who showed evidence of onset of breast development were more likely to consume diets that were high in animal protein and sugar and low in vegetable protein, dietary fiber, and phytoestrogens.
- Selected chemical exposures appear to be associated with pubertal status at the first followup exam. Girls who showed evidence of onset of breast development appear to have lower urinary excretion levels of PCBs and phytoestrogens.
- Further analysis is being conducted to account for covariates and to include data from the other two BCERC epidemiology studies.
- As an example of the unique capabilities of the KPNC-based cohort, preliminary analyses were conducted using data from the electronic birth records of these girls. These analyses confirmed earlier findings that birthweight is directly correlated with BMI at age 6 or 7 years, when the girls entered the study (**Figure 18**).

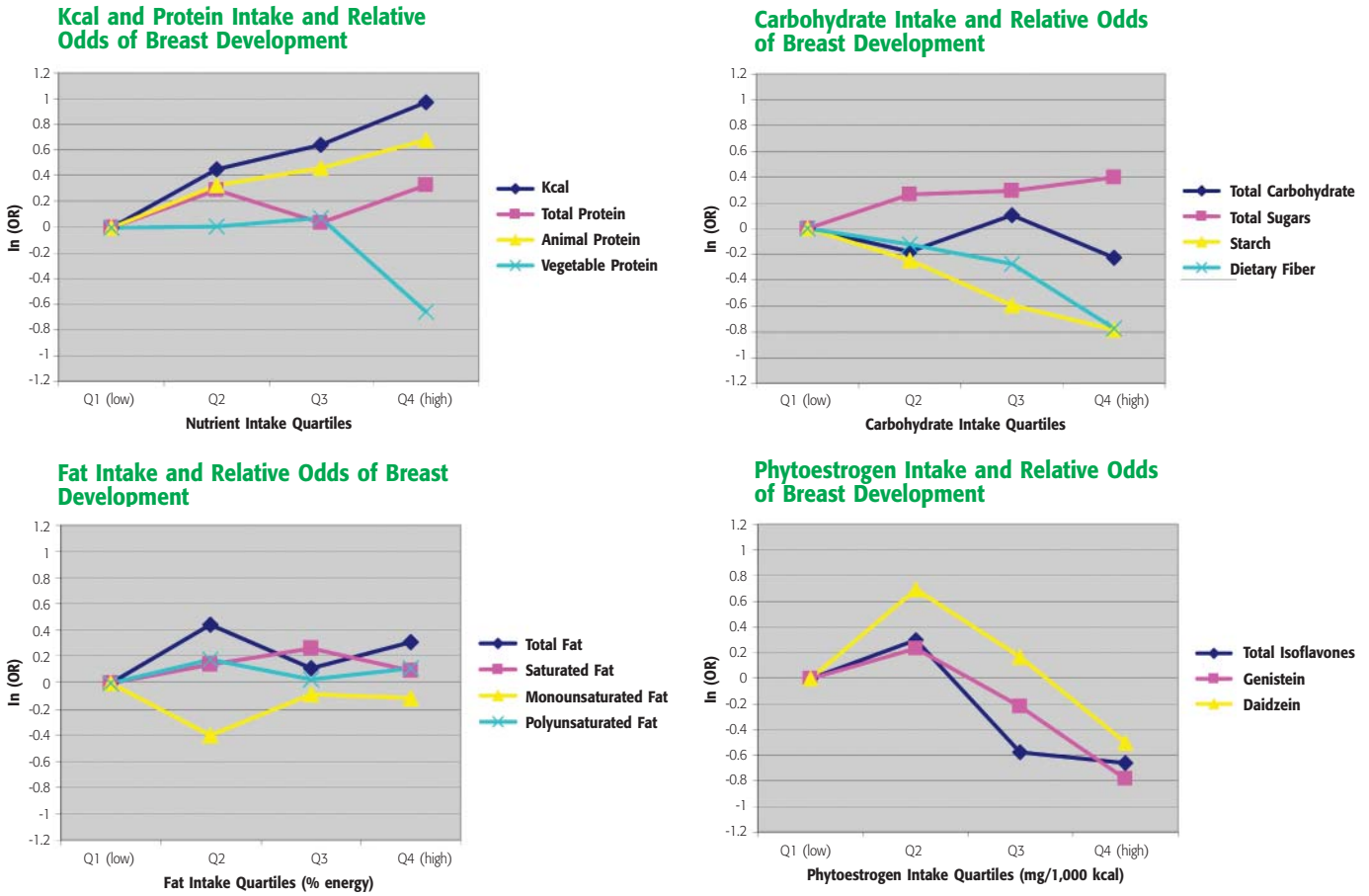


Figure 17. **Relative odds of breast development at Year 2 examination according to baseline nutrient intake, Bay Area BCERC Epidemiologic Study (CYGNET Study).**

4. **A formal evaluation of the community-based participatory research approach followed by the Bay Area BCERC COTC revealed that the interaction with community advocates has had multiple positive effects on the research process.**

   - Results of the evaluation, carried out using surveys as well as individual and group interviews with Bay Area BCERC scientists, COTC, and community members, confirmed that translation and dissemination of science to the public increased advocates' and lay community members' understanding of the scientific process and its importance.
   - The community-based participatory approach created better relationships among diverse stakeholders, augmented knowledge generation, improved the sensitivity and relevance of the research, and increased community support for the research.[36]

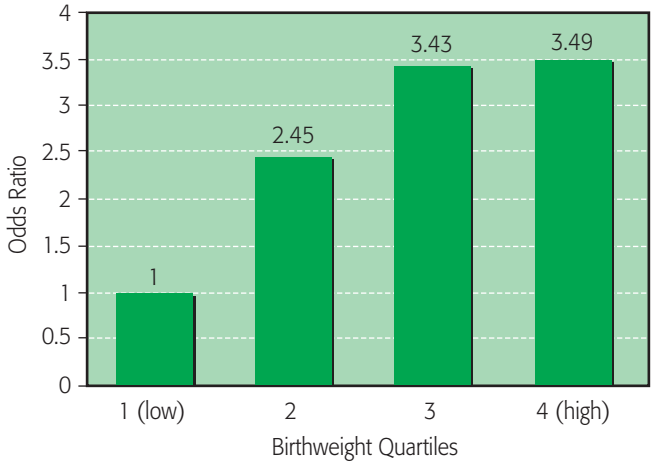


Figure 18. **Association of birthweight with body mass index above the age-specific 85th percentile estimated during the baseline examination, girls age 6–8 years, Bay Area BCERC Epidemiologic Study (the CYGNET Study). Baseline examination was conducted in 2005–2006 with height measured using a fixed stadiometer and weight measured using a calibrated scale; birthweight was obtained from Kaiser Permanente birth records, 1997–2000.**

- The COTC also has increased the knowledge and understanding of the methods for successfully engaging community members and community partners in the ongoing design, implementation, and dissemination of the Bay Area BCERC's basic science and epidemiology research studies.
- Through this community participatory process, COTC members, in partnership with Center researchers and community advocates, have:
  - Sponsored four community meetings: three focus groups in the African-American community and two with adolescent girls and mothers. The proceedings informed research protocols and dissemination/translation strategies.
  - Conducted annual town hall meetings designed to bring together researchers, advocates, public health professionals, and public policymakers. More than 150 individuals attend each year. Program reach is extended via availability of Web site videos, CDs, online and printed monographs, and Public Access TV. Evaluation results influence future programs and educational/outreach materials.
  - Promoted retention and communication of study findings to CYGNET families via biannual newsletters and "tea talks" that bring together investigators, study staff, and participants and their families to discuss the CYGNET study and related health and developmental topics (Figure 19). The themes reflect topics of interest identified by parents through evaluations.
  - Developed and distributed community educational and outreach tools and materials, including a 45-minute educational DVD *Of Mice and Women: Modeling Breast Cancer and the Environment*, which describes why and how different types of mouse models are used to study various aspects of breast cancer biology and is accompanied by a glossary of scientific terms and other educational aids for the community. The educational tool kit was distributed to 98 institutions and individuals, accessed via Web site 279 times, and shown six times on Public Access TV. Other educational and outreach materials developed and accessed via the Web site include: DVD and written proceedings from a community forum on biomonitoring, environmental fact sheets, *The Mind-Body Connection–Onset of Puberty in Girls*, and *Tanner Staging*. More than 1,500 printed copies of the above educational and outreach materials were distributed via information tables during the past 4 years.

Figure 19. CYGNET study girls attending a "tea talk" are learning about the ecosystem at the Bay-Delta Model Museum in Sausalito. "Tea talks" provide educational activities for CYGNET study girls while parents attend a presentation related to the BCERC study.

University of Cincinnati

Biological and Environmental Modifiers of Pubertal Maturation in Girls and Mammary Gland Development and Susceptibility to Breast Cancer in Rodents

The Cincinnati BCERC's working research model is based on the premise that prepubertal obesity—driven by environmental, psychosocial, and genetic factors—leads to a pathway of pubertal development in which estrogen and insulin-like growth factor stimulate breast development as the initial manifestation of puberty; and that girls with a family history of breast cancer are more likely to develop through this pathway. Such a pathway leads to earlier menarche, as well as other physiologic states, such as central adiposity, that increase the susceptibility of the mammary gland to carcinogenic insults (Figure 20). Dietary fatty acids, through modification of estrogen synthesis, metabolism, and signaling, might modify rates of mammary gland maturation and impact the timeframe of mammary gland susceptibility to initiation. The Cincinnati BCERC's Biology Project and Epidemiology Project are designed to examine and test this model.

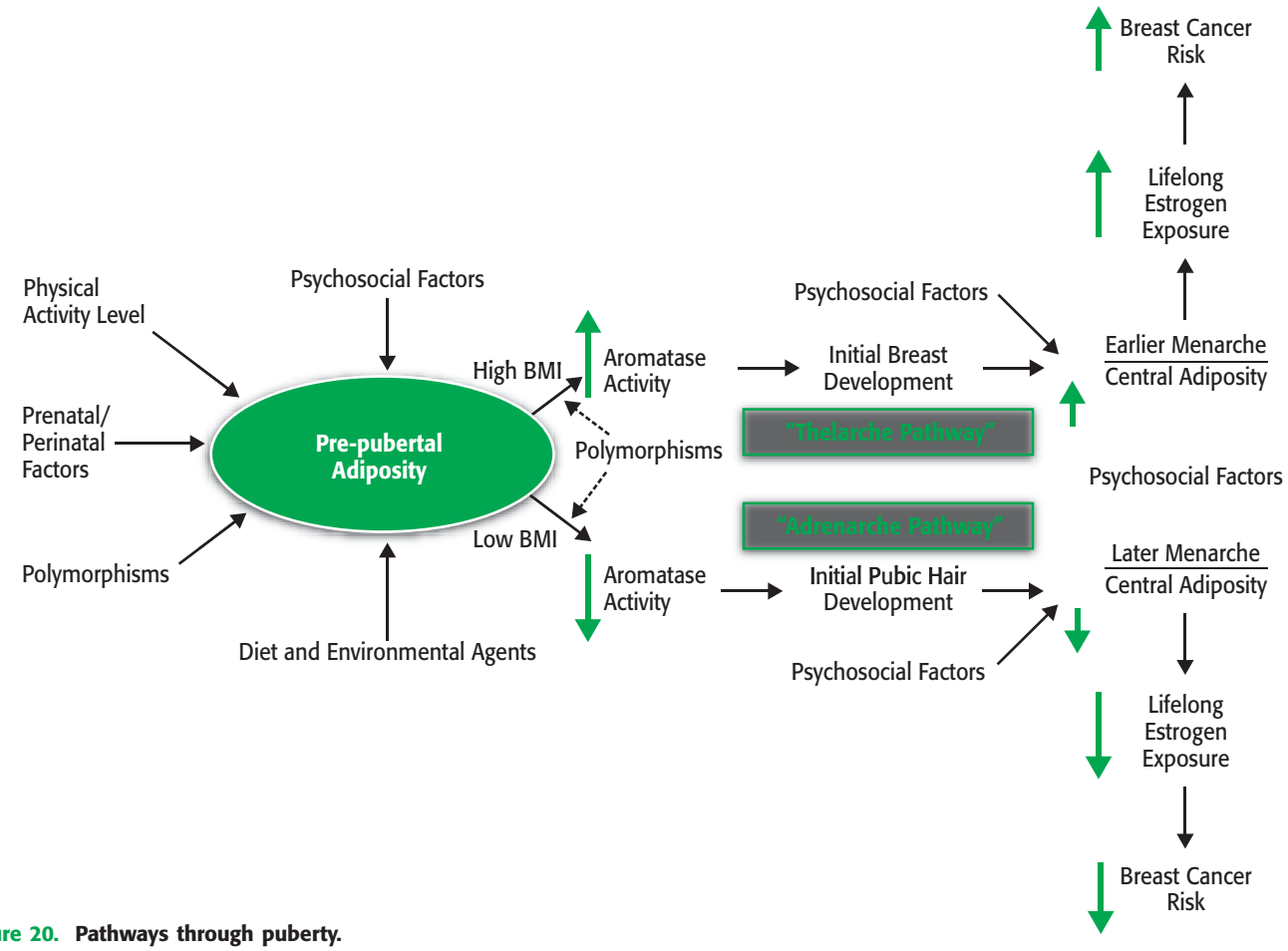


Figure 20. **Pathways through puberty.**

The primary goal of the Biology Project is to characterize the effect of specific dietary fatty acids—such as palmitic, oleic, linoleic, omega-3, and omega-6 fatty acids—as modifiers of age-specific visceral adiposity, pubertal maturation, hormonal balance, mammary gland development, gene expression, and susceptibility to carcinogenesis using a rat model that closely resembles breast cancer progression in humans[37] (**Figure 21**). The first set of rodent studies sought to evaluate the most popular fats found in Western diets (olive oil, safflower oil, fish oil, soy oil, and butter) as well as parallel the human pattern of maternal breast feeding and feeding through childhood and adolescence. These studies followed female rat pups from inhouse breeding consuming one of these diets, which also had been consumed by their dams (mothers) before and throughout gestation and lactation. Cincinnati BCERC investigators followed these female rat offspring and evaluated the mentioned parameters at key time points in the animal's lifetime (weaning/prepubertal, adolescence, and adulthood) that correspond to important milestones in human development and maturation.[38]

The Cincinnati Epidemiology Project defined a group of specific aims: (1) identify pathways into puberty, (2) compare and contrast girls with and without a family history of breast cancer, (3) examine environmental factors that interact with adiposity and the proposed pathway of pubertal development, (4) evaluate the impact of the social environment on the pathway and timing of pubertal onset, and (5) identify genetic variants associated with the pubertal development pathway.

To examine these aims, the researchers recruited 379 girls between the ages of 6 and 7 years to be seen at their schools or in the General Clinical Research Center (GCRC) of Cincinnati Children's Hospital as part of the Growing Up Female study. Girls were recruited between August 2004 and July 2007 and were seen every 6 months. As of November 3, 2008, 2,078 clinical

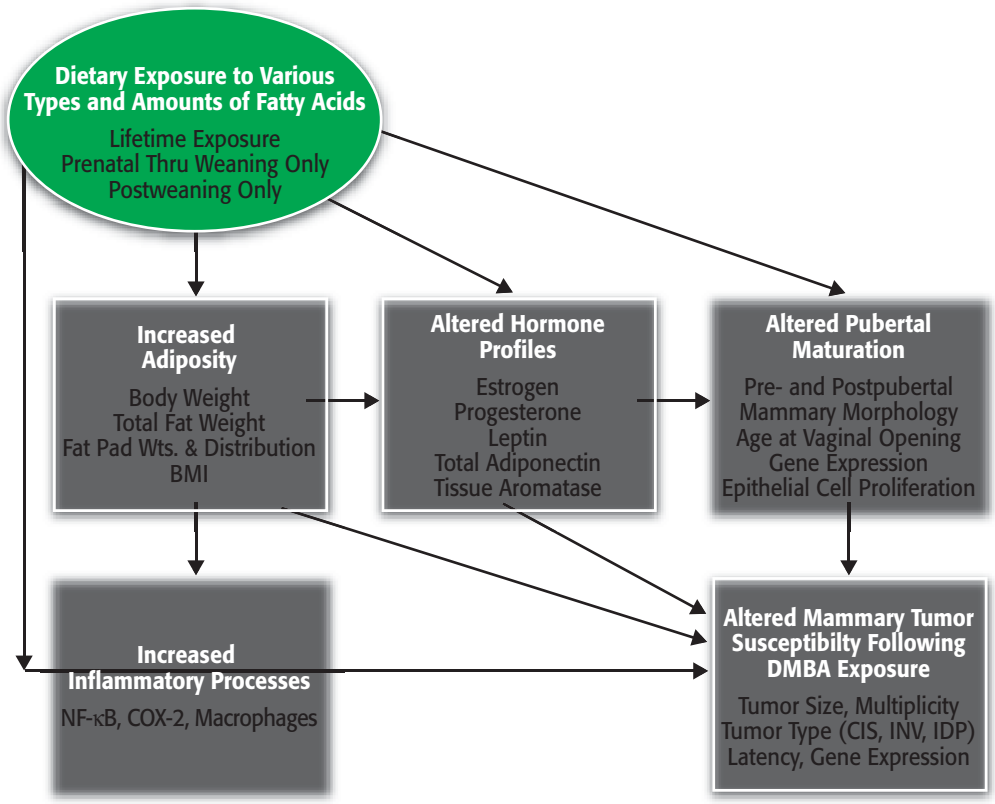


Figure 21. Conceptual model of the Cincinnati BCERC Biology Project.

assessments had been generated from the original group of 379 participants.

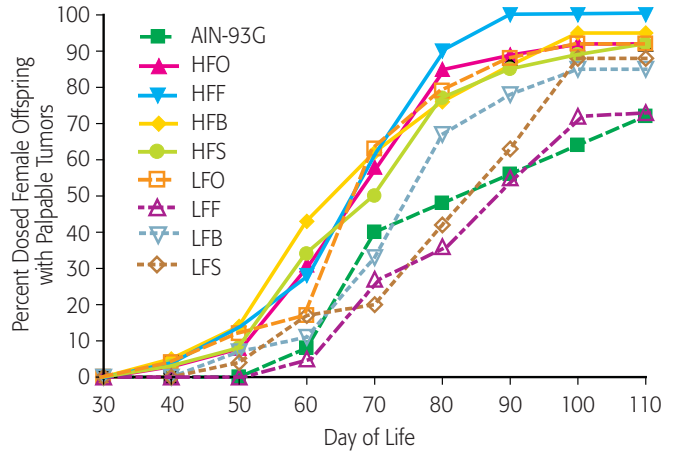
During the clinical visits, girls receive several evaluations: blood pressure, height, weight, skinfold measurements (triceps, suprailiac, subscapular), waist and hip circumference, bioelectrical impedance, pubertal maturation, and foot length. Blood is drawn at every visit and once a year, urine and blood are obtained for selected biomarkers. Parents receive a phone call every 3 months for a 24-hour diet recall. Adherence to the protocol has been very high, with greater than 90 percent retention. Blood was obtained at more than 89 percent of visits, urine at more than 95 percent of scheduled visits, and study questionnaires at more than 97 percent.

The Cincinnati BCERC COTC developed a volunteer program for community advocates to serve as Study Helpers with the Growing Up Female study. Of note, Study Helpers are present at each visit of four or more participants. The vast majority of the Study Helpers are breast cancer advocates as well as COTC members. Study Helpers attend each study visit, assist with registration, chat with the girls to allay their anxieties about blood collection, serve refreshments after fasting blood draws, and engage the girls in pastime activities and conversation. Thirty-nine individuals have been trained as Study Helpers. They travel to study sites on weekday and Saturday mornings without remuneration.

Major findings from the three components of the Cincinnati BCERC at this time are:

1. The percentage of total caloric intake from dietary fat and types of fatty acids consumed influence mammary gland development, pubertal maturation, and mammary tumor formation in female rat offspring.

- Consumption of high-fat diets (40% of kcal from olive oil, safflower oil, fish oil, or butter) from before gestation through adulthood leads to a significant ($P \leq 0.02$) increase in mammary tumor number and tumor size, primarily through increasing carcinoma-in-situ lesions,[37] when female rat pups are treated with the mammary carcinogen DMBA at weaning (Figure 22). When DMBA is administered to female pups during puberty, there is a significantly ($P \leq 0.03$) greater incidence of mammary adenocarcinomas in rats consuming the 40 percent safflower, 10 percent butter, and 40 percent butter diets compared to the reference AIN-93G diet. The association between individual fatty acids and mammary tumorigenesis is the highest for palmitic acid when the pups are administered the carcinogen at weaning or during puberty.
- When diet is the sole modifying agent in female rat offspring at the time of adolescence, a sig-

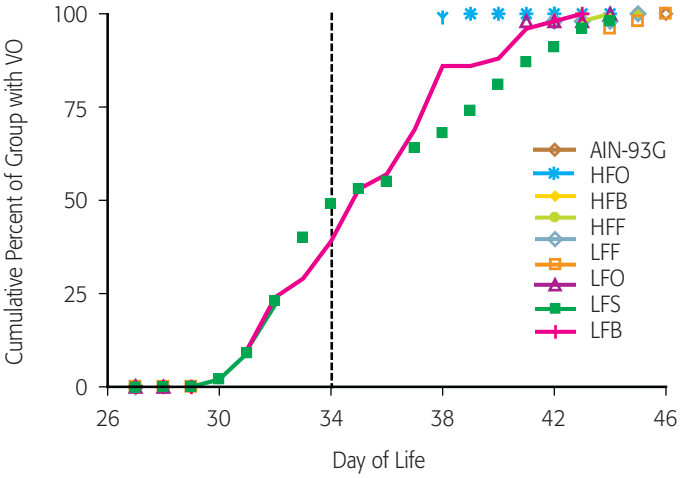


KEY: AIN-93G, reference diet; HFO, high-fat olive oil; HFF, high-fat fish oil; HFB, high-fat butter; HFS, high-fat safflower oil; LFO, low-fat olive oil; LFF, low-fat fish oil; LFB, low-fat butter; LFS, low-fat safflower oil.

Figure 22. Mammary tumor palpation data from female rat offspring consuming the same diets as their dams from before gestation through their lifetimes dosed with DMBA (34.1 mg/kg) at weaning.

nificant negative correlation exists between oleic, linoleic, and omega-3 fatty acid intake and the day of vaginal opening (pubertal maturation; Figure 23) and a positive correlation with percent of epithelium present in the developing mammary gland (mammary gland maturation). Interestingly, oleic acid was the only fatty acid significantly correlated with visceral adiposity (central adiposity) at this age.

- Consumption of all six fatty acid diets increases the proliferation of mammary epithelial cells (MEC) to differing degrees, according to the microarray data. A highly significant enriched "mitotic cell cycle" gene ontology was observed compared to the AIN control diet. The increase in proliferation for control, high-fat, and low-fat diets was confirmed further by immunohistochemical Proliferative Cell Nuclear Antigen (PCNA) antibody staining of proliferating MECs, which revealed a significant increase of the proliferation rate relative to the AIN control diet.[39,40] Relaxed criteria of a 1.5-fold increase of differential gene expression and P values ≤ 0.01 additionally identify an immune and a sterol biosynthesis cluster of genes at these ages.



KEY: AIN-93G, reference diet; HFO, high-fat olive oil; HFB, high-fat butter; HFF, high-fat fish oil; LFF, low-fat fish oil; LFO, low-fat olive oil; LFS, low-fat safflower oil; LFB, low-fat butter.

Figure 23. Cumulative frequency of young pups exhibiting vaginal opening. Animals on the HFO diet reach puberty earlier as measured by vaginal opening (VO) than those on the reference diet. VO was found to be negatively correlated (r = -.51; $P < 0.001$) with oleic acid content in the diet and to have a weaker association (r = -16; $P = 0.03$) with linoleic acid.

2. **BMI and pathways into puberty were associated significantly in the girls participating in the epidemiological study. Those with gonadarche or with undefined pathway had higher BMI values than those with pubarche ($P < 0.0001$). Notably, an earlier study[41] showed that girls in whom breast development occurred first had younger ages of menarche and greater bone mineral density, both of which have been associated with increased risk of breast cancer in epidemiologic studies.**

- At age 8 years, 29 percent of white, 48 percent of African-American, and 33 percent of Hispanic girls recruited into the study were Tanner Scale breast stage 2 or greater; these proportions are significantly greater than those published in 1997 by Herman-Giddens and colleagues.[34] Consistent with other studies,[42–44] advanced maturational status was associated with increased BMI and race (African American more advanced than white). A "pathway" (breast or pubic hair development, without any maturation of the other characteristic) could be defined in 85 percent of participants.
- Among those with a defined "pathway," 71 percent had initial breast development ("gonadarche"), and 29 percent had initial pubic hair development ("pubarche") (Figure 24).
- The ratio of estradiol to estrone was significantly greater at 6 months prior to onset of puberty, and

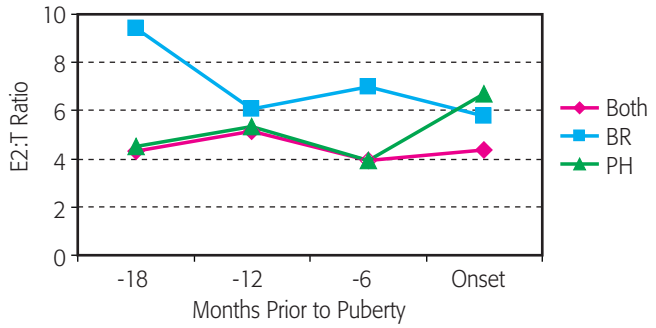


Figure 24. Estradiol:testosterone ratio by pathway. Pathway is defined as breast development (BR, n = 70) or pubic hair development (PH, n = 29), without maturation of the other characteristic. "Both" (n = 18) indicates that the participant had simultaneous maturation of both characteristics when exams were conducted every 6 months. Hormone analyses were based on 117 participants; about 30% of the eventual data. Longitudinal analyses: pathway *P* = 0.061; at 6 months prior to puberty, BR vs. both, *P* = 0.047; BR vs. PH, *P* = 0.078.

the ratio of estradiol to testosterone was greater in longitudinal analyses across the 18 months prior to onset of puberty, with the ratio greater in those with the gonadarche pathway.

- The higher hormone ratios (estradiol to estrone and estradiol to testosterone) are consistent with greater activity of aromatase and 17-dehydrogenase, both of which are increased in those with greater adipose tissue.

3. The Cincinnati BCERC COTC has implemented programs to promote interaction with and education of study participants, family members, and members of the community.

- Study Helpers assisted at more than 400 study sessions and in the office as well, saving grant dollars. The COTC is scheduled to evaluate the Study Helper program in winter 2009. A poster about the Study Helper program, entitled *Study Helpers: Breast Cancer Advocates Assist With the Growing Up Female Study of Young Girls*, was presented at the 4th Annual Early Environmental Exposures Conference in Cincinnati in November 2007.
- The Cincinnati BCERC COTC has conducted annual public education forums since 2005 called *Looking Upstream for Environmental Links to Breast Cancer*. In addition to hosting a nationally recognized keynote speaker, the program includes updates from Cincinnati BCERC researchers and presentations by area experts on topics such as water quality, endocrine disruptors, breast cancer genetics, and biomarkers. The program is evaluated each year and Nursing Continuing Education Units (CEUs) are available. More than 100 people attend each program.
- The Cincinnati BCERC COTC has worked with researchers and staff to promote the communication of study findings to the Growing Up Female study families. Meetings with the families were conducted in 2007 and 2008 and included general study updates, detailed review of the clinical examination conducted during the study visits, and time for questions and answers. Unexpected study findings have been presented, and families received their child's results, along with reference data.
- In fall 2008, the COTC surveyed the Growing Up Female study families about the information they would like to receive about or related to the study and how they would like to receive the information. Fifty-four percent of the 324 families completed the anonymous questionnaire. In response, a biannual newsletter is being planned to present health topics and study updates, and procedures for reporting individual study results to families are being assessed. A poster about the survey entitled *Family Viewed: The Survey Says* won First Place in the COTC category at the 5th Early Environmental Exposures Conference in Birmingham, AL, in November 2008.
- The Cincinnati BCERC COTC developed a coloring book that explains the different activities involved in a Growing Up Female study sessions, e.g., height and weight measurement, blood collection (**Figure 25**). The coloring book was distributed to the first young girls enrolled in the Growing Up Female study; it was later used in the recruitment of new participants. The coloring book was adapted for use in the CYGNET study at KPNC. A poster about the coloring book, entitled *Working Together in Cincinnati: Explaining the Growing Up Female Study to Young Girls*, was presented at the 2nd Annual Early Environmental Exposures Conference in E. Lansing, MI in November 2005.
- In 2006 the Breast Cancer Alliance (BCA) of Greater Cincinnati received the National Breast

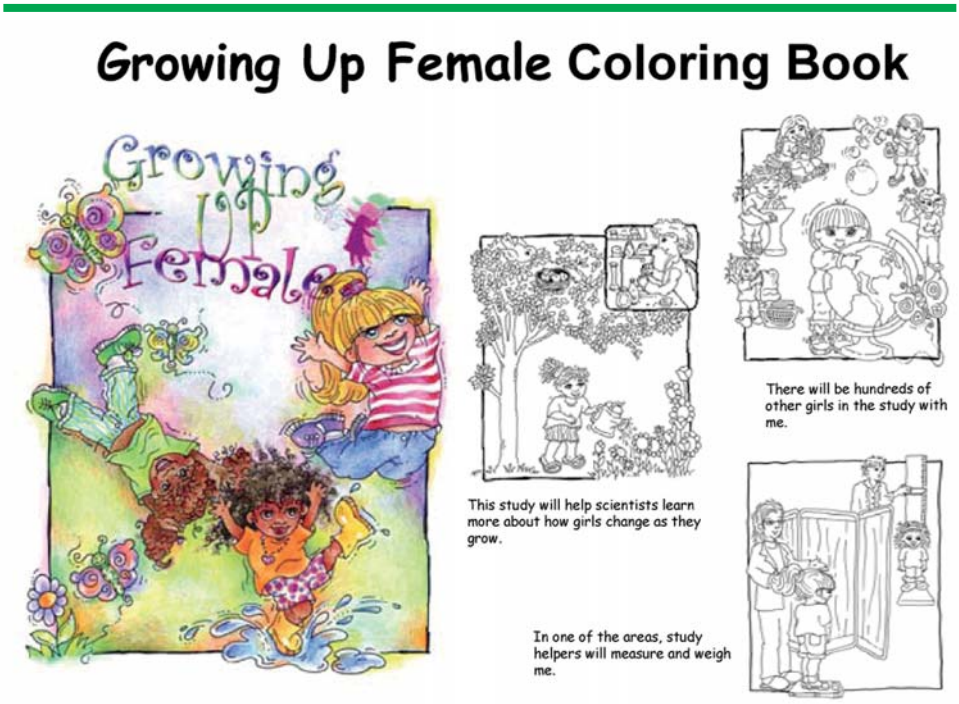


Figure 25. Breast cancer advocates active in the Cincinnati BCERC COTC created a coloring book to explain the Growing Up Female study to the initial study participants and future recruits.

Cancer Coalition Fund's Best Practices in Breast Cancer Advocacy Award in recognition of their Advocacy in Research Program. With this award, the BCA has worked with the Cincinnati BCERC COTC and Epidemiology and Biology Projects in the development and conduct of ART: Advocate Research Training, a series of multimedia workshops for advocates to learn about scientific concepts and research methods. The workshops have included tours of wet and dry labs, lectures, demonstrations, hands-on exercises, and discussions. Posters about ART have been presented at the 4th Annual Early Environmental Exposures Conference in Cincinnati in November 2007, at the National Breast Cancer Coalition's Annual Advocacy Research Training Conference in Washington, DC, in April 2007, and at the DOD Breast Cancer Research Program Era of Hope Meeting in Baltimore, MD, in June 2008.

- The Cincinnati BCERC COTC has maintained a Web site (http://eh.uc.edu/growingupfemale) since working with the Greater Cincinnati breast cancer advocacy community on the development of the funding application. The Web site includes BCERC background, research project hypotheses, progress reports, copies of publications by Center researchers with lay abstracts, photos, links to resources, and more than 30 videos for online viewing. Nearly 2,500 visitors visit the Web site each year. In 2008, visitors to the site viewed three times as many of the Web pages as visitors in the previous year. In 2009, Growing Up Female study families are being introduced to the Web site; new family-friendly pages are being created so they can access study information and related health topics. ●

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SECTION

IV

Future Scientific Opportunities

The BCERC data and biospecimens will serve as a rich resource for scientists studying environmental exposures, lifestyle factors, and intermediate phenotypes related to pubertal development and future breast cancer risk.

A transdisciplinary approach to science requires that investigators from the multiple disciplines involved in a scientific enterprise join together in tackling a problem under a common conceptual framework.[1] As described above, BCERC investigators and advocates from four research consortia across the United States have developed a common conceptual framework linking events during puberty and other key periods during the life span with breast cancer development. Within this framework, the BCERCs have conducted extensive animal and tissue culture experiments, designed and implemented a three-site longitudinal epidemiologic study of puberty in girls, and engaged in extensive two-way interaction with the community. Each of these endeavors adds value to the others. Putative environmental factors found to affect either normal breast development or carcinogenesis in animals can be explored in studies with the girls in the BCERC cohort. Discoveries of factors associated with pubertal endpoints in the girls can be tested in animal models and human tissue culture systems developed in the laboratory. Topics of public concern raised by community advocates can be incorporated into the laboratory and epidemiologic research programs, and findings from the BCERC studies can be disseminated to study participants and broader audiences through community outreach.

The conceptual framework described above has characterized the BCERC project to date and can continue to do so as new scientific opportunities present themselves. The investigation of emerging environmental agents and etiologic factors during pubertal development and the continued study of known environmental toxicants across the continuum ranging from *in utero* exposures to the postmenopausal years is an important next step. The existing BCERC transdisciplinary framework serves as a novel model for future studies of other health topics, including those of greatest concern to community members.

Capitalizing on Investment

Over the course of this cooperative agreement, the BCERC project has become known for changing the paradigm from studying environmental influences on breast cancer in adult women to studying the effects of environmental exposures much earlier in life. New information on normal mammary gland development provides the opportunity to develop new intermediate markers of changes and to understand how they might be influenced by exposure to a variety of agents. In 2001, when this program was conceived, little information existed on the pubertal window of mammary gland development and factors that might change the timing of structural, functional, and hormonal changes during maturation in young girls. It is important to continue to learn more about this unique window of breast development and determine how exposures during this time period might change the risk of breast cancer in later life.

The BCERCs are uniquely positioned to identify determinants of pubertal and developmental milestones associated with long-term risk of breast cancer. Currently only 30 percent of study participants have reached onset of menses. Although the BCERCs anticipate having adequate followup under the current funding period to examine associations with onset of breast or pubic hair development, continued followup will be necessary to enable investigation of other developmental milestones and factors linked to the underlying hypotheses, including

age at menarche, maximum growth velocity, and bone mineralization. Additionally, the BCERCs will continue to add value to the scientific community and the general public in the following ways:

- Utilizing the biospecimens and BCERC data, this project will serve as a rich data resource for future generations of scientists studying environmental exposures, lifestyle factors, and novel intermediate phenotypes that are related to pubertal development and future breast cancer risk (see Appendix D for examples of data already available).

- The BCERC project has strengthened ties with national and regional advocacy organizations such as the Avon Foundation. These are valuable relationships that should continue to be cultivated (see Appendices B and C).

- The COTCs are developing key messages for the dissemination of BCERC findings tailored to a variety of target audiences. These efforts are critical both because of the inherent challenges associated with translating basic science findings to lay audiences and because some of the BCERCs' environmental findings may have broad impact and affect public policy.

The Next Steps for the BCERCs

Operating under a shared conceptual framework, advances in human and animal studies focusing on pubertal development and future breast cancer risk can be enhanced and expedited through the transdisciplinary nature of the BCERC project. Examples of future opportunities to improve understanding of environmental influences on breast cancer risk include the following:

- New environmental chemicals and biomarkers of both community and scientific interest can be studied to determine how mammary glands are perturbed when challenged with additional environmental agents and exposures.

  - Measuring biomarkers in girls at multiple points in time could provide information about the persistence of chemicals of interest.

  - Collection of serial urine samples potentially could provide an estimate of intraindividual variation in sex hormone levels and identification of times of peak hormone levels and ovulatory cycles in girls.

The BCERCs have conducted extensive animal and tissue culture experiments, designed and implemented a longitudinal epidemiologic study of puberty in girls, and engaged in extensive interaction with the community. Each of these endeavors adds value to the others.

- Genomic and epigenetic changes, altered signaling pathways, and changes in cellular composition of the mammary gland identified from *in vivo* and *in vitro* animal studies will be indicative of changes in humans that can then be used as markers of exposure, susceptibility, or refractoriness to early puberty and breast cancer.

  - The BCERC Epidemiology Project has collected biospecimens (see Appendix D, Tables D4, D5, and D6) that can be used to test this hypothesis in humans.

  - Proteomic and epigenomic signatures can be explored using human blood specimens.

- The combined effects of multiple environmental exposures on pubertal development can be explored in animals and humans.

  - Animal studies can look at how specific compartments of the mammary gland (epithelium, stroma) and cell types (stem/progenitor cells) are affected by different environmental exposures individually and in environmentally relevant combinations based on the exposure assessment results from the epidemiologic study.

- Interactions between the Epidemiology and Biology Project investigators have resulted in increased interest in central adiposity and body fat distribution and the interaction between obesity and exposure to endocrine disrupting chemicals in the epidemiologic studies.

- Animal models can be used to conduct studies of the interaction between these factors.

- The feasibility of expanding biospecimen collections would enable investigation of markers of metabolic syndrome, such as fasting insulin and blood lipids, inflammatory cytokines, and related biomarkers.

✦ Bioinformatics tools that identify genetic patterns leading to earlier onset of puberty and breast cancer development can be explored.

Recently, the Breast Cancer and the Environment Research Act of 2008 was signed into law. This law directs the Secretary of Health and Human Services to create a committee of individuals with diverse talents and expertise who are knowledgeable about the science and impact of the environment on the risks of breast cancer. The bill mandates a multidisciplinary dialogue between academic scientists and clinicians, government scientists, and breast cancer advocates to flesh out a research agenda for the future. Contributions from the BCERCs will be part of the discussions that will ensue.

The BCERC program has been an excellent resource and has the potential to continue contributing significantly to the field of cancer research. Through continued followup of the epidemiology cohort, the findings in humans will inform the direction of relevant exposure studies in animal models; at the same time, the findings from animal studies will provide insight into the potential mechanisms by which the environmental exposures and genetic factors under investigation might affect humans. The involvement of multiple breast cancer advocacy organizations at multiple levels will continue to play an integral role in prioritizing exposures for data collection and analysis, in supporting the followup and retention of study participants, in translating information about environmental exposures to different audiences, and in determining methods for dissemination of results to both study participants and their families and to the broader community, but most importantly to continue to keep all involved focused on the impact that the burden of breast cancer has on women's lives. Working together and moving forward, the scientific advances produced from this program and similar work in the future will help prevent breast cancer in future generations. ●

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Appendices

Appendix A. Areas of Expertise of BCERC Staff and Collaborators

Fox Chase Cancer Center BCERC

Biology Project (P1)	*Expertise*
Coral Lamartiniere, Ph.D.	Toxicology: animal models, environmental exposures, endocrine disruptors, mammary carcinogenesis, dietary chemoprevention; proteomics
Irma H. Russo, M.D.	Endocrinology: hormones, normal mammary gland development and mammary tumor biology; pathology
Jose Russo, M.D.	Human and experimental pathology; molecular and cell biology; endocrinology; oncology
Robert Beck, M.D., Ph.D.	Bioinformatics
Ricardo Lopez, Ph.D.	Molecular biology and genetics
Julia Pereira, Ph.D.	Molecular biology and genetics
Eric Ross, Ph.D.	Biostatistics
Suraj Peri, Ph.D.	Bioinformatics
Mark Carpenter, Ph.D.	Biostatistics
James Mobley, Ph.D.	Mass spectrometry, proteomics
Isam Eltoum, M.D.	Pathology
Angela Betancourt, D.V.M., Ph.D.	Proteomics, animal models, toxicology

Epidemiology Project (P2)	*Expertise*
Mary Wolff, Ph.D.	Environmental epidemiology
Susan Teitelbaum, Ph.D.	Epidemiology
Barbara Brenner, Dr.P.H.	Community relations and research
Julie Britton, Ph.D.	Epidemiology
James Wetmur, Ph.D.	Molecular biology and genetics
Maida Galvez, M.D.	Pediatrics and community research
Nita Vangepurum, M.D.	Pediatrics (obesity and asthma)
Lisa Boguski, M.D.	Pediatrics
Warria Esmond, M.D.	Pediatrics
Lisa Handwerker, M.D.	Pediatrics
Adam Aponte, M.D.	Pediatrics
Joel Forman, M.D.	Pediatrics
Suzanne Miller, Ph.D.	Behavioral psychology
Carolyn Fang, Ph.D.	Behavioral psychology
Antonia Calafat, Ph.D.	Toxicology
Christine Pfeiffer, Ph.D.	Toxicology
Dana Barr, Ph.D.	Toxicology
Laura Liao, M.S.	Biostatistics
Jia Chen, Sc.D.	Molecular biology and genetics

COTC	*Expertise*
Luz Claudio, Ph.D.	Community research and outreach
Sarah Williams, M.P.H.	Community research
Donna Duncan	Breast cancer advocacy
Theresa McGrath	Breast cancer advocacy

Advocates	*Affiliation*
Ann Fonfa	President and Founder, Apple Seed Foundation
Maryellen Delapine	Program Director, Advocacy, Linda Creed Breast Cancer Foundation; Field Coordinator, National Breast Cancer Coalition

Michigan State University BCERC

Biology Project (P1)	*Expertise*
Sandra Z. Haslam, Ph.D.	Endocrinology: hormones, normal mammary gland development, and mammary tumor biology
Susan E. Conrad, Ph.D.	Molecular biology: cell cycle/estrogen receptor/breast cancer
Richard Miksicek, Ph.D.	Molecular biology: steroid hormone receptors/bioinformatics
Karl Olson, Ph.D.	Endocrinology: metabolic regulation/diet/diabetes
Richard Schwartz, Ph.D.	Immunology: inflammation
Chengfeng Yang, Ph.D.	Toxicology: environmental exposure, mammary carcinogenesis/cancer cell signaling

COTC	*Expertise*
Charles Atkin, Ph.D.	Mass communication; health campaigns: strategies and implementation
Pam Whitten, Ph.D.	Telecommunication: telemedicine, webpage design and evaluation
Kami Silk, Ph.D.	Health communication; message design
Sandi Smith, Ph.D.	Persuasion and communication theory
Janet R. Osuch, M.D.	Surgical oncology, epidemiology, breast cancer risk assessment

Advocates	*Affiliation*
Latecia Matthews, B.S.	Faith Access to Community Economic Development
Christine Pearson, B.A.	Lansing Affiliate, Susan G. Komen Breast Cancer Foundation
Lana Pollack. B.A.	Michigan Environmental Council
Victoria Rakowski, R.N.	American Cancer Society, Great Lakes Division
Rebecca Cwiek	Michigan Breast Cancer Coalition

Bay Area BCERC

Biology Project (P1)	*Expertise*
Zena Werb., Ph.D.	Developmental biology
Joe Gray, Ph.D.	Genetics
Mary Helen Barcellos-Hoff, Ph.D.	Cell biology
Paul Yaswen, Ph.D.	Cell biology

Epidemiology Project (P2)	*Expertise*
Lawrence Kushi, Sc.D.	Epidemiology – nutrition/diet, cancer
Gayle Windham, Ph.D.	Epidemiology – environmental, reproductive
Robert Hiatt, M.D., Ph.D.	Epidemiology – cancer
Louise Greenspan, M.D.	Pediatric endocrinology
Julianna Deardorff, Ph.D.	Child psychology
Barbara Sternfeld, Ph.D.	Epidemiology – physical activity
Christine Erdmann, Ph.D.	Epidemiology – environmental
Christine Ambrosone, Ph.D.	Epidemiology – molecular, genetic
Charles Quesenberry, Ph.D.	Biostatistics
Bill Lasley, Ph.D.	Sex hormone metabolism

COTC	*Expertise*
Janice Barlow, M.S.N., CPNP	Advocacy: national/state/local; community and public health; community-based participatory research; SPORE grant reviews; organizational management; community outreach and education
Karen Pierce, J.D.	Community advocate, law, environmental justice
Kathy Koblick, M.P.H.	Public health, health educator
Fern Orenstein, M.Ed.	Breast cancer survivor; public health specialist/health education/training; advocacy: state/local
Neena Murgai, M.P.H.	Epidemiologist, public health, community assessment, planning, education and evaluation

Susan Samson	Breast cancer survivor, advocacy, DOD reviews, health care policy
Brynn Taylor, M.P.H.	Environmental health, legislative advocacy, program management
Kaya Balke	Program management, communications

BCERC Coordinating Center	*Expertise*
Susan Stewart, Ph.D.	Biostatistics
Kaya Balke	Program management, communications

P2 – Ancillary Studies and Collaborators	*Expertise*
Irene Yen, Ph.D.	Social epidemiology/built environment
Dejana Braithwaite, Ph.D.	Social and breast cancer epidemiology
David Rehkopf, Ph.D.	Social epidemiology

University of Cincinnati BCERC

Biology Project (P1)	*Expertise*
Marshall Anderson, Ph.D.	Cancer biology
Robert Bornschein, Ph.D.	Environmental toxicology
Debbie Clegg, Ph.D	Dietary regulation of hormones and obesity
Shuk-mei Ho, Ph.D.	Epigenetics
Ronald Jandacek, Ph.D.	Dietary fats and xenobiotic absorption
Mario Medvedovic, Ph.D.	Bioinformatics and microarray data analyses
Kenneth Setchell, Ph.D.	Phytoestrogen biology
Paul Succop, Ph.D.	Biostatistics and longitudinal data analyses
Patrick Tso, Ph.D.	Animal models of obesity

Epidemiology Project (P2)	*Expertise*
Frank Biro, M.D.	Adolescent medicine
Susan Pinney, Ph.D.	Cancer epidemiology and exposure biomarkers
Robert Bornschein, Ph.D.	Environmental epidemiology
Kim Dietrich, Ph.D.	Developmental psychology
Lorah Dorn, Ph.D.	Female puberty
Paul Succop, Ph.D.	Biostatistics and longitudinal data analyses

COTC	*Expertise*
Wendy Anderson	Breast cancer survivor; human resources; writing/science translation
Kathleen Ball	Advocacy: national; breast cancer survivor; clinical research; DOD reviews; health care policy; health care services; oncology nursing; organizational management
Robert Bornschein	Environmental epidemiology; research administration
M. Kathryn Brown	Environmental epidemiology; writing/science translation
Paulette Cunningham	Breast cancer survivor; laboratory sciences
Jim Flessa	Writing/science translation
Gail Greenburg	Advocacy: state/local; health care services; patient advocacy
Ann Hernick	Advocacy: national; advocacy: state/local; breast cancer survivor; business management; organizational management; writing/science translation
Andrea Ice	Advocacy: state/local; breast cancer survivor; business management; health care policy; patient advocacy
Mary Justice	Advocacy: state/local; breast cancer survivor; clinical research; DOD grant reviews
Peggy Monroe	Breast cancer survivor; business management
Carole Price	Advocacy: state/local; clinical research; DOD grant reviews; health care policy, health care services; writing/science translation
Veronica Ratliff	Program management
Jennifer Ruschman	Clinical research; genetics/genetic counseling; health care services; laboratory sciences; writing/science translation

Appendix B. BCERC Scientific Publications

2008

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Appendix C. COTC Publications and Events

Mount Sinai School of Medicine

Educational Activities

2005

New York City Parks Foundation – Offered workshop entitled "A Day in the Park," conducted at the Dana Discovery Center. Fifteen project participants attended the workshop.

Green Girls Science After School Program – Dr. Claudio and Ms. Mennuti conducted a science workshop in Dr. Claudio's laboratory. The hands-on workshop was entitled "You and Your Genes" and included a laboratory session in which minority girls extracted DNA and discussed genetics and heredity.

TRUCE – Developed partnership with Laura Vural, director of TRUCE, to conduct a multimedia project for girls enrolled in the Epidemiology Project. Participating girls created their own multimedia presentations about the environment in East Harlem. The first workshop at TRUCE took place on April 25, 2005.

Lasker Skating Rink – Twelve participants in the study attended a skating event that served to encourage physical activity and use of outdoor recreational facilities in the community.

Little Sisters of the Assumption – An evening workshop was conducted in which five mothers were enrolled and consented to participate in the study. In addition, a healthy cooking class took place at Little Sisters on March 31, 2005.

Mount Sinai Office of Multicultural Affairs – The COTC provided an informational table for the Community Health Fair organized by Mount Sinai and scheduled for April 9, 2005.

Julia De Burgos Community Center – Presented at the Heart Healthy Fair organized by the Mount Sinai Office of Community Relations. Close to 100 community residents attended the February 14, 2005 event.

Green Guide – Planned a series of articles on children's environmental health that will appear in upcoming issues of this publication.

Cornell Regional Cancer Environment Forum – Presented a talk entitled "Communicating Environmental Health Sciences Information to Minority Communities."

2006

New York City Parks Foundation – Environmental educators offered three "Make Your Own Nature Journal" workshops at the Mount Sinai Growing Up Healthy Lab. Children learned about forest ecology, then learned about keeping field notes by handcrafting individualized "nature journals."

New York City Parks Foundation – Environmental educators introduced children to the hidden wonders of Central Park, equipping them with magnifying glasses and bug boxes and then taking them on a scavenger hunt in the park.

Growing Up Healthy Staff – Springtime gift bags were distributed. Each gift bag included sidewalk chalk, hopscotch instructions, a jump rope, a jacks set, a printed map of walking paths in Central Park, spring-themed recipes, a coloring book, crayons, and tips for keeping children healthy.

Little Sisters of the Assumption – Mold abatement expert Ray Lopez presented two multimedia workshops to parents at East Harlem elementary school P.S. 50, in English and Spanish. Topics covered included how to identify dangerous molds in the home, how to properly clean mold, and which authorities to contact if mold damage is too extensive for standard cleaning techniques.

2007

Julio De Burgos Community Center – Hip hop dance party (January 15, 2007). Growing Up Healthy worked with a local dance group to have a hip hop dance party, with the focus on diabetes and obesity prevention. The kids had a fun time learning that dance was a form of exercise and that exercise can be fun.

Julio De Burgos Community Center – Art Day (August 11, 2007; 31 attendees [nine participants and 22 others]). Growing Up Healthy held an art day where participants and their families were able to make such crafts as masks, puppets, and key chains, and decorate their own gift bags.

Growing Up Healthy Staff – Back-to-School Giveaway (August 28-August 30, 2007). As of October 10, 2007, 291 backpacks were given to participants. The bags contained school supply items (notebook, pencil case, glue, scissors, erasers, pencil sharpener, folder, yo-yo, and jump rope).

Mount Sinai School of Medicine – Scary Potter's Broomstick Bash: Healthier way to celebrate Halloween (October 26, 2007). One hundred forty kids were in attendance (62 participants and 78 others). Participants dressed in costume and were able to participate in such activities as Halloween crafts and costume cardio.

Growing Up Healthy Staff – Holiday Photo Studio (December 10-December 14, 2007). One hundred six kids were in attendance (29 participants and 67 siblings/friends). Kids and family members are able to take holiday photos against a Christmas background. The pictures are printed for them as they decorated their own picture frames.

2008

Winter Hat, Scarf, and Glove Set Giveaway (January 21-January 25, 2008) – As of January 26, 67 hats were given out (giveaway continued beyond January 25 on an appointment basis). Seventeen follow-up appointments were made due to this giveaway. Thirty-one hats were given away by project. Two and six lost participants were recovered.

The Young Scientist Club Presents: The Magic School Bus Explores the Human Body (February 18, 2008-February 22, 2008). Kids watched The Magic School Bus episodes and did science experiments based upon the television show.

Peace On The Streets-Ultimate Karate Event (April 22, 2008-April 25, 2008). Worked with local karate center to allow our participants to take free introductory karate classes and expose them to alternative ways of exercise.

Little Sisters of the Assumption-Growing Up Healthy Summer Picnic (July 3, 2008). GUH worked with the members of Little Sisters to put on a healthy cooking event in the local community garden. The recipes were based from the Go Green East Harlem Cookbook. There was a MSSM nutritionist there to talk to participants about healthy eating and how to read the nutrition label. Each participant was given a copy of the Go Green East Harlem Cookbook donated by Manhattan Borough President Scott Stringer.

Growing Up Healthy Staff-Back to School Backpack Giveaway (August 26-August 28, 2008). Book bags were given out to participants and contained school supply items (notebook, pencil case, glue scissors, erasers, pencil sharpener, and folder).

Print Communications: The COTC also makes additional contacts with study participants using original print materials created especially for this population. The communications include a bi-annual newsletter that informs participants of the progress of the Center studies and fact sheets that take one topic in pediatric environmental or community health and present its relevance to the community. These mailings are often accompanied with materials specifically designed to reinforce the environmental health messages for participants. In addition, fact sheets and other materials created by the Mount Sinai COTC have been distributed broadly to the advocacy community.

Materials Produced by MSSM COTC

2006

A listing (with map) of Farmers' Markets in Upper Manhattan, including all of Harlem and the Bronx, with days and hours of operation for each market.

Phthalates: an introduction to these chemicals, where they are found, and how they can be avoided.

Quick Guide to Plastics, an easy-to-understand guide to the numbering system used by plastics manufacturers, mailed along with a wallet-sized card for easy reference while shopping.

Volume 2 of the *East Harlem Kids in Action News* newsletter. Highlights included photos and re-cap of activities offered by Growing Up Healthy, a healthy recipe, a listing (with map) of markets in East Harlem that have fresh fruits and vegetables available, and a listing of primary care and recreational facilities for children.

2007

Fact Sheets:
- Artificial Turf
- Chemicals in Cosmetics with compact mirror
- Lead in Toys
- Mercury in Fish with shopping list
- Pesticides in Food
- Pesticides in Your Home
- Sunblock

Other Material:
- Free summer activities guide
- Growing Up Healthy newsletter
- 2007 Growing Up Healthy calendar

2008

Fact Sheets:
- Camphor
- Know Your Hospital with telephone pad
- Lead in Candy
- Healthy Bones

Other Material:
- Growing Up Healthy results newsletter
- Free summer activity guide
- 2008 Growing Up Healthy calendar

MSSM COTC Publications

2007

Chace, R. Growing Up Healthy in East Harlem and the Bronx, New York. 2007; The Ribbon 12:9-12.

2008

Claudio, L. Green My Health: A clear look at water bottles. Prevention Magazine, September 2008, pp 201-202.

Number of Requests for MSSM COTC Materials as of December 2008

2007

- 956 plastics wallet cards
- 40 Chemicals in Cosmetics fact sheets
- 40 Pesticides in Your Home fact sheets

2008

- 3,970 plastics wallet cards
- 520 safe plastics fact sheets
- 20 Sunblock fact sheets
- 520 Mercury in Fish fact sheets
- 20 Pesticides in Food fact sheets
- 500 Chemicals in Cosmetics fact sheets

FCCC Advocacy Activities

Advocacy Workshops, Fox Chase Cancer Center, Philadelphia, PA

April 24, 2007
April 7, 2008
October 6, 2008
October 17, 2008

Michigan State University

Conference Presentations, Papers and Posters

Atkin C. (November 2008). *Reforming food advertising and marketing practices in response to childhood obesity concerns.* Plenary presentation at the 5th Annual Meeting of the BCERC, Birmingham, AL.

LaPlante C, Smith SW, Nazione S, Kotowski MR. (November 2008). *The effects of the framing of memorable breast cancer messages on leading people to engage in detection or prevention behaviors.* Presented at the 5th Annual Meeting of the BCERC, Birmingham, AL.

Silk K, Atkin C, Yun D, Bowman N, Johnson J, Osuch J, Pierce K. (November 2007). *Persuading mothers to perform breast cancer prevention practices with their pre-adolescent daughters: a pilot message study.* Poster presented at the BCERC annual meeting in Cincinnati.

Whitten P, Smith SW, Munday S, LaPlante C. (November 2007). *Guidelines for breast cancer websites*. Paper presented at the Annual Meeting of the BCERC, Cincinnati.

Smith SW, Munday S, LaPlante C, Kotowski MR, Atkin CK, Skubisz CM, Stohl C. (November 2007). *Types and sources of memorable breast cancer messages and their impact on prevention and detection behaviors*. Paper presented at the Annual Meeting of the BCERC, Cincinnati.

Munday S, LaPlante C, Smith SW, Atkin CK. (November 2007). *Annotated bibliography of relevant journals for possible publication of advocate and communication research*. Paper presented at the Annual Meeting of the BCERC, Cincinnati.

Whitten P, Smith S, Munday S, LaPlante C. (November 2006). *Evaluating the design and information of the top 185 breast cancer websites*. Poster presented at the Third Annual Meeting of the BCERC, Berkeley, CA.

Smith S, Atkin C, Munday S, Skubisz C. Ferguson, V. (November 2006). *The types and sources of meaningful messages about breast cancer*. Poster presented at the Third Annual Meeting of the BCERC, Berkeley, CA.

Silk KJ. (November 2006). Message Testing Study Concept. Presentation for the COTC at the BCERC meeting, Berkeley, CA.

Atkin C, Lapinski, M. (November 2005). *Appropriate messages about diet relating to breast cancer*. Plenary presentation at annual symposium of Breast Cancer and the Environment Research Centers, East Lansing, MI.

Winn B, Whitten P. (November 2005). *Techniques for designing user-friendly and informative websites.* Presentation at COTC Workshop at Second Annual Meeting of BCERC, East Lansing, MI.

Silk KJ, Atkin CK. (November 2005). *Communication campaigns: social marketing practices and dissemination of breast cancer risk information to the lay public*. Presentation at COTC Workshop at Second Annual Meeting of BCERC, East Lansing, MI.

Smith S, Wagner S. (November 2005). *Principles of persuasive communication for influencing policy-makers and constituencies*. Presentation to Advocate Education Workshop at Second Annual Meeting of BCERC, East Lansing, MI.

Atkin C, Barlow, J. (November 2004). *News content and audience responses: Michigan and Marin*. A plenary presentation at the First Annual Meeting of the BCERC, Princeton, NJ.

Silk KJ. (November 2004). *The role of health literacy and numeracy in cancer communication*. A plenary presentation at the First Annual Meeting of the BCERC, Princeton, NJ.

Smith S, Atkin C. (November 2004). *Content analysis of breast cancer news coverage*. A plenary presentation at the First Annual Meeting of the BCERC, Princeton, NJ.

Educational Materials

Each of the presentations above is available for free downloads on the bcerc.msu.edu website. In addition, MSU produced and disseminated the following:

2008 Multidimensional method to evaluate health websites – provided to advocates and others at the 2008 BCERC meeting to assist them in evaluating their own websites and improving them.

2007 Annotated bibliography of relevant journals for possible publication of advocate and communication research – used in a training for advocates in a writing workshop.

2006 Coding scheme to assess breast cancer information news with particular emphasis on assessing prevention information – provided to interested advocates and used as a guide in a Bay Area BCERC project assessing a local newspaper's information.

Bay Area

Poster Sessions at Meetings

Barlow J. (September 2005). Portland, Oregon, Northwest Health Foundation Conference, Poster Presentation: *Enhancing Community Participation in the Research Process*

Barcellos-Hoff/Barlow/Pierce/Balke/Koblick/Lee/Marks/Ornstein/Johnson. (October 2007). San Francisco, UCSF Helen Diller Family Comprehensive Cancer Center Specialized Program of Research Excellence (SPORE) Breast Oncology Program Scientific Retreat, Poster Presentation: *Of Mice and Women: Modeling Breast Cancer and the Environment*

Barlow J. (November 2004). New Jersey, 1st Annual NIEHS Early Environmental Exposures Conference, Poster Presentation: *Enhancing Community Participation in the Research Process*

Schwartz/Barlow. (November 2005). Michigan, 2nd Annual NIEHS Early Environmental Exposures Conference, Poster Presentation: *Adolescent Peer Education Breast Cancer Awareness Project*

Barlow/Orenstein/Koblick/Pierce. (November 2005). Michigan, 2nd Annual NIEHS Early Environmental Exposures Conference, Poster Presentation: *Evaluating the Effectiveness of Community Forums*

Schwartz/Barlow. (November 2006). California, 3rd Annual NIEHS Early Environmental Exposures Conference, Poster Presentation: *Adolescent Breast Cancer Prevention, Risk Reduction and Education Project*

Barcellos-Hoff/Barlow/Pierce/Balke/Koblick/Lee/Marks/Ornstein/Johnson. (November 2006). California, 3rd Annual NIEHS Early Environmental Exposures Conference, Poster Presentation: *"Of Mice and Women": An Innovative Educational Kit to Engage the Community on Why Mice Models Are Used in Breast Cancer Research*

Steingraber/Taylor. (November 2007). Ohio, 4th Annual NIEHS Early Environmental Exposures Conference, Poster Presentation: *The Falling Age of Puberty in US Girls: What We Know, What We Need To Know*

VanOlphen/Ottoson/Green/Barlow/Hiatt. (November 2007). Ohio, 4th Annual NIEHS Early Environmental Exposures Conference, Poster Presentation: *Evaluation of a Community-based, Participatory Research Approach in BABCERC (revised)*

Barlow/Landaverde/Ergas/Mirabedi/Ferguson/Dickinson/Kushi. (November 2008). Alabama, 5th Annual NIEHS Early Environmental Exposures Conference, Poster Presentation: *Successful Retention Strategies in Trans-disciplinary Research: The Cygnet Study*

VanOlphen/Ottoson/Green/Barlow/Hiatt. (November 2008). Alabama, 5th Annual NIEHS Early Environmental Exposures Conference, Poster Presentation: *Evaluation of a Community-based, Participatory Research Approach in BABCERC (revised)*

Schwartz/Guerra/Deardorff/Barlow. (November 2008). Alabama, 5th Annual NIEHS Early Environmental Exposures Conference, Poster Presentation: *Latina Adolescent Outreach Project*

BCERC Presentations

Barlow. (November 2004). New Jersey, 1st Annual NIEHS Early Environmental Exposures Conference, Presentation: *Media Coverage of Breast Cancer from the Advocate and Consumer Perspective: The Marin County Experience*

Barlow. (November 2005). Michigan, 2nd Annual NIEHS Early Environmental Exposures Conference, Presentation: *Critical Issues in Biomonitoring*

Barlow. (November 2006). California, 3rd Annual NIEHS Early Environmental Exposures Conference, Presentation: COTC: *Linking Scientists and the Community through the Research Process*

Barlow. (November 2007). Ohio, 4th Annual NIEHS Early Environmental Exposures Conference, Presentation: *Unlocking the Laboratory: Introducing Breast Cancer Advocates to Bench-Top Research*

Other BCERC-Related Presentations

October 2004, San Rafael, Kaiser San Rafael Pediatric Staff, *Presentation/Bay Area Breast Cancer and the Environment Research Center (BABCERC)*

January 2005, San Francisco, UCSF Breast Cancer Oncology Lecture Series: televised to medical and postdocs. Researchers at UCSF, Buck Institute for Age Research, and Lawrence Berkeley National Laboratory, *Presentation: Breast Cancer and the Environment*

January 2005, San Rafael, Marin County Breast Cancer Coordinating Council, *Presentation/BABCERC*

March 2005, Novato, CA, Stanford University VIA American and Japanese Medical Students at the Buck Institute for Age Research, *Presentation/BABCERC*

July 2005, San Francisco, African American Coalition for Health Improvement and Empowerment, *Presentation/BABCERC*

January 2006, Sacramento, Committee on Environmental Safety and Toxic Materials, State of California, *Presentation/BABCERC*

January 2006, San Francisco, Bay View Hunters Point Health and Environmental Resource Center, *Presentation/BABCERC*

February 2006, San Francisco, Wednesday Morning Women Leaders' Symposium, *Presentation/BABCERC*

March 2006, San Rafael, YMCA Wellness Lecture, *Presentation/Breast Cancer and the Environment*

August 2006, San Francisco, Days of Dialogue, National Sisters Network, *Presentation/Environmental Contributions to Breast Cancer*

November 2006, San Antonio, TX, National Communications Association Conference/Health Communications Division, *Panel Presentation/The Breast Cancer and the Environment Research Centers: Communication Research and Advocacy Efforts of the Community Outreach and Translation Core*

February 2007, New York, Cornell University/Department of Communications, *Webcast Presentation: Community Involvement in Environmental Decision Making*

May 2007, San Francisco, International CommunicationAssociation/Health, Risk and Crisis Communication Education Division, Panel Presentation/*Risk Communication Activities of the BCERC: Two Community Outreach Exemplars*

May 2007, San Francisco, UCSF Comprehensive Cancer Center, *Presentation: Bay Area Breast Cancer and the Environment Research Center: Community Outreach and Translation Core*

October 2007, UCSF Comprehensive Cancer Center, Bay Area Breast Cancer Forum, *Presentation/Environmental & Lifestyle Factors: What Do They Have To Do With Breast Cancer? What Do We Know… and Directions for the Future*

October 2007, Public Library South San Francisco, CA, So. San Francisco Library Monthly Lecture Series, *Presentation/Environmental and Lifestyle Factors: What Do They Have To Do With Breast Cancer? What Do We Know… and Directions for the Future*

October 2007, San Rafael, YMCA Wellness Lecture, *Presentation/Environmental and Lifestyle Factors: What Do They Have To Do With Breast Cancer? What Do We Know… and Directions for the Future*

December 2007, San Francisco UCSF Center of Excellence Reproductive Health Program, *Presentation/Toxic Tour of Bay View Hunters Point*

February 2008, San Rafael, Dominican University, The Promise of Stem Cell Research in Human Health Stem Conference, *Breast Cancer and the Environment Research Centers*

October 2008, Marin, Marin Cancer Institute, *Breast Cancer Prevention*

COTC-Sponsored Resource Tables at Conferences

July 2006, San Francisco, Avon Foundation Breast Cancer Walk,

October 2006, San Francisco, Bay View Hunters Point Health and Environment Resource Center Annual Luncheon

December 2006, Oakland, African American Health Summit: *Strengthening Our Relationships*

January 2007, San Francisco, UCSF-CHE Summit on Environmental Challenges to Reproductive Health and Fertility

January 2007, San Rafael, Community Educational Forum: *Positive Effects of Physical Activity on Breast Cancer*

March 2007, San Francisco, Northern California Cancer Center Breast Cancer Conference for Survivors, Family, Friends and Medical Professionals

May 2007, San Francisco, Third Annual African American Breast Cancer Conference: *Each One Reach One: Working Together to Make Change*

May 2007, Berkeley, Tenth Annual Northern California Tobacco, Alcohol, Drug Education and Youth Health and Development

May 2007, San Francisco, UCSF Breast Oncology Program Scientific Retreat

September 2007, Los Angeles, California Breast Cancer Research Program (CBCRP) 3rd Annual Symposium, *From Research to Action: Breaking New Ground*

October 2007, Mills College Oakland, Women's Cancer Resource Center 12th Annual Swim-A-Mile for Women with Cancer

October 2007, UCSF Laurel Heights, *African American Women Taking Care of Business: Getting to Know the System*/Susan G. Komen for the Cure, SF Bay Area

October 2007, Bayview Opera House San Francisco, Bay View Hunter's Point Health and Environmental Resource Center 7th Annual Women's Luncheon

October 2007, Southeast Community Facility San Francisco, Sisters Network San Francisco Chapter Health Fair

October 2007, UCSF Comprehensive Cancer Center, Bay Area Breast Cancer Forum

October 2007, Cornerstone Community Church Marin City, Marin City Health and Wellness Center Community Health Fair/*Women's Breast Cancer Awareness and Festivity Day*

October 2007, San Francisco, UCSF Helen Diller Family Comprehensive Cancer Center Specialized Program of Research Excellence (SPORE) Breast Oncology Program Scientific Retreat

October 2007, SFSU, San Francisco, Susan G. Komen, *Komen on the Go*

October 2007, Public Library, So. San Francisco, Lecture, Zero Breast Cancer and *BCERC, BABCERC and Adolescent Peer Education Project*

November 2007, Cincinnati, BCERC 4th Annual Meeting, *Emerging Topics in Breast Cancer and the Environment Research Posters*

December 2007, San Rafael, Marin County School Nurse Assoc. Meeting, *Breast Cancer and the Environment Peer Education Tool Kit*

December 2007, Mission Bay San Francisco, 1st Annual Cancer Survivorship Conference/*Now What? The New Normal of Cancer Survivorship After Treatment*

February 2008, Dominican University, San Rafael, Stem Cell Conference, *The Promise of Stem Cell Research in Human Health*

February 2008, Houston, TX, Avon Foundation Breast Cancer Forum: Advancing Prevention and Access to Treatment

March 2008, San Francisco, Breast Cancer Fund

March 2008, Oakland, BABCERC 3rd Annual Town Hall Meeting, *Translating Breast Cancer and Environmental Research Into Action*

July 2008, San Francisco, National Latino Cancer Summit, *Science Meets Service: Moving Forward Together*

COTC-Sponsored Community Events

February 2004, *BABCERC community meeting in San Francisco*, 55 attendees, Small Groups, Discussion Notes

June 2004, *BABCERC community meeting in San Rafael*, 42 attendees, Small Groups, Discussion Notes

September 2004, *BABCERC community meeting in San Rafael*, 36 attendees, Small Groups, Discussion Notes

October 2004, *Critical Issues in Biomonitoring – A Community Forum*, 112 attendees, DVD Summary of Proceedings available at the following websites: http://www.bcerc.org/pubs.htm, http://www.bayarea.bcerc.org/pubs.htm

May 2005, *BABCERC community meeting in Alameda*, 28 attendees, Small Groups, Discussion Notes

January 2006, 1st Annual Town Hall Meeting: *Communities Coming Together to Explore Environmental Links to Breast Cancer,* 182 attendees, Distributed to media list: articles ran in the *Marin Scope*, *Bay Area Business Woman*, *Marin IJ*. Program available on following websites: http://www.bcerc.org/pubs.htm http://www.bayarea.bcerc.org/pubs.htm

March 2006, Two Discussion Groups With African American Breast Cancer Survivors in Bay View Hunter's Point on Breast Cancer and the Environment, six attendees per group, transcription available

May 2006, Discussion Group with African American Breast Cancer Survivors in Alameda on Breast Cancer and the Environment, five attendees, Transcription available

March 10, 2007, 2nd Annual Town Hall Meeting: *Environmental Influences on Girls' Development During Puberty,* 94 attendees, program and presentation summaries are available on the following websites: http://www.bcerc.org/pubs.htm http://www.bayarea.bcerc.org/pubs.htm

March 1, 2008, 3rd Annual Town Hall Meeting: *Translating Breast Cancer & Environmental Research Into Action: Integrating Biological, Human and Community-Based Research*, 96 attendees, distributed proceedings via Podcasts, Public Access TV, and newsletters. Slideshow presentations and E-Zine available on following websites: http://www.bcerc.org/pubs.htm http://www.bayarea.bcerc.org/pubs.htm

Educational and Outreach Activities Related to CYGNET Retention

CYGNET "Tea Talks"

March 29, 2006, History of the CYGNET Study and the Impact on Girls' Health, Larry Kushi, Sc.D., Division of Research, Kaiser Permanente, Oakland

June 7, 2006, History of the CYGNET Study and Its Impact on Girls' Health, Larry Kushi, Sc.D., San Rafael Kaiser, Terra Linda Campus, San Rafael, 44 attendees

November 8, 2006, Introduction to the Community Outreach and Translation Core (COTC), History of the CYGNET Study and Its Impact on Girls' Health, Janice Barlow and Bob Hiatt, M.D., UCSF Laurel Heights Campus, San Francisco, 36 attendees

March 9, 2007, Measuring Environmental Exposures and Research on their Health Effects, Gayle Windham, Ph.D., Hall of Health Museum, Berkeley, 54 attendees

October 13, 2007, Speaking to Your Daughter about Puberty: It's Never Too Early to Talk, Louise Greenspan, M.D., Bay-Delta Model Museum, Sausalito, 47 attendees

April 5, 2008, Keeping Physically Active Throughout Life, Barbara Sternfeld, Ph.D., Lawrence Hall of Science, Berkeley, 52 attendees

October 11, 2008, Positive Parenting, Julie Deardorff, Ph.D., San Francisco Exploratorium, San Francisco, 107 attendees

Bi-Annual CYGNET Newsletters (Distribution: 440 Study Families) (copies available on BABCERC website)

- Spring 2006, CYGNET Newsletter – Making a Difference: Local and National Levels
- Summer 2006, CYGNET Newsletter – The CYGNET Study: Part of a National Research Effort
- Holiday 2006, CYGNET Newsletter – Progress on the CYGNET Study
- Spring 2007, CYGNET Newsletter – The CYGNET Community
- Summer/Fall 2007, CYGNET Newsletter – Greetings from the CYGNET Study
- Winter/Spring 2008, CYGNET Newsletter – Greetings from the CYGNET Study

BCERC and BABCERC Educational Materials produced by BABCERC COTC (See http://www.bcerc.org/pubs.htm and http://www.bayarea.bcerc.org/pubs.htm)

BABCERC

January 2007, Target audience: general public, Brochure (English), *Bay Area Breast Cancer and the Environment Center,* Purpose: to provide information about the Center and the research being done

August 2007, Target audience: general public, Conference Board, *Bay Area Breast Cancer and the Environment Center,* Purpose: to provide overview of BCERC, BABCERC, Project 1 &2, and to display outreach materials

July 2008, Target audience: Latino population, Brochure (Spanish), *Centro de Investigacion del Medio Ambiente & el Cancer de Seno del Area de la Bahia,*

Purpose: to provide information about the Center and the research being done

Educational and Outreach Materials Related to the Biology Project

June 2006, Target audience: breast cancer advocates, science students, and general public, DVD accompanied by scientific glossary, *Of Mice and Women: Modeling Breast Cancer and the Environment*, Purpose: to further communities' understanding of why mouse models are used to study breast cancer etiology and environmental exposures

June 2008-August 2008, Target audience: breast cancer advocates and general public, Lay abstracts of BABCERC's to date scientific publications (26), Purpose: to further communities'understanding of the center's research findings

Educational and Outreach Materials Related to the Epidemiology Project

October 2004, Target audience: breast cancer advocates, researchers, public health professionals, general public; DVD Summary of Proceedings, *Critical Issues in Biomonitoring*, Purpose: to facilitate a transdisciplinary dialogue on important issues related to biomonitoring

August 2007, Target audience: lay: CYGNET families, breast cancer and environmental health advocates, Educational Brochure, *Phthalates – The Everywhere Chemical*, Purpose: To describe the chemical phthalate and its various sub-categories, products made with phthalates, BABCERC research, and tips on how to avoid products containing phthalates

October 2007, Target audience: lay - CYGNET families, breast cancer and environmental health advocates, Educational Brochure, *The Mind-Body Connection – Defining Onset of Puberty in Girls*, Purpose: to define puberty and its different stages, Tanner staging, and BABCERC research.

November 2007, Target audience: general public, breast cancer and environmental health advocates, policy makers, Timeline, *California Bay Area Breast Cancer and the Environment Research Center: Advocates and Researchers Work Together 1990–2010*, Purpose: To visually represent the creation of BABCERC in relation to the San Francisco Bay Area environmental breast cancer movement.

November 2007, Target audience: researchers, breast cancer and environmental health advocates, policy makers, Fact Sheets, *BCERC Scientific Fact Sheets: Early Life Exposure and Breast Cancer Risk in Later Years: Chemicals Perfluoralkyl Acids, Phenols, Phthalates, and Phtyoestrogens Daidzein, Genistein, and Enterolactone (Version 1)*, Purpose: to provide information about compounds being measured and examined by the BCERC epidemiology studies, sources of exposures, effects on puberty, effects in the body, and research studies looking at the compounds as being associated with breast cancer risk.

February 2008, Target audience: lay: CYGNET families, breast cancer and environmental health advocates, Educational Brochure, *What is the Tanner Staging System?* Purpose: to define Tanner staging, and BABCERC research

Media Communications

TV and Radio Publicity

October 2004, KGO TV (ABC 7), "Beyond the Headlines" with Cheryl Jennings, Purpose: aired in the Bay Area re: BABCERC

November 2004, CNN News (Channel 57), "Headliner News" interview with Jack Hanson, Purpose: aired throughout the Bay Area during the month of November re: BABCERC

November 2004, Radio Canal, "Breast Cancer and the Environment" Interview with Rosamaria Hayden, Purpose: aired in the Bay Area targeted to the Latino population re: BABCERC

December 2004, T48 Telemundo, "Breast Cancer and the Environment" Interview with Pilar Niño, Purpose: aired in the Bay Area targeted to the Latino population

February 2006, KNBR/KFOG/KWMR Radio, 1 hour radio interview, Purpose: to promote the Breast Cancer and the Environment Research Center

May to November, 2008, Public Access TV, "Of Mice and Women: Modeling Breast Cancer and the Environment", Purpose: aired proceedings

May-November, 2008, Public Access TV, 3rd Annual Town Hall Meeting: *Translating Breast Cancer & Environment Research into Action: Integrating Biological, Human and Community-Based Research*, Purpose: aired proceedings of the BABCERC town hall meeting

Zero Breast Cancer Newsletter Articles (Circulation: 5,000)

- Spring 2004, An Environment Breast Cancer Research Center Comes to the Bay Area
- Fall 2005, Environmental Research and Prevention: A Priority for the Bay Area Breast and the Environment Research Center

- Fall 2006, Importance of Hormones in Breast Cancer: Summary of Dr. Valerie Beral's Presentation at 3rd Annual Early Environmental Exposures Meeting
- Spring 2007, Are Girls Entering Puberty Earlier?
- Spring 2008, Translating Breast Cancer & Environmental Research into Action
- Fall 2008, Environmental and Genetic Determinates of Puberty: A Mid-Project Report

Newspaper Publicity

- October 2003, Environmental links will be focus of studies at UCSF, *Marin Independent Journal*
- October 2003, UCSF picked for breast cancer study, *San Francisco Bay Area*
- October 2003, Breast Cancer Research Center Launched, *Marin Independent Journal*
- June 2004, Study focused on answers to breast cancer, *Marin Independent Journal*
- May 2005, Future of breast cancer research, *Marin Independent Journal*
- October 2005, Environmental factors could be linked to breast cancer, *Marin Scope Newspapers*
- January 2006, Marin forum: environmental links to breast cancer, *Coastal Post*
- January 2006, Forum looks at environmental links to breast cancer, *Marin Scope Newspapers*
- January 2006, Possible breast cancer links explored, *Contra Costa Times*
- June 2006, Timing is everything-and the time is now, *Bay Area Business Woman*
- February 2008, Phthalates risk is everywhere, *Marin Independent Journal*

Magazine Publicity

- December 2003, Cancer Puzzle, *Pacific Sun Magazine*
- Fall 2006, The Geography of the Breast, *MS Magazine*
- March 2007, Puberty, Obesity, Environment and Breast Cancer, *UCSF Today*

UCSF Website and CCC Reports

- August 2006, Discovering How Environment Contributes to Breast Cancer, *UCSF Today*
- Fall 2007, Fewer breast cancers are still too many, UCSF CCC *Report*
- September 2008, Early Puberty and Early Exposure to Breast Cancer Risks: A Conversation with Robert Hiatt, online publication

University of Cincinnati

Community Education Publications

Event Program, 4th Annual BCERC Early Environmental Exposures Conference, Cincinnati, OH, November 8-9, 2007.

Glossary of Scientific and Medical Terms for advocates participating in the 4th annual public forum, Looking Upstream for Environmental Links to Breast Cancer, May 2008. 3rd ed. Available at http://www.bcerc.org/COTCpubs/Cinc_glossary_032108.pdf

Cincinnati BCERC informational brochure, 2008. Available at http://www.eh.uc.edu/growingupfemale/pdfs/CINTI%20BCERC%20brochure_final.pdf

Body Mass Index (BMI) Fact Sheet, June 2008. Available at http://www.bcerc.org/COTCpubs/Cinc_FactSheet_BMI.pdf

Growing Up Female Study Protocol Fact Sheet, 2008.

Online video of presentations from the Looking Upstream for Environmental Links to Breast Cancer forums, May 14, 2005, May 13, 2006, May 12, 2007, May 17, 2008. Available at http://www.eh.uc.edu/growingupfemale/events.asp.

Online video of presentations from the 4th Annual BCERC Early Environmental Exposures Conference, Cincinnati OH, November 8-9, 2007. Available at http://www.eh.uc.edu/growingupfemale/events.asp.

Cincinnati COTC Support Network committee (Croucher L, Brown MK, Stautberg M, et al.). Growing Up Female Coloring Book for Project 2 study girls. 2005. Available at http://www.eh.uc.edu/growingupfemale/pdfs/COTC_GUF%20coloring%20book.pdf

Matrix template of Project 1 study designs of the four BCERCs. July 2006.

Flessa J. Lay abstract of Biro FM, Khoury P, Morrison JA. Influence of obesity on timing of puberty. Int J Androl. 2006;29:272-8. Available at http://eh.uc.edu/growingupfemale/pdfs/P2_PUBS_LAY%20ABS_Biro&Flessa%20'06.pdf

Flessa J. Lay abstract of Clegg DJ and Heffelfinger S. Obesity: its influence on breast cancer susceptibility. Women's Health. 2006 Jul;2(4): 577-85. Available at http://eh.uc.edu/growingupfemale/pdfs/P1_PUBS_LAY%20ABS_Clegg&Flessa%20'07.pdf

Flessa J. Lay abstract of Heffelfinger SC. The Renin angiotensin system in the regulation of angiogenesis. Curr Pharm Design. 2007;13:1215-29. Available at http://eh.uc.edu/growingupfemale/pdfs/P1_PUBS_LAY%20ABS_Heffelfinger&Flessa%20'07.pdf

Gear RB, Nikolaides L. Lay abstract of Gear RB, Yan M, Schneider J, Succop P, Heffelfinger SC, Clegg DJ. Charles River Sprague Dawley Rats lack early age-dependent susceptibility to DMBA-induced carcinogenesis. Intl J Bio Sci. 2007;3:408-16. Available at http://eh.uc.edu/growingupfemale/pdfs/P1%20PUBS_LAY%20ABS_Gear&Nikolaides%20'08.pdf

Pinney SM, Yaghjyan L, Beatty J. Biomarker description database. Series of .pdf files posted on BCERC intranet, September 2006.

Educational Programs

K. Ball, F. Biro, R. Bornschein, M.K. Brown and S. Heffelfinger presented the educational program at the BCA's Annual Spring Educational Forum; their presentations focused on the study hypotheses, research methods, and educational activities of the Cincinnati BCERC. The program was entitled *Looking Locally for Answers about Breast Cancer and the Environment*. Approximately 100 people attended on May 11, 2004.

F. Biro, M.K. Brown, P. Cunningham, G. Greenburg, A. Hernick, C. Price presented an advocate Mentoring Session at the 4th Annual BCERC Early Environmental Exposures Conference, Cincinnati, OH, November 8-9, 2007.

F. Biro, R.L. Bornschein, K. Dietrich, S. Pinney, L. Yaghjyan, and the CCHMC Volunteer Department conducted a half-day training program for advocates to be Study Helpers with the Growing Up Female study on September 15, 2007.

R. Bornschein presented *Environment and the Links to Breast Cancer* at the Celebration of Life Program hosted by the Parish Nurse Program of Greater Emanuel Apostolic Church at Greater New Hope Missionary Baptist Church on October 30, 2004.

R. Bornschein presented *In Our Midst: Environmental Exposures in Everyday Life* at the Cincinnati BCERC's second annual Looking Upstream for Environmental Links to Breast Cancer forum, May 13, 2006.

R. Bornschein presented *Biomarkers of Environmental Chemical Exposures* at a meeting of the Greater Cincinnati Water Works Advisory Committee, February 23, 2007.

R. Bornschein presented *An Update from the Cincinnati Breast Cancer and the Environment Research Center* at the program Upfront About Breast Cancer 2007, March 17, 2007.

The Breast Cancer Alliance of Greater Cincinnati and Cincinnati BCERC conducted the half-day *ART* (Advocate Research Training) program at the UC Genome Research Institute (GRI) on August 18, 2007. Cincinnati BCERC members participating included K. Ball, S. Benoit, M.K. Brown, D. Clegg, R. Gear, H. Hendrix, A. Hernick, R. Jandacek, M. Mistry, S. Pinney, and J. Schneider.

M.K. Brown, with J. Brody and S. Snedeker, conducted a 3-hour Writing Workshop for COTC advocates and NIH personnel at the 4th Annual BCERC Early Environmental Exposures Conference, Cincinnati OH, November 8-9, 2007.

M.K. Brown presented *Community-based Participatory Research* for the workshop Knowledge for Improving the Community's Health: An Introduction to Medical and Health-Related Research, at The Conference on Closing the Health Gap in Greater Cincinnati, November 13-14, 2003.

The COTC Education Committee sponsored a public seminar and private, working luncheon with Electra Paskett, Ph.D., Marion N. Rowley Professor of Cancer research at Ohio State University on May 25, 2004. The title of her talk was *The Robeson County Outreach Screening & Education (ROSE) Project*, presented on the campus of the University of Cincinnati College of Medicine.

The Growing Up Female (Project 2) Study Team presented a Study Update for participant families on May 12 and 13, 2008 at Cincinnati Children's Hospital and a local elementary school.

The Growing Up Female (Project 2) Study Team presented a Study Update for participant families on May 14 and 15, 2007 at Cincinnati Children's Hospital and a local elementary school.

Appendix D. Data From the Epidemiology Project

TABLE D1. Numbers of Participants With Data or Biospecimens, by Type of Data Collected, According to Wave of Data Collection and Epidemiology Project Site, as of August–October 2008.

	Cohort or in FU	Pubertal Stages	Anthropometry	Questionnaires	Urine	Blood	Buccal/ Saliva	Pedometer	Diet, 1+ Recall	Psychosocial
BASELINE										
KPNC	444	441	444	444	422	227	209	342	440	380
MSSM	416	416	414	416	415	16	413	393	367	N/A
Cin	379	379	379	313	335	345	N/A	N/A	352	327
Total	**1239**	**1236**	**1237**	**1173**	**1172**	**588**	**622**	**735**	**1159**	**707**
FU1-YR02										
KPNC	440	407	408	414	405	286	N/A	242	N/A	392
MSSM	344	305	305	305	304	4	304	146	114	297
Cin	330	330	330	315	306	288	N/A	N/A	330	315
Total	**1114**	**1042**	**1043**	**1034**	**1015**	**578**	**304**	**388**	**444**	**1004**
FU2-YR03										
KPNC*	436	395	402	402	395	15	N/A	N/A	N/A	389
MSSM	370	174	174	173	173	1	96	67	46	N/A
Cin	229	229	229	169	190	199	N/A	N/A	233	169
Total	**1035**	**798**	**805**	**744**	**758**	**215**	**96**	**67**	**279**	**558**
FU3-YR04										
KPNC*	436	98	97	98	97	N/A	59	N/A	N/A	41
MSSM	389	57	57	56	54	0	49	17	7	N/A
Cin	153	153	153	65	69	142		N/A	150	66
Total	**978**	**308**	**307**	**219**	**220**	**142**	**108**	**17**	**157**	**107**
Grand Total	**4366**	**3384**	**3392**	**3170**	**3165**	**1523**	**1130**	**1207**	**2039**	**2376**

*Psychosocial numbers are from August 2008.
Baseline "cohort" numbers are those consented with complete data and eligibility (definitions vary by site). Followup numbers are those not withdrawn for KPNC, not withdrawn or not out of the FU window for MSSM, and those seen or in progress for Cin. Six girls enrolled by MSSM were found to be ineligible.

TABLE D2. Demographic and Developmental Characteristics of the BCERC Epidemiology Project Participants, Including Age, Race/Ethnicity, Tanner Stage for Breast Development, and Body Mass Index. Data Are From Baseline Data Collection Unless Otherwise Noted, 2004-2007.

	MSSM		CINCINNATI		KPNC		TOTAL	
	N	%	N	%	N	%	N	%
Age at Consent (years)								
6	167	41	188	50	105	24	460	37
7	128	31	190	50	332	75	650	52
8	121	29	1	0.3	7	1.6	129	10
Total	**416**		**379**		**444**		**1239**	
Race/Ethnicity								
White			236	62	182	41.0	418	33.8
Asian			5	1	49	11.0	54	4.4
Hispanic	248	60	10	3	107	24.1	365	29.5
Black & Hispanic-Black	168	40	127	34	96	21.6	391	31.6
Other					10	2.2	10	0.8
Total	**416**		**378**		**444**		**1238**	
Breast Tanner Stage								
Baseline								
B1	329	79	324	86	408	93	1061	86
B2+	87	21	54	14	33	7	174	14
Total	**416**		**378**		**441**		**1234**	
Follow-Up 1								
B1	195	66	221	67	341	84	757	73
B2+	102	34	107	33	66	16	275	27
Total	**297**		**328**		**407**		**1032**	
Follow-Up 2								
B1	62	41	115	50	226	61	403	53
B2+	90	59	114	50	147	39	351	46
Total	**152**		**329**		**373**		**754**	
BMI Percentile								
<50	117	28	142	37	153	34	412	33
50 to <85	135	32	125	33	165	37	425	34
85 to <95	65	16	59	16	60	14	184	15
95+	99	24	53	14	66	15	218	18
Total	**416**		**379**		**444**		**1239**	

BMI percentile is calculated from NHANES sex/age-specific data, 2000. Body size category definitions: Underweight: < 5th percentile; Normal, 5th to < 85th percentile; At risk of overweight, 85th to < 95th percentile; At risk of obesity, ≥ 95th percentile.

TABLE D3. Mean Baseline Measures of Physical Activity and Dietary Phytoestrogen Intake According to Tanner Stage for Breast Development, at Baseline (MSSM Site Only) and First Annual Follow-up Exams (MSSM and KPNC Epidemiology Project Sites)

	MSSM *(data as of October 2007)* Baseline Breast Stage		MSSM *(data as of October 2007)* Follow up Year 1 Breast Stage		KPNC *(data as of October 2007)* Follow up Year 1 Breast Stage	
	B1	**B2+**	**B1**	**B2+**	**B1**	**B2+**
Physical activity	**N = 253**	**N = 71**	**N = 167**	**N = 92**	**N = 224**	**N = 38**
Pedometer steps per day	10,087	9,311	10,308	10,026	11,074	9,887
Dietary intake	**N = 273**	**N = 76**	**N = 159**	**N = 86**	**N = 341**	**N = 64**
Sum of isoflavones (μg/d)	738	500	343	328	1911	643**
Biochanin A	27	11.1	9.6	2.3	54	46
Coumestrol	46	48.2	49	55.9	27	19
Daidzein	263	156	108	91.7	741	309**
Formononetin	6.4	9.6	2.8	10.2	2.0	0.9
Genistein	335	244	159	153	886	402**
Glycitein	60.6	30.9	14.4	15.1	201	65**
Energy (kcal/d)	1458	1481	1407	1496	1542	1633*

No comparisons differed for MSSM, $P > 0.2$; KPNC: $^{**}P > 0.01$ $^{*}P = 0.05$, energy adjusted.

TABLE D4. Distribution of Phthalate, Phenol, and Phytoestrogen Biomarker Concentrations in Urine of Girls at Three BCERC Epidemiology Project Sites Combined, as of September 25, 2008

Analyte	N	LOD	% >LOD	Range (low-high) µg/L	Median µg/L	Median µg/g Cr
Phthalate monoesters						
LoMWP*	1227	.	.	0.0 – 4925.9	2.96	1.28
MEP	1227	0.70	100.00	2.0 – 17500.0	98.00	138.16
MBP	1227	0.60	99.43	0.3 – 6330.0	37.40	49.96
MiBP	1227	0.30	98.45	0.2 – 988.0	11.50	14.78
MCPP	1227	0.20	99.10	0.1 – 585.0	5.20	5.46
DEHP**	1227	.	.	0.0 – 2482.0	1.53	0.62
MECPP	1227	0.60	99.92	0.4 – 2780.0	57.40	77.82
MEHHP	1227	0.70	99.76	0.5 – 1860.0	36.70	48.96
MEHP	1227	1.20	81.99	0.6 – 358.0	3.70	4.57
MEOHP	1227	0.70	99.59	0.5 – 1066.5	23.20	30.20
HiMWP***	1227	.	.	0.0 – 2492.7	2.02	0.86
MBzP	1227	0.30	99.59	0.1 – 2790.0	22.90	30.21
Phenols						
Benzophenone-3	1227	0.40	98.61	0.2 – 46100.0	27.50	18.45
*bis*Phenol A	1227	0.40	95.03	0.3 – 116.0	2.20	2.68
2,4-Dichlorophenol	1227	0.20	93.07	0.1 – 642.0	1.00	1.81
2,5-Dichlorophenol	1227	0.20	98.29	0.1 – 27200.0	9.00	25.55
Butyl Paraben	1137	0.20	50.66	0.1 – 901.0	0.20	0.36
Methyl Paraben	1137	1.00	99.65	0.7 – 8390.0	60.60	79.16
Propyl Paraben	1137	0.20	94.55	0.1 – 2360.0	7.70	9.48
Triclosan	1227	2.30	83.29	1.6 – 4550.0	13.60	18.11
Phytoestrogens						
Daidzein	1228	0.30	100.00	1.3 – 29500.0	95.15	101.49
Enterolactone	1228	0.30	100.00	2.1 – 18200.0	447.00	451.96
Genistein	1228	0.30	100.00	0.4 – 13900.0	43.25	47.17

* LoMWP is the molar sum of MEP, MBP, MiBP, MCPP.
** DEHP is the molar sum of MECPP, MEHHP, MEHP, MEOHP.
*** HiMWP is DEHP + MBzP.

TABLE D5. Distribution of Serum Organohalogen Biomarker Concentrations (ng/g lipid) for KPNC Girls, Including PBDEs, PCBs, and Organochlorine Pesticides, as of November 2008.

Analyte	N	N <LOD (%)	Median	Min	Max
Polybrominated Diphenyl Ether Congeners					
PBDE28	343	49 (14.3%)	2.0	0.28	33.2
PBDE47	343	5 (1.5%)	45.3	1.13	855.0
PBDE85	343	103 (30.0%)	0.9	0.28	16.5
PBDE99	343	6 (1.7%)	10.4	0.64	382.0
PBDE100	343	4 (1.2%)	10.7	0.42	154.0
PBDE153	343	4 (1.2%)	15.2	1.34	220.0
PBDE154	342	67 (19.6%)	1.1	0.28	31.8
Polychlorinated Biphenyl Congeners					
PCB74	342	115 (33.6%)	2.4	0.64	36.5
PCB99	344	21 (6.1%)	1.9	0.28	17.3
PCB105	344	135 (39.2%)	0.7	0.21	10.7
PCB118	344	13 (3.8%)	2.9	0.35	39.4
PCB146	344	125 (36.3%)	1.1	0.21	26.6
PCB156	344	122 (35.5%)	1.4	0.21	60.5
PCB170	344	68 (19.8%)	2.75	0.21	47.4
PCB180	344	19 (5.5%)	6.65	0.28	133.0
PCB187	343	100 (29.2%)	1.8	0.21	36.4
PCB138_158	344	13 (3.8%)	7.2	0.35	126.0
PCB196_203	344	125 (36.3%)	0.95	0.21	44.3
Organochlorine Pesticides					
HCB	342	14 (4.1%)	9.6	1.98	62.4
p,o'_DDE	343	3 (0.9%)	165.0	32.2	8010.0
t-nonachlor	343	89 (25.9%)	6.7	0.35	55.0
Oxychlordane	343	132 (38.5%)	4.5	0.35	53.6

Data include pilot data, <LOD values imputed as LOD/√2).

TABLE D6. Distribution of Serum Perfluorinated Compound Biomarker Concentrations (ng/mL) for KPNC and Cincinnati Girls Combined, as of 11/03/2008.

Analyte	N	%>LOD	Range (low-high)	Median	Geometric Mean (GSD)
Et-PFOSA-AcOH	615	15	<LOD – 3.1	<LOD	0.0 (27.9)
Me-PFOSA-AcOH	615	96	<LOD – 16.8	0.80	0.6 (7.7)
PFDeA	528	75	<LOD – 1.2	0.30	0.0(55.6)
PFHxS	612	99.8	<LOD – 192.0	3.30	3.8 (3.2)
PFNA	615	99.8	<LOD – 15.5	1.50	1.5 (1.9)
PFOSA	615	16	<LOD – 1.6	<LOD	0.0 (16.5)
PFOS	615	99.8	<LOD – 104.0	13.40	13.5 (2.1)
PFOA	615	99.8	<LOD – 55.9	6.40	6.7 (2.0)

Table D7. Detailed Questionnaire Content, Clinical Exam Content, and Other Special Procedures

Center	MSSM				KPNC				Cincinnati							
Data Collection	Baseline Y01	Y02	Y03	Y04	Baseline Y01	Y02	Y03	Y04	Baseline Y01		Y02		Y03		Y04	
									a	b	a	b	a	b	a	b
Questionnaire Domains																
Demographic information	X				X					X						
Socioeconomic status	X	X	X	X	X	X	X	X		X		X		X		X
Physical activity	X	X	X	X	X	X	X	X		X		X		X		X
Product use	X	X	X	X	X	X	X	X		X		X		X		X
Environmental exposures	X	X	X	X	X	X	X	X		X		X		X		X
Health history	X	X	X	X	X	X	X	X		X		X		X		X
Household characteristics	X	X	X	X	X	X	X	X		X		X		X		X
Neighborhood characteristics	X	X	X	X		X	X	X				X		X		X
Residential and school history	X	X	X	X	X	X	X	X		X		X		X		X
Psychosocial assessments		X			X	X	X	X		X		X		X		X
Family environment		X			X	X	X	X		X		X		X		X
Clinical Exam Components																
Height	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Waist, hip circumference	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Skinfolds									X	X	X	X	X	X	X	X
Bioelectrical impedance analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tanner staging	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood pressure	X	X	X	X					X	X	X	X	X	X	X	X
Biospecimen Collection																
Blood (for biomarkers and DNA)	X*	X*	X*	X*	X	X*	X*		X	X	X	X	X	X	X	X
Blood (for endogenous factors**)									X	X	X	X	X	X	X	X
Urine (for biomarkers)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine (for endogenous factors***)					X*	X	X	X								
Saliva (Oragene™ kit for DNA)			X	X	X			X								
Buccal swabs (for DNA)	X	X														
Other Procedures																
Pedometer log	X	X*	X*	X*	X	X					X		X		X	
24-hour dietary recall	X	X*	X*	X*	X	X*			X	X	X	X	X	X	X	X

* Assessment or collection among a subset of participants.
** Includes hormones, lipids, and other factors associated with insulin resistance or metabolic syndrome.
*** For hormones.

Table D8. Environmental Agents Assayed by the Centers for Disease Control and Prevention and Major Sources of Exposure

Agent	Abbreviation	Major Sources of Exposure
Urinary Biomarkers		
Phthalate monoesters		
Monoethyl phthalate	MEP	Metabolite of diethyl phthalate (DEP); Shampoo, scents, soap, lotion, cosmetics, industrial solvent, medications
Monobutyl phthalate	MBP	Metabolite of dibutyl phthalate (DBP); Adhesives, caulk, cosmetics, industrial solvents
Mono-isobutyl phthalate	MiBP	Metabolite of di-isobutyl phthalate (DiBP); Adhesives, caulk, cosmetics, industrial solvent
Mono(3-carboxypropyl) phthalate	MCPP	Metabolite of di-n-octyl phthalate (DnOP); Soft plastics
Mono(2-ethylhexyl) phthalate	MECPP	Metabolite of diethylhexyl phthalate (DEHP); Soft plastics including tubing, toys, home, products, food containers, packaging film, especially polyvinyl chloride (PVC, as sometimes present in clear food wrap)
Mono(2-ethyl-5-oxohexyl) phthalate	MEHHP	Metabolite of DEHP
Mono(2-ethyl-5-carboxypentyl) phthalate	MEHP	Metabolite of DEHP
Mono(2-ethyl-5-hydroxyhexyl) phthalate	MEOHP	Metabolite of DEHP
Monobenzyl phthalate	MBzP	Metabolite of benzylbutyl phthalate (BzBP); Vinyl flooring, adhesives, sealants, industrial solvent
Phenols		
Benzophenone-3	BP-3	Sunscreen
*bis*Phenol A	BPA	Polycarbonate containers and coatings (cans, cups), dental sealant
2,4-Dichlorophenol	2,4-DCP	Herbicide
2,5-Dichlorophenol	2,5-DCP	Metabolite of 1,4-DCB; Mothballs, room deodorizers
Butyl Paraben	B-PB	Preservative in personal care products
Methyl Paraben	M-PB	Preservative in personal care products
Propyl Paraben	P-PB	Preservative in personal care products
Triclosan	TRCS	Microbicide in cleaning fluids, including hand sanitizers
Phytoestrogens		
Daidzein	DAZ	Isoflavone in selected plant foods, especially soy products, including soy added to processed meats, meat substitutes, breads, and protein food bars
Enterolactone	ENL	Metabolite of lignans in selected plant foods (e.g., rye, flax seeds)
Genistein	GNS	Isoflavone in selected plant foods, especially soy products, including soy added to processed meats, meat substitutes, breads, and protein food bars
Cotinine		Metabolite of nicotine; tobacco
Blood Biomarkers		
Brominated Flame Retardants (BFRs), including polybrominated diphenyl ether (PBDE) congeners		Generally, exposure from dust, but now found in air and water, as well as diet. Commercial products are mixtures of congeners.
2,2′4-tribromodiphenyl ether	BDE17	
2,4,4′-tribromodiphenyl ether	BDE28	
2,2′,4,4′-tetrabromodiphenyl ether	BDE47	
2,3′,4,4′-tetrabromodiphenyl ether	BDE66	
2,2′,3,4,4′-pentabromodiphenyl ether	BDE85	"Penta" product usually contains BDE-47, -99, -100, -153, and -154 (plus low levels of hexa-, trace levels of tri- and hepta-BDEs); Used in polyurethane in furniture foam and carpet padding, mostly in U.S.; use now being phased out.
2,2′4,4′,5-pentabromodiphenyl ether	BDE99	
2,2′,4,4′,6-pentabromodiphenyl ether	BDE100	

Table D8. Environmental Agents Assayed by the Centers for Disease Control and Prevention and Major Sources of Exposure *(continued)*

Agent	Abbreviation	Major Sources of Exposure
2,2′,4,4′,5,5′-hexabromodiphenyl ether	BDE153	"Octa" product contains hexa- to nona-brominated congeners; Used in hard plastics, such as TV and computer casings.
2,2′,4,4′,5,6′-hexabromodiphenyl ether	BDE154	
2,2′,3,4,4′,5′,6-heptabromodiphenyl ether	BDE183	
2,2′,4,4′,5,5′-hexabromobiphenyl	BB153	Older BFR, production discontinued in mid-1970's
Polychlorinated Biphenyl Congeners (PCBs)		Generally, PCBs were used in electrical insulating and heat-exchange fluids, such as in transformers. Banned in U.S. after 1979, but still found in the environment (and wildlife). Exposure currently from diet, as PCBs concentrate in high-fat foods such as dairy, eggs and animal fat, some fish. Infants exposed from breast-feeding as well.
2,4,4′-trichlorobiphenyl	PCB28	Many congeners are not commonly detected in serum samples.
2,2′,3,5′-tetrachlorobiphenyl	PCB44	
2,2′,4,5′-tetrachlorobiphenyl	PCB49	
2,2′,5,5′-tetrachlorobiphenyl	PCB52	
2,3′,4,4′-tetrachlorobiphenyl	PCB66	
2,4,4′,5-tetrachlorobiphenyl	PCB74	
2,2′,3,4,5′-pentachlorobiphenyl	PCB87	
2,2′,4,4′,5-pentachlorobiphenyl	PCB99	
2,2′,4,5,5′-pentachlorobiphenyl	PCB101	
2,3,3′,4,4′-pentachlorobiphenyl	PCB105	
2,3,3′,4′,6-pentachlorobiphenyl	PCB110	
2,3′,4,4′,5-pentachlorobiphenyl	PCB118	
2,2′,3,3′,4,4′-hexachlorobiphenyl	PCB128	
2,2′,3,4′,5,5′-hexachlorobiphenyl	PCB146	
2,2′,3,4′,5′,6-hexachlorobiphenyl	PCB149	
2,2′,3,5,5′,6-hexachlorobiphenyl	PCB151	
2,2′,4,4′,5,5′-hexachlorobiphenyl	PCB153	
2,3,3′,4,4′,5-hexachlorobiphenyl	PCB156	
2,3,3′,4,4′,5′-hexachlorobiphenyl	PCB157	
2,3′,4,4′,5,5′-hexachlorobiphenyl	PCB167	
2,2′,3,3′,4,4′,5-heptachlorobiphenyl	PCB170	
2,2′,3,3′,4,5,5′-heptachlorobiphenyl	PCB172	
2,2′,3,3′,4′,5,6-heptachlorobiphenyl	PCB177	
2,2′,3,3′,5,5′,6-heptachlorobiphenyl	PCB178	
2,2′,3,4,4′,5,5′-heptachlorobiphenyl	PCB180	
2,2′,3,4,4′,5′,6-heptachlorobiphenyl	PCB183	
2,2′,3,4′,5,5′,6-heptachlorobiphenyl	PCB187	
2,2′,3,4,4′,5′-hexachlorobiphenyl and 2,3,3′,4,4′,6-hexachlorobiphenyl	PCB138-158	

Table D8. Environmental Agents Assayed by the Centers for Disease Control and Prevention and Major Sources of Exposure *(continued)*

Agent	Abbreviation	Major Sources of Exposure
Persistent Organochlorine Pesticides		All are persistent in the environment and concentrate in lipid, so still found in diet, breast milk.
Hexachlorobenzene	HCB	Used primarily as fungicide until 1984.
β-Hexachlorocyclohexane	β-HCCH	Technical grade HCCH contains 4 isomers, of which β-HCCH is one. Only lindane has insecticidal activity, while others are fungicidal or by-products of producing lindane. Most uses cancelled in 1985. Lindane is still in limited use for treating seeds pre-planting.
γ-Hexachlorocyclohexane (Lindane)	γ-HCCH	
Oxychlordane	Oxychlor	Metabolite of chlordane, which was used on crops, lawns and in buildings until 1988.
Trans-Nonachlor	t-NONA	Component of technical-grade formulation of chlordane
2,2-bis(4-chlorophenyl)-1,1-dichloroeth-ene	p,p′-DDE	Metabolite of DDT in the body
2-(4-chlorophenyl)-2-(2-chlorophenyl)-1,1,1-trichloroethane	o,p′-DDT	DDT is an insecticide used against mosquitoes, now banned in U.S., but used in other countries against malaria.
2,2-bis(4-chlorophenyl)-1,1,1-trichloro-ethene	p,p′-DDT	
Mirex	same	Used to control fire ants and as flame-retardant additive. Not used in U.S. since 1977.
Perfluorinated Compounds		
2-(N-ethyl-perfluorooctane sulfonamido) acetate	Et-PFOSA-AcOH	By-product of stain- and grease-proof coatings on food packaging, couches, carpets
2-(N-methyl-perfluorooctane sulfon-amido) acetate	Me-PFOSA-AcOH	By-product of stain- and grease-proof coatings on food packaging, couches, carpets
Perfluorodecanoate	PFDeA	By-product of stain- and grease-proof coatings on food packaging, couches, carpets
Perfluorohexane sulfonate	PFHxS	Fire-fighting foams and post-market carpet treatment applications
Perfluorononanoate	PFNA	Produced during the production of stain-resistant carpets, clothing and food packaging
Perfluorooctane sulfonamide	PFOSA	Produced during the production of stain-resistant carpets, clothing and food packaging
Perfluorooctane sulfonate	PFOS	Surface protection of carpets, textiles and leather, coatings on paper, cardboard, food packaging materials
Perfluorooctanoate	PFOA	Surface-active agent in the production of fluoropolymers, used in non-stick cookware; waterproof, breathable textiles; and electronics

Appendix E: Breast Cancer and the Environment Working Group of the National Advisory Environmental Health Sciences Council

Julia G. Brody, Ph.D.
Executive Director
Silent Spring Institute
Newton, MA

Diana Chingos
Young Survival Coalition
Culver City, CA

Dale Eastman
Alamo Breast Cancer Foundation
San Antonio, TX

Suzanne Fenton, Ph.D.
Reproductive Toxicology Division
Environmental Protection Agency
Research Triangle Park, NC

Maria Carolina Hinestrosa, M.A., M.P.H.
National Breast Cancer Coalition
Washington, DC

Marc Hurlbert, Ph.D.
Senior Consultant, Grants and Partnerships
Avon Foundation Breast Cancer Crusade
New York, NY

Marion Kavanaugh-Lynch, M.D., M.P.H.
Director, California Breast Cancer Research Fund
University of California
Oakland, CA

Karen Miller
Huntington Breast Cancer Action Coalition
Huntington, NY

Suzanne Snedeker, Ph.D.
Assistant Professor, Environmental Toxicology and Health
Division of Biological Sciences
Cornell University
Ithaca, NY

Bruce Trock, Ph.D.
Breast Cancer Program
Lombardi Comprehensive Cancer Center
Georgetown University
Washington, DC


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