Breast Cancer and the Environment Research Centers

3rd annual

Early Environmental Exposures Meeting

November 2-3, 2006

Berkeley, California

Summary of scientific presentations

Funding for the Breast Cancer and the Environment Research Centers is provided by the National Institute of Environmental Health Sciences (NIEHS), and the National Cancer Institute (NCI), NIH, DHHS.

Effects of exposure to ionizing radiation provide important clues to breast cancer

Ionizing radiation heightens the risk of breast cancer, and Mary Helen Barcellos-Hoff, Ph.D., of the Lawrence Berkeley National Laboratory, is conducting research to learn more about radiation-induced effects, including breast cancer initiation and progression. She presented "Mechanisms and Actions of Ionizing Radiation as a Breast Carcinogen" at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC.

Barcellos-Hoff's research team is studying how certain exposures to ionizing radiation influences both mammary glands in mouse models and cultures of normal human epithelial cells. "There's a pretty straight-forward dogma about how radiation instigates cancer, which is that ionizing radiation causes DNA damage in individual cells, DNA damage can cause mutations, and these mutations are the precursors to cancer. So over the course of time, those (mutated) cells then expand and increase in number, and acquire additional events that will give them the malignant potential, which is the ability to escape the barriers of the epithelium and move to surrounding tissue." She noted that this progression occurs on the time scale of years to decades, depending on the tissue involved. "With breast cancer, there seems to be pretty good evidence it's about 40 years between exposure and increased risk of developing cancer."

She is especially interested in the target of ionizing radiation, and is placing much of her emphasis on tissue-specific stem cells. Unlike embryonic stem cells, which can develop into a diversity of tissue types, tissue-specific stem cells are restricted to becoming only one kind of tissue. She explained the foundation for her work: "What we are saying is that if radiation causes mutations in stem cells, those are the cells that have the greatest potential to expand and possibly acquire the additional mutations that would lead to cancer in the long run," she said. "That's why we spend so much time trying to identify not only stem cells in the mammary gland, but also where the stem cells are located so we can begin to look at the interaction as those cells acquire more malignant potential."

Specifically, she and her research group believe that carcinogens, like ionizing radiation, not only alter the genomic sequence of the stem cell, but also affect the regulation of the stem cell, including its ability to multiply. "What's clear from a variety of different experimental models of mouse and rat is there are a number of signals that impinge on the stem cell to tell it whether to proliferate, to divide, to stay here, to move, or to differentiate into daughter cells. The idea is that these same signals that regulate stem cells are misregulated during carcinogenesis."

Over the past 15 years, Barcellos-Hoff has identified a certain protein, a cytokine called transforming growth factor-ß 1 (TGF-ß) as one of those signals. Under normal circumstances, the cytokine is important in the development and function of mammary tissue. When exposed to ionizing radiation, however, the cytokine's regulation is altered, and this alteration eventually becomes associated with the progression of cancerous tumors through the initiation of a pathway known as epithelial-to-mesenchymal transition

or (EMT). In this pathway, cells become motile and invasive, which leads to the spread of cancer to other areas of the body.

Barcellos-Hoff and her research group tested the impact of TGF-ß on cancer progression by irradiating stem cells, treating them with active TGF-ß, and monitoring the results. They soon observed disorganized clusters of cells that were indicative of a proliferative effect, she said. In addition, they also found that these cells became very highly motile. She asserted, “The cells irradiated with TGF-ß were undergoing epithelial–to-mesenchymal transition.”

She added, “EMT is important because it is a characteristic of an invasive phenotype that is required to allow cells to escape their epithelial barriers, and to permit the metastatic progression of breast cancer cells and their ability to move out into distant organs. And we think that radiation can actually predispose certain cells to undergo this alteration.”

Interestingly, she said, it is not only these predisposed cells, but also their microenvironment — the reaction of the surrounding stromal cells — that play a part in carcinogenesis. In other words, "The microenvironment dictates the neoplastic potential." This presents another research opportunity, she noted, because if the stromal cells can be reoriented so they revert to their normal behavior, the potential exists to combat malignancy.

Overall, she added, “Ionizing radiation action as a carcinogen allows us to understand the dynamics of radiation-induced effects, and that helps us to understand better what is capable of suppressing cancer in normal tissue.”

Summary of Dr. Barcellos-Hoff’s presentation at the November 2006 BCERC annual meeting: Mechanisms and Actions of Ionizing Radiation as a Breast Carcinogen

Mix of scientists, advocates in COTCs bring research to life

"One of the things that's unique about the four Breast Cancer and the Environment Research Centers (BCERC) is that each of them has a Community Outreach and Translation Core that joins breast cancer and environmental advocates with scientists in the research process, and also comprises the essential link that connects potential users of the research findings with those who generate it," said Janice Barlow, R.N., of Zero Breast Cancer in San Rafael, Calif. Barlow, who is also principal investigator of the Community Outreach and Translation Core (COTC), Bay Area Center, presented "Community Outreach and Translation Cores: Linking Scientists and Communities through the Research Process" at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC.

She described four long-term objectives of the COTCs:

1) to develop and implement strategies to ensure ongoing community input into the research process.
2) to translate the scientific findings from the centers into information that is understandable, relevant and useful for the public and policy makers.
3) to determine whether these strategies are effective; and
4) to develop and communicate "key public health messages" based on research findings and precautionary principles, designed to educate young girls and women who are at high risk for breast cancer about the role of environmental stressors in breast cancer initiation and progression.

"Although the models of community involvement differ in each COTC, all are based on active participation of breast cancer advocacy groups and other relevant community groups in the conceptualization, planning, implementation and dissemination of research findings," she said. For example, breast cancer and environmental advocates have been actively involved in selecting and framing the research agenda ever since the National Institute of Environmental Health Sciences and National Cancer Institute jointly announced the creation of the BCERC in 2003. "Once the grants were awarded to the four centers, the COTCs held community educational forums and town hall meetings, and took other steps to involve community members in the research process."

Now that the research is well under way, Barlow said, the majority of COTCs' activities center around enhancing communications, interaction and dissemination of information about BCERC research activities to the public and policy makers. She noted that COTCs have developed and are continuing to generate newsletters, websites and other educational materials, have helped orchestrate national scientific conferences, and have held local forums and conferences to help participants in the community understand the scientific process and build a foundation of knowledge that will provide a context for new research findings.

In some cases, these educational efforts are designed to reach specific audiences, she said. "The Mount Sinai School of Medicine, for instance, has done a really good job of

targeting many materials to their communities, which are largely Spanish-speaking. Another targeted program here in the Bay Area is the Adolescent Prevention, Risk Reduction and Education Program. The long-term objective of this program was to create a persuasive, developmentally appropriate peer-education model that can be used with adolescents to inform them about breast cancer and breast cancer risk, and to motivate them to adopt healthier lifestyles and minimize their exposures to known and suspected environmental carcinogens."

In addition, the COTCs are beginning to develop a bank of peer-reviewed publications. These publications, the traditional route for scientific findings, not only make findings available to the scientific community, but also help to spur additional research on translational science.

This combined advocate-scientist format of the COTCs is already proving to be a successful one, Barlow said. "The goal of doing research is that it actually gets used." She added, "The centers are built in part to disseminate the study results, so that the research findings will have an impact on policy decision or on intervention programs; they will stimulate the development of new products and technologies; they will advance the understanding of breast cancer; and most importantly, they will bring about positive health benefits in individuals and in communities."

"In this case, the positive health benefits we are seeking are to learn ways to prevent breast cancer, to decrease breast cancer mortality, and to reduce environmental exposures in our communities."

Summary of Ms. Barlow's presentation at the November 2006 BCERC annual meeting: Community Outreach and Translational Cores: Linking Scientists and Communities through the Research Process.

Hormones play a key role in breast cancer

To make better inroads into the prevention of breast cancer, researchers should begin placing their focus on the entire battery of hormones that are involved in reproduction, said Valerie Beral during her closing keynote address, “Hormonal Factors in Breast Cancer” at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC. Beral is the director of the Cancer Research UK Epidemiology Unit in Oxford.

The importance of hormones to breast cancer incidence is unmistakable in what she described as “overwhelming evidence” gleaned from numerous research projects. The Million Women Study is one of them. This joint study between Cancer Research UK and the National Health Service Breast Screening Programme included 1.3 million U.K. women aged 50 to 64. In addition, the Collaborative Group of Hormonal Factors in Breast Cancer, has compiled a wealth of additional data, she said. “The group began about 10 years ago and involves more than 100 studies from about 40 countries, and about 100,000 women with breast cancer and 200,000 controls.”

In one of the major findings from the Million Women Study, Beral and other researchers described a heightened breast cancer risk among women who used hormone replacement therapy (HRT). The risk was even more pronounced with combined HRT, which includes both estrogen and progesterone, she said. “HRT with estrogen alone showed a 30 percent increase (in risk), but combined HRT had a 100 percent increase — a doubling.” The same risk spread occurred whether they tested estrogen delivered by a patch, orally or through other methods, she said. “In every study, combined HRT had a bigger risk than estrogen alone.”

In another key finding, this time from the Collaborative Group on Hormonal Factors in Breast Cancer, data revealed that the addition of hormones in HRT or in oral contraceptives did not pose a permanently high breast cancer risk. Beral explained, “Use of both oral contraceptives and menopausal hormones increased the risk of breast cancer, but only in current and recent users with no persistent effects.” This finding raised more than a few eyebrows, she said, but the data were clear. “There was no persistent effect.”

The great breadth of recent research has also allowed for comparisons between breast cancer incidence in different countries, and not only highlighted an increased risk in women from North America compared to those from Europe, but also pointed out the most likely cause for the disparity: obesity. She explained that a body mass index of 30 or more is considered obese. In comparing studies from both geographic regions, obesity was present in 45 percent of the participants in the North American Women’s Health Initiative study, whereas obesity was only present in 18 percent of participants in the Million Women Study. Obesity amplifies the level of estradiol in a woman’s system, she said, and produces a risk rate equivalent to the use of estrogen-only hormone replacement therapy. “So, body mass index matters in postmenopausal women,” she summarized.

Although many of the studies to date have centered on estrogen and/or progesterone, future research should cast a wider net, she said. “One thing that is really important is that

the effect of childbearing accounts for much of the difference in breast cancer rates around the world," Beral said. Incidence of the disease is about 6 percent in developed countries, where women frequently have no or few children, but only about 1 percent in developing countries, where women have numerous offspring. This ratio is mimicked in historical comparisons between nuns, who remain childless, and non-nuns, she said. While some of this difference is probably attributable to obesity or other causes, she said, "child-rearing is the big, big player in breast cancer."

The data also show a clear and continuing reduction in a woman's risk of breast cancer with each birth and each year of breastfeeding, she said. "Every birth gives a woman about a 10 percent reduction in breast cancer throughout life. Likewise, the longer you breastfeed, the lower the risk of breast cancer, and this, too, is persistent." She noted, "So we've got a pattern of something very profound: Birth only lasts nine months, and yet something that happens over such a short time can give you a persistent effect."

Beral added, "We have to think about this. During pregnancy, it's not just one or two hormones that change. It's a whole slew of hormones. It's very unlikely that it's only estrogen that affects breast cancer risk, so I think we should start looking at other hormones, and should look beyond puberty to childbirth and lactation to understand exactly which hormones involved in reproduction lead to long-term protection against breast cancer."

Summary of Dr. Beral's presentation at the November 2006 BCERC annual meeting: Hormonal Factors in Breast Cancer

Why are girls growing up earlier?

Early sexual maturity among females appears to heighten the risk of developing breast cancer later in life, a finding that carries special concern now that numerous studies show girls are maturing earlier than they have in past generations. At the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC, Frank Biro, M.D., discussed the physical changes and timing of puberty in girls during his talk titled "A Presentation on Puberty to Promote Project-wide Proposals."

Puberty can be traced back much further most people suspect, Biro said. "The first puberty occurs in the third trimester in the fetus. At that time, the fetus already has a functioning LHRH (luteinizing hormone-releasing hormone) pulse generator." The LHRH pulse generator is a group of nerve cells, or neurons in a portion of the hypothalamus in the brain that integrates information about nutritional status, body composition, and other factors. Later in life, the hypothalamus will use this information to direct the pituitary gland to develop the ovaries in females or the testes in males.

This initial activity of the LHRH pulse generator turns off between the ages of six and 12 months, and remains off until the onset of puberty later in life. Much of this inactive phase results from a direct inhibition of the part of the hypothalamus called the medial basal hypothalamus, where the LHRH pulse generator is specifically located. "One of the major inhibitory factors in the brain is a neurotransmitter called GABA, or gamma amino butyric acid," Biro said. "It appears from some studies in animals that it is the reduction in the inhibition of the medial basal hypothalamus that is the critical factor leading to the reactivation of the pulse generator to initiate puberty."

When the pulse generator turns back on, a girl's body begins a rapid sequence of changes, he said. One of the first and most noticeable differences is the acceleration of height gain. "Before puberty, the child is growing between 3 to 4.5 cm a year, but once she hits puberty, growth really takes off. This is because of stimulation of the sex hormones, growth hormone, as well as some other factors on the skeleton, and it occurs well before she has what is traditionally called the onset of puberty, which is the appearance of secondary sexual characteristics."

Biro outlined the four primary stages of puberty and their associated changes in the order in which they occur:

1) Adrenarche —the activation of the adrenal medulla, a portion of the adrenal gland that produces adrenal androgens, which help serve as the building blocks for the male and female sex hormones testosterone and estrogen
2) Pubarche — the appearance of pubic hair
3) Thelarche — the appearance of breast tissue
4) Menarche — the first occurrence of menstruation.

Current studies suggest that a younger-age menarche can heighten the risk for the eventual development of breast cancer, he said. While the major factors in the onset of the pubertal stages are genetic or heritable in nature, he noted that exposure to certain

chemicals in the environment may also hasten development. These comprise a long list of endocrine disruptors, which range from “androgen skin cream and shampoo with placenta extract” to “a DDT-like pesticide that was found in the Everglades and was linked to feminization of male alligators,” he said. Exposure to other chemicals may also have connections to breast cancer. He pointed out several candidates, including a group of chemicals known as polybrominated biphenyls (PBBs), which until 1976 were manufactured as fire retardants in plastics. Research has shown that PBB exposure can cause liver cancer in mice and rats, and the U.S. Department of Health and Human Services has identified it as a potential human carcinogen.

Not all exposures are bad, he noted. Some, like certain foods, may have a protective effect. An example is a diet high in phytoestrogens. Research suggests that these estrogen-like plant compounds may effectively block human estrogen from taking action in the body. Since a high level of estrogen activity is associated with earlier puberty and an increased breast cancer risk, phytoestrogens may be able to counter these effects.

Although research studies have already made many connections between environmental exposures and breast cancer, much additional work is needed, he said. The BCERC is answering the call with a large, longitudinal study that will follow more than 1,000 6-, 7-, and 8-year-old girls as they grow and mature. “The study is being done at three different sites: New York City, Cincinnati, and the San Francisco Bay Area,” he said. “We’re looking at nutritional intake and physical activity, we’re asking families to fill out product-use surveys, we’re evaluating psychosocial factors in these girls, and we’re getting specimens on which to do genetic analyses.” The specimens from some of the sites will also provide data on levels of various hormones as well as growth factors in the girls. There are parallel studies in the laboratory investigating some of these issues, at UAB, Fox Chase Cancer Center, University of Cincinnati, Michigan State University. and UCSF.

Together, information gathered from the three-site study will help researchers to study many aspects of the progression toward maturity, including pertinent trends, and connections between lifestyles, environmental exposures, and pubertal development, Biro said. He concluded, “Through this study, we hope to look at the interactions between the many contributing factors to get a better idea of why girls are growing up earlier.”

Summary of Dr. Biro’s presentation at the November 2006 BCERC annual meeting: A Presentation on Puberty to Promote Project-wide Proposals

Where are the mammary gland stem cells and what do they look like?

"Stem cells may be targets for carcinogenesis and we want to know how stem cells are affected by carcinogens. The first thing we need to know is where those cells are located," said Rodrigo Fernandez-Gonzalez, Ph.D., of the Cancer Biology Department at Lawrence Berkeley National Laboratory. He presented "Mouse Mammary Gland Stem Cells: Location, Location, Location!" at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC.

To pinpoint the stem cells' exact whereabouts, he is using a method based on an understanding of how the cells reproduce. Most cells reproduce by doubling their DNA and then randomly divvying it up, so half goes to each of two daughter cells. This works perfectly as long as the DNA's doubling is error-free. If it is not, a mutation may occur. Adult stem cells reproduce differently. Instead of doubling and then randomly distributing their DNA, they retain the intact DNA template. Not only does this greatly minimize the possibility of passing mutations on to their progeny, it also provides a method for determining which cells are adult stem cells.

It works like this: By labeling the parental, template DNA in a cell with an easily identifiable chemical marker, scientists can check the progeny to see whether 1) the marker eventually becomes so diluted that it is undetectable, as would occur with randomly dividing DNA; or 2) the marker is still present, as would happen if the cells conserve the DNA template. Scientists call the latter "label-retaining cells," or LRCs, because they hold onto the chemical label. Fernandez-Gonzalez remarked, "Label-retaining cells have been used to characterize adult stem cells in several tissues, like small and large intestine, epidermis, prostate, muscle."

His research group is using novel computational microscopy techniques to find label-retaining cells, and therefore putative stem cell populations, in mammary tissue, and is also working to characterize the differences between label-retaining cells and already-differentiated mammary epithelial tissue.

The researchers have learned or confirmed several details about label-retaining cells. For example, they have found that mammary label-retaining cells have a distinct set of features that set them apart from already-differentiated mammary epithelial cells. For one thing, the label-retaining cells' nuclei are smaller than those of the differentiated cells, and they have a dissimilar shape, he said. "LRC nuclei are more elliptical than non-LRC ones," he said. In addition, the research group compared the texture of the chromatin (a mass of DNA and proteins inside the nucleus) and found that LRC chromatin was less rigidly patterned than that of the differentiated mammary epithelial cells. He described, "We found that the chromatin pattern is more heterogeneous in the label-retaining cells."

They have also discovered where most of the label-retaining cells are located. A clustering of label-retaining cells resides in larger ducts of mouse mammary gland tissue, Fernandez-Gonzalez said, and most of them occur in one specific area. The researchers found a higher number of the label-retaining cells — two to four times more — in portions of ducts nearer the nipple compared with portions more distant, suggesting that adult stem cells are located in the proximity of the nipple, the site of embryonic origin of the gland.

Other results, including cell transplantation experiments in mice, appeared to corroborate this

notion, he said. "We can surgically remove the epithelial bud (the developing ductwork) from the breast tissue when the animal is three weeks old, creating a tissue without epithelium. This is called a cleared fat pad. Amazingly, and attesting to the plasticity of epithelial cells, this tissue can be injected with mammary epithelial cells from another animal at any point in the animal's life, and the new donor cells will repeat the developmental pattern, grow and fill the fat pad."

For the next steps, they removed mammary gland tissue from other mice, divided it into fragments that were either proximal to or distal from the nipple, and transplanted it into the cleared fat pads, he explained. "Among proximal epithelial cells, one in 6,000 could grow a mammary gland. In the distal tissue, only one in 15,000 cells had that potential," Fernandez-Gonzalez said. Since stem cells carry the instructions and are therefore required for the production of new mammary gland tissue, he said, "This confirms the presence of a proximal stem cell zone."

The findings and the methodology behind them should be especially useful to the many research groups that hope to decipher the role played by adult stem cells in the development of breast cancer.

Summary of Dr. Fernandez-Gonzalez's presentation at the November 2006 BCERC annual meeting: Mouse Mammary Gland Stem Cells: Location, Location, Location!

Animal studies are valuable sources of information

Animal studies can provide insight that human studies cannot, because scientists can precisely control even the often-minute details of the animals' lives. "In animal studies, we're able to bypass the extremely complicated interactions between risk factors that you've seen in human epidemiological studies and get causal information by doing experimental studies," said Mari Golub, Ph.D., of the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment and the University of California, Davis.

She described how this research has helped shed light on chemical exposures and their effect on sexual development in her talk, "Environmental Agents and the Timing of Puberty: Information from Animal Studies," at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC.

"It has been established that early menarche is a risk factor for breast cancer — both for incidence and for mortality — while late menarche is protective," she said. "There has been a lot of concern about the temporal trend in breast cancer incidence and also the temporal trend in chemical exposure, with the idea being that environmental chemicals are linked to earlier menarche and this could in turn influence breast cancer rates." Through animal studies, Golub said, researchers are taking a closer look at the influence of chemicals, and especially those that disrupt the normal hormonal (endocrine) system that regulates female development.

In rodents, which are some of the most commonly used laboratory animals, researchers identify puberty by a measure known as vaginal opening, she explained. "In rats, the vaginal membrane is a membrane that covers the orifice of the vagina. This membrane dissolves at puberty and is no longer present, and this dissolution of the epithelial membrane covering the vaginal opening is directly under the influence of estrogen," she said. This measure serves as a definitive marker of the onset of puberty in many mammals, although she remarked that it is not present in humans.

In fact, she said, the U.S. Environmental Protection Agency uses rodent vaginal opening as an assay for onset of puberty in their standard toxicology protocols for various chemical substances. To validate rodent vaginal opening as a pubertal assay, she added, researchers have shown that estrogens and other substances can change the timing of puberty.

She pointed out several rodent studies that have recorded the timing of the onset of puberty in the presence of various chemical exposures, and listed some of their results. "Estrogenic agents all accelerated vaginal opening, meaning that it occurred earlier," she said. These agents include estradiol, the predominant form of estrogen produced by the ovaries in women; tamoxifen, which is used in breast cancer treatments; and diethylstilbestrol (DES), a therapeutic hormone sometimes administered to girls to limit height growth deemed to be a psychosocial drawback. In addition, naturally occurring

plant estrogens, or phytoestrogens, had a similar effect on pubertal timing. She said, "Two of the phytoestrogens, genistein (from soy) and flaxseed oil, both accelerated vaginal opening. Now you have to remember that the vaginal membrane is directly responsive to estrogen, so whether this represents an opening of the vaginal membrane only, or a real acceleration of all the steps of puberty, it is difficult to know from this data."

Beyond the onset of puberty, some researchers are now becoming interested in whether estrogens also lead to earlier onset of the estrous cycle or ovarian cycling, or to more regular estrous cycles over the lifespan. She said, "These are some of the features that are associated with breast cancer in humans." Already, studies have indicated a correlation between earlier onset of estrous cycling in rats and exposure to either DES or tamoxifen, she said.

Despite the significance of rodent studies, she said, they can only go so far. "Do we have vaginal opening in humans? No. Can we biologically extrapolate from vaginal opening to menarche? Not really." Golub and her research group are beginning to close that gap with an animal model that more closely approximates humans. They studied how monkeys' menstrual cycles and pubertal growth were altered when the animals were treated in the peripubertal period (six months before and after the expected age at menarche) with DES and another estrogenic agent called methoxychlor. "We found that DES delayed the age of first menses in nonhuman primates, and the days with menses were also shortened from 16 days to 9 days," she reported. Methoxychlor had similar, but smaller effects.

Each of these studies, whether on rodents or primates, have provided important information about the impact environmental chemicals can have on the onset of puberty, she said, and may also provide a valuable understanding of the effect of various substances on human puberty and possibly breast-cancer risk.

Summary of Dr. Golub's presentation at the November 2006 BCERC annual meeting: Environmental Agents and the Timing of Puberty; Information from Animal Studies

BCERC investigators and their colleagues work together to reach broad goals

Transdisciplinary science is at the core of the Breast Cancer and Environment Research Centers (BCERC) research efforts, and is combining the widely ranging expertise of scientists and advocates to work toward mutual goals and outcomes. They are:

1. to study the impact of prenatal-to-adult environmental exposures that may predispose a woman to breast cancer;
2. to investigate mammary gland development in animals and young girls to determine vulnerability to environmental agents in the pre-pubertal period that may influence breast cancer development in adulthood; and
3. to develop public health messages designed to educate young girls and women who are at high risk of breast cancer about the role(s) of specific environmental stressors in breast cancer and how to reduce exposures to them.

Robert A. Hiatt, M.D., Ph.D., of the University of California, San Francisco provided an overview of the BCERC and its work during his talk, "Introduction, Goals, and Transdisciplinary Nature of BCERC" at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC.

He began by describing those participating in transdisciplinary science as collaborators who "work jointly using a shared conceptual framework that draws together discipline-specific theories into a new synthesis of concepts, methods, measures and approaches to address a common problem."

Through its four centers at the University of Cincinnati, Fox Chase Cancer Center, the University of California, San Francisco, and Michigan State University, the BCERC has embraced transdisciplinary science, Hiatt said. Using the example of the Bay Area BCERC, he showed a listing of researchers and advocates from many institutions and agencies who are bringing to the BABCERC effort their expertise in such disciplines as developmental biology, genetics, cell biology, epidemiology (ranging from nutrition/diet, minority studies/diet, physical activity, and reproductive, environmental, and genetic areas), pediatric endocrinology, oncology, biostatistics, community advocacy in nursing or law, and public health. This interdisciplinary mix is repeated with only slight variation in the other three centers.

"This type of collaborative, multicenter research can often accomplish objectives not possible in one center," he said, noting that a single institution may not have the diversity of expertise required to tackle broad research questions. "Collaborators learn from one another, proposals are often stronger if based on partnerships, and transdisciplinary science, by crossing disciplinary boundaries, is likely to generate questions not considered within a discipline," he explained.

With all of its benefits, however, transdisciplinary science does present its own set of challenges, Hiatt said. Different institutional and disciplinary cultures, varying personal and institutional agendas, paperwork and bureaucratic inconsistencies, and logistical issues all can add difficulty to projects that include multiple researchers, and multiple institutions or agencies. Nonetheless, he said, researchers and funding agencies recognize that transdisciplinary science is the wave of

the future. In fact, many funding agencies already appreciate its great potential and are demanding such collaborations in grant proposals.

The opportunities for transdisciplinary science are particularly notable in three major hypotheses relating to the goals and outcomes of the BCERC, Hiatt said. The three are:

1. Endogenous hormones (those produced by the body) that are associated with adiposity, or fat-associated weight, in pre-pubertal girls stimulate breast development and predict the occurrence of an earlier first menstrual period.
2. Exogenous environmental factors, especially endocrine disruptors (substances that interfere with endogenous hormones) and hormonally active agents, influence puberty and consequently breast carcinogenesis. Endocrine disruptors are in the body.
3. Multipotent stem cells in the breast, at least in adult mice, are targets for radiation carcinogenesis. (A multipotent stem cell can give rise to one type of cell. This differs from a pluripotent stem cell, which is able to develop into numerous types of cells.)

In addressing these hypotheses and related topics, Hiatt said, BCERC investigators and their colleagues continue to engage in transdisciplinary science. Many of those researchers were on hand at the annual meeting to discuss their work. He added, "This meeting will demonstrate our multiple and increasing integrated approaches to the problem of understanding environmental influences on puberty that may lead breast cancer in adult life."

The 2006 annual meeting was the BCERC's third. The 2007 meeting will be held in Cincinnati. Details of that meeting will be posted at www.bcerc.org as they become available.

Summary of Dr. Hiatt's presentation at the November 2006 BCERC annual meeting: Introduction, Goals and Transdisciplinary Nature of BCERC

A new study has begun

Do plant estrogens, like soy, have an impact on puberty?

"A one-year decrease in age at menarche is associated with a 10 percent increase in breast cancer risk," said Pamela Horn-Ross, Ph.D., of the Northern California Cancer Center. "This is generally attributed to the earlier establishment of regular ovulatory cycles and increased estrogen levels."

Horn-Ross explained how phytoestrogens, which are plant forms of estrogen, may impact menarche in her talk "Phytoestrogen Exposure and Pubertal Development" at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC.

While genetics may explain some of the ethnic differences that occur in average age of menarche, other factors clearly are at play, she said. Studies now suggest that physical activity, body fat, and height are some of those factors. Diet — including phytoestrogens — may also play a role. Phytoestrogens are weak estrogens that can bind or plug into, the sites on cells (called receptors) where estrogen would normally reside, she said. "Phytoestrogens can have anti-estrogenic effects, because they can competitively bind to the estrogen receptors and therefore diminish binding by stronger endogenous (naturally occurring) estrogens. This means that they bind to the estrogen receptors, but they don't produce the estrogen's effects and thus may reduce breast cancer risk."

Phytoestrogens come in two major types, isoflavones and lignans, Horn-Ross said. "Isoflavones are predominantly found in soy beans; soy-based foods are generally consumed with greater frequency in Asian populations. Lignans are more ubiquitous in the non-Asian populations and have sources predominantly in whole grains, nuts, fruits, vegetables and legumes."

"We don't know much about the effects of soy exposure in puberty, but we do know something about soy exposure and adult breast cancer risk," she said. "Most of the studies have been with Asians or Asian-Americans, and they are fairly consistent in suggesting that the greater the soy consumption, the lower the breast cancer risk." The research on non-Asian women is less conclusive. In many of these studies, results indicate no connection between soy and reduced risk, she said.

Horn-Ross is hoping to help clarify the understanding of soy's impact on women's health through her project, which is designed to explore whether isoflavone consumption affects onset of menarche and pubertal development, to determine if certain forms of hormone-controlling genes influence the onset of menarche; and to establish a data resource for future research.

She and her research group have already enrolled 230 girls aged 10 to 13 in their study, determined the girls' isoflavone consumption — less than 4 mg per day (the standard Western diet), or 12 or more mg per day (the typical Asian-American diet), and gathered such data as height, body fat, body fat distribution, physical activity and other lifestyle factors through interviews with the girls and their mothers. They have also collected blood, DNA and urine

samples. “In addition, every month between interviews, the girls will fill out a short questionnaire and keep a journal of their menstrual cycles once they begin,” she said.

The researchers will perform full analyses of the collected data in the coming months, but Horn-Ross was able to divulge some of their preliminary results. “Based on just the first 160 girls enrolled and adjusting for all the pertinent factors, such as body composition, we have seen no effect between isoflavone level and earlier onset of menarche,” she reported. She and her research group have, however, found that girls who have a body type that is proportioned with larger hips and smaller waist rather than the opposite have earlier menarche, and also confirmed long-established reports linking greater height and increased body fat with an earlier first period. In addition, she said, “Although we are not seeing an association between soy consumption and breast development, we are seeing that less isoflavone exposure is associated with delayed public hair growth during pubertal development.”

She cautioned that these results are based on less than 70 percent of the girls recruited for the study, and that these preliminary findings may not pan out as the girls who joined the study later are different in terms of soy consumption and ethnicity than the girls joining earlier. She hopes to report the cross-sectional results of her full study in the coming months. The more powerful longitudinal analyses will be available after the end of the two-year follow-up period.

Summary of Dr. Horn-Ross’s presentation at the November 2006 BCERC annual meeting: Phytoestrogen Exposure and Pubertal Development

BCERC research provides insight into breast, breast cancer risk

Through the research at the four Breast Cancer and the Environment Research Centers (BCERC), the scientific community is increasing its knowledge about the breast and the risk for breast cancer, about the potential impact on both from environmental chemicals and other substances. Four researchers from the BCERC described some of the ongoing work during a joint presentation, "Role of Endogenous and Environmental Agents on Mammary Gland Development and Cancer Susceptibility," at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC.

BCERC/MSU

"One of the things that we've been doing is trying to understand how the two hormones progesterone and estrogen function in the normal mammary gland," said Sandra Haslam, Ph.D., from the Michigan State University center, or BCERC/MSU. "We have looked at which genes are regulated by progestin in the pubertal vs. the adult mammary gland; we've looked at progestin action in two different animal models, the rat and the mouse; and we are interested in genetic factors that determine susceptibility." As a result of their studies, they have identified previously unknown genes that "are regulated by progestins in both the pubertal and the adult mammary gland and are related to ductal development, carcinogen activation and the immune response," she said. "Maybe as we study these further, we will have additional insight into that window of susceptibility in the pubertal gland."

They are also investigating the surprisingly large number of genes with connections to calcium, she remarked. "We're not exactly sure how this is important in normal development and function, but it's an interesting new set of genes that we had not known about before."

Other animal studies have also proved intriguing, she said. "The current thinking is that estrogen is required to get a progesterone response, but we found that when we have progesterone all by itself, it has a very potent proliferative effect on the mammary gland." She noted that the magnitude of this effect is determined in part by genes, so genetic variation may cause the breast to respond differently and therefore increase or decrease cancer susceptibility.

The BCERC/MSU is continuing its wide range of animal research, she said. "Our studies of two different species – the rat and the mouse – have given us some really interesting and novel insights that may be very relevant to understanding progesterone action in the human breast."

UCBCERC

At the University of Cincinnati center (UCBCERC), Deborah Clegg, Ph.D., is mainly exploring whether factors in the human diet, specifically different types of fatty acids, alter the susceptibility to carcinogenesis. "We're looking at fatty acids, obesity and carcinogenesis to see if there's some level of interaction between them. Using rats, we are

exploring the contribution of the maternal diet on her offspring and what impact this has on the offspring's susceptibility to carcinogenesis. To do this, we are exposing the mothers to one of nine different diets to see if her diet leads to increased carcinogenesis in her daughters." Clegg's research group is testing diets that are high in the following types of fat: olive oil, safflower oil, butter oil, or fish oil (omega-3 fatty acids). The diets are either high in fat, (40 percent of the calories coming from fat, similar to the typical American diet), or low in fat (20 percent of the calories coming from fat). These diets are compared to the standard, "rat chow," which is also low in fat.

"Our overall hypothesis is that maternal exposure to diets that are high in fat increases the susceptibility of female offspring to carcinogenesis," she said. After tracking the weight of the offspring, they dosed the animals with carcinogens to determine their susceptibility to cancer. "The animals exposed to the high-fat fish, the high-fat olive, and the high-fat safflower diets had the highest levels of carcinogenesis, and these were also the animals that weighed the most at weaning," Clegg described.

Besides these results, she said, they found that the high-fat olive oil diet accelerated puberty, an especially noteworthy link in light of other research that has shown a connection between early puberty and higher breast cancer risk. "Additionally, we found that the high-fat fish, high-fat safflower and high-fat olive-oil diets clearly increased carcinogenesis, but the butter-oil diet did not appear to enhance carcinogenesis." She cautioned, however, that the study is in its preliminary stages. "I'm not telling you all to go home and eat butter, but what I am saying is that at the end of these experiments, we may actually be able to make some level of nutritional recommendations for mothers on certain types of diets during their pregnancy."

Bay Area BCERC

Researchers at the Bay Area BCERC are placing their emphasis on how exposure to environmental estrogens during puberty alters mammary epithelial and stromal cells, and may lead to a disruption in the regulation of stem cells, which is a particularly critical part of carcinogenesis, said Mary Helen Barcellos-Hoff, Ph.D., of the Lawrence Berkeley National Laboratory. Besides the work of her research group, which she and Rodrigo Fernandez-Gonzalez described in separate presentations at the conference, Barcellos-Hoff provided a brief overview of two other, major, ongoing studies at the center.

One is in the laboratory of Zena Werb of the University of California, San Francisco. "She has actually compared the gene expression (which genes are turned on and active, and which are not) in ductal epithelial cells vs. the terminal end buds, which is the growing portion of the pubertal mammary gland," Barcellos-Hoff related. Werb's research group has also learned more about a transcription factor, which is a protein that controls gene expression, called GATA-3. "They discovered that GATA-3 is the most highly expressed transcription factor in the mammary epithelium, and it is localized specifically to the luminal epithelial cells (which line the mammary ducts)," she said. In addition, since their experiments show that halting the expression of GATA-3 during puberty causes the epithelial cells to die, they believe that GATA-3 is critical for mammary development.

A second primary research group of the Bay Area BCERC is in the lab of Paul Yaswen, Ph.D., of the Lawrence Berkeley National Laboratory. One of the interests of his group is whether environmental carcinogens alter the genome, which is an organism's complete set of genetic information, and the way the genome is expressed. His group is placing a special focus on a tumor suppressor gene called p16. Normally, Barcellos-Hoff explained, normal cells proliferate only until they reach a plateau, which is called stasis. When the p16 gene is silenced, however, proliferation continues unabated and cancer develops. "Paul's group is studying how p16 mediates this major barrier to malignant transformation."

Fox Chase BCERC

The fourth scientist to describe BCERC work was Coral Lamartiniere, Ph.D., of the University of Alabama at Birmingham. He is part of the Fox Chase BCERC research group. One of the aims of the center is to observe the effects of exposure to endocrine disruptors, or substances that interfere with normal hormone pathways during the perinatal period, he said.

The specific endocrine disruptors under study include the commonly used plasticizer butylbenzylphthalate (BBP), the herbicide agent 2,3,7,8-tetrachlorodbenzo-p-dioxin (TCDD), and the plastic-related compound bisphenol A. The scientists are especially interested in how a dose of the compounds administered before puberty affects cell proliferation and development in mammary glands, and if they alter lifelong breast cancer risk.

The researchers have also looked into gene expression (when the genes are turned on and active) in mice. By comparing untreated rats and those treated with environmental chemicals, they are beginning to learn which genes are turned on, or upregulated, at certain stages in a rat's development, and which are turned off, or downregulated. Further research into the function of each gene is giving them a picture of the exact consequences of exposure to the chemicals. For instance, he said, "We have now examined the potential of prepubertal bisphenol A and whether it can cause an increase in carcinogenesis. We have found that when we give bisphenol A to the mother from days one to 21, and then the carcinogen, dimethylbenz(a)anthracene, it will result in the offspring having more tumor development." Lamartiniere added, "These results are preliminary, but they suggest that endocrine disruptors given at one critical period of development can result in making offspring more susceptible to mammary cancer."

This research at the Fox Chase BCERC, and the many other projects at the MSU, UC, and Bay Area centers, crosses disciplines and institutions, he said. "The four centers are now engaging in collaborative transdisciplinary research projects as a means of breaking new grou Phytoestrogen Exposure and Pubertal Development nd and extending our fundamental understanding of breast biology and how environmental agents alter susceptibility for breast cancer."

Large-scale studies reveal connections between radiation and breast cancer

A wide variety of studies have shown and continue to show a clear connection between radiation exposure and cancer risk, and many of these note a particularly close tie to breast cancer, according to Dale Preston, Ph.D., former chief of the Radiation Effects Research Foundation's statistics department and now a researcher at HiroSoft International Corp. in California. He presented "Epidemiology of Breast Cancer Risk Following Radiation Exposures" at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC.

"Radiation is one of the most widely studied cancer risk factors, in part because there are a number of large populations in which we can characterize (exposure) doses. So we can actually carry out large-scale epidemiological studies," Preston said.

Survivors of the 1945 atomic-bomb blasts in the cities of Nagasaki and Hiroshima, Japan, during World War II are the primary source of quantitative data about radiation exposures for several reasons, he said. "With the A-bomb survivors, we have a large population that had some potential for radiation exposure. We have good-quality dose estimates, and we have very long-term active follow-up of mortality and cancer-incidence." Over the past several years, researchers have begun to collect information from additional populations, including patients who have undergone medical radiation treatments, as well as nuclear-plant workers, X-ray technologists, and other employees who have experienced occupational radiation exposures. All have helped provide a window into radiation exposures and effects.

To provide an idea of the units of radiation exposure and their range, he said a person from an economically advanced population receives an average annual radiation dose of about 0.001 gray (the equivalent of 0.1 rad) from medical diagnostic tests. In comparison, he said, "The average dose received by A-bomb survivors was about 0.2 gray, ranging up to 4 or more gray. A dose of 1 gray of acute whole-body exposure causes immediate effects. Most people would temporarily lose their hair and experience a disruption of their hematopoeitic system (which produces blood cells). Those symptoms generally would go away, but your cancer risk would be increased by about 50 percent." Higher doses of 4–6 gray are almost certainly fatal, he said. "Everyone who was exposed at Chernobyl to 6 gray died." The accident at the Ukraine-located Chernobyl Nuclear Power Plant occurred in 1986.

In addition, he said, radiation exposure is linked to higher cancer risk, a connection made clear from studies of A-bomb survivors. "About 80,000 individuals were in the cities at the time of atomic bomb, so they had some potential for exposure, although many of them had extremely low doses. During the more-than-40 years of incidence follow-up in A-bomb survivors from 1958-98, there have been about 17,500 cases of solid-tumor cancers, and we estimate that 853 of those cases were associated with radiation exposure."

Of the 853 radiation-associated, solid-tumor cancers in male and female A-bomb survivors, the most common was stomach cancer with 150 instances, he said. Exposed women also experienced a significant increase in the occurrence of breast cancer. "Breast tissue is in many ways the most radiosensitive tissue," he said. "If we look at breast cancer risk in the A-bomb survivors there's clear evidence of a dose response. At the the highest radiation doses, we see that 90 percent of the cases seem to be associated with the radiation exposure. If we look at the dose response (overall), it is quite linear: Thus, radiation at any dose seems to be increasing the risk of breast cancer, however these increases are small at low doses.

A broader view of radiation-associated breast cancer became available in 2002 when Preston, then at the Radiation Effects Research Foundation, and a team of internationally based researchers published a pooled analysis of eight populations from around the world that had received varying doses and types of radiation.

In addition to A-bomb survivors, the pooled analysis included data collected from U.S. women who received repeated fluoroscopic X-rays in treatments for tuberculosis, U.S. infants who underwent radiation therapy for enlarged thymus, U.S. women subjected to radiation for acute post-partum mastitis, women in Stockholm who had been treated with radiation for benign breast disease, and Swedish infants who had been treated for hemangioma (a type of reddened skin lesion) with gamma rays.

While the pooled analysis proved to be an enormous analytical challenge and did not reveal a blanket description for the impact of radiation on breast tissue, it did provide a few generalizations about radiation and breast cancer:

- the higher the radiation dose, the greater the breast cancer risk;
- the current age of the individual as well as age at exposure have an impact on disease incidence; and
- high-dose-rate exposures — either acute or fractionated (meted out) — are associated with a similar increase in breast cancer risk, and that increase is much greater than the risk related to low-dose-rate, protracted exposures.

He said, "The idea was that breast cancer is one of the most commonly studied cancers for radiation effects and that there were many good epidemiologic studies that provide some useful risk data to complement A-bomb survivors." The full publication, "Radiation Effects on Breast Cancer Risk: A Pooled Analysis of Eight Cohorts" is available in the August 2002 issue of *Radiation Research*.

Using Policy, Marketing Campaigns Effectively

“Try going up to somebody and saying, ‘We need to reform the way chemicals are managed in this country,’ and you’ll get blank stares back,” said Gretchen Lee, program and policy coordinator for the Breast Cancer Fund. Yet, her organization has been highly successful in drawing the interest and the influence of the public and decision makers to the topic of chemical exposures, their possible connections to breast cancer, and how to reduce potential health risks. Together with Jeanne Rizzo, R.N., executive director of the Breast Cancer Fund, the two women presented “Addressing Chemical Exposures Through Policy and Market-Based Campaigns” at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC.

One of the most well-known efforts is the Campaign for Safe Cosmetics, put together by a broad-based coalition of environmental, health and faith-based groups, and launched in 2003. Rizzo said the coalition targeted personal care products not only because of their chemical content, which is mainly unregulated in the United States, but also because they are discretionary-purchase items that are heavily marketed to women. “The Environmental Working Group started first with gathering the information,” she said. Currently, they have gathered data on 14,500 products representing more than 10,000 individual ingredients, and compiled it into an interactive database called Skin Deep, she said. The database includes descriptions of the chemicals in each of those products and, she added, “what we know and what we don’t know about them.”

It also includes a consumer feature, called the Skin Deep Report. By accessing the report at www.ewg.org/reports/skindeep/, an individual can search various shampoos, gels, nail polishes and cosmetics by brand name and learn about the chemicals they contain, Rizzo explained. “There have been one million searches per month on the Skin Deep database, which indicates the public interest in and concern about this kind of information.”

In addition, she said, the coalition has called upon U.S. and international companies that produce personal-care items to participate by signing the Compact for Global Production of Safe Health and Beauty Products. Companies that sign the compact pledge “not to use chemicals that are known or strongly suspected of causing cancer, mutation or birth defects in their products and to implement substitution plans that replace hazardous materials with safer alternatives in every market they serve.”

Rizzo remarked, “We have good news. We now have close to 500 companies that have signed the compact and are working closely with us.”

Besides the Campaign for Safe Cosmetics, the Breast Cancer Fund has also joined forces with other organizations to promote policy change. Lee highlighted two of the policy campaigns. “The first is the California Safe Cosmetics Act of 2005, which we co-sponsored with our friends at Breast Cancer Action and the National Environmental Trust,” she said. The bill, which has become law, requires all companies selling products in California to disclose ingredients linked to cancer and/or birth defects, she said.

The policy campaign did more than promote the act, she said. It also provided an avenue, often through the media, for informing legislators and the public about the possible significance of low-dose exposure, synergistic effects between chemicals, cumulative impacts of exposure over time, and the importance of timing of exposure. She added, "Another great thing about doing a policy campaign is that it educates the public about research that's being done, and it also helps the public demand more and better research that can serve the public good."

She also singled out a second piece of legislation called the California Environmental Contaminant Biomonitoring Program. "This bill took five years and four versions, but it was finally signed on the 29th of September of this year." She explained, "It is a statewide and community-based program that takes samples of blood, urine, breast milk, hair and saliva, and measures them for the presence of toxic chemicals. It will do a statewide base exposure measure every two years, and on the off years will look at people living in communities of concern."

The Breast Cancer Fund put its focus on biomonitoring following the strong recommendations of numerous bodies, including the International Summit on Breast Cancer and the Environment held in 2002, but also after a poll that the Breast Cancer Fund commissioned in 2004, she said. "We found that 97 percent of the public agree that chemical exposure is related to health problems, more than 90 percent believe it's important to know what toxic chemicals we have in our bodies, and nearly 80 percent supported a California biomonitoring program." She commented, "This is a great example of modeling policies in response to the interests and concerns of the public."

Like the campaign for the Safe Cosmetics Act, she noted, the run up to the biomonitoring legislation provided an excellent way "to educate legislators and the general public about the need for chemical policy reform."

In summary, Rizzo and Lee offered the following advice to others hoping to mount policy or market-based campaigns about chemical exposure:

- Make the information easily understandable and accessible to the general public.
- Form a coalition for a more powerful effect.
- Join forces with diverse constituencies, including environmental health and justice organizations.
- Remember that media coverage can be a meaningful tool for public education.

Although banned in the U.S., DDT still poses threat

Although the pesticide DDT has been banned in the United States since 1971, it is still a worthy research subject because of its potential connection to current-day breast cancer incidence, its lingering presence in humans and its continued use in some areas of the world, said Walter J. Rogan, M.D., of the epidemiology branch of the national Institute of Environmental Health Sciences. He presented "DDE Associations With Some Hormonal Risk Factors for Breast Cancer: Adolescent Weight Gain, Puberty, and Duration of Lactation" at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC.

Rogan and other researchers are concerned about DDT for a variety of reasons. One is that DDT breaks down into DDE (dichlorodiphenyl dichloroethylene), which is "all but indestructible," Rogan said. "Everybody alive in the world has some DDT residue in their bodies." DDE is also fat-soluble, he said, and appears in both breast milk and mammary tissue. "We originally got interested in doing this work because we wanted to know whether there were consequences for the health of infants in being exposed to PCBs (another environmental contaminant) and DDTs in breast milk."

Rogan and his research group were especially interested in the pesticide's link to breast milk because DDE — specifically known as o,p,DDE — has a chemical conformation similar to estrogen, the reproductive hormone that is critical to the normal development of a female, including mammary gland development and milk production. The possibility then arises that DDE is acting as an estrogen by binding to estrogen receptors, and therefore interfering with normal development and lactation, he said.

"During pregnancy, the breast structure is undergoing a process of increasing its internal surface area, and it does so under the influence of estrogen, progesterone and other hormones, including prolactin. Estrogen inhibits the ability of prolactin, which is required for promote full milk synthesis," he said. When the estrogen level drops, as it normally does at childbirth, the prolactin stimulates milk production. In other words, he explained, "Most women don't go into full lactation until the estrogren levels drop and prolactin can act on its own." This is where DDE comes in, he said. "We thought that a weak but persistent estrogen like o,p,DDE hanging around in women might do the same thing — that is to say, it might antagonize the effects of prolactin and result in early weaning."

This estrogenic activity of DDE may also have a connection to breast cancer through the same route, he said. DDE's chemical similarity to estrogen may allow it to act as an estrogen in the body, and "although studies of DDT/DDE as a cause of breast cancer have been inconsistent, DDT/DDE exposure is associated with several of the established risk factors for breast cancer, perhaps through a mechanism involving occupancy of the estrogen receptor," he said.

Rogan and his research group conducted two studies to learn whether DDT/DDE exposure affected the duration of breastfeeding. One was the 780-child North Carolina

Infant Feeding Study, which checked the DDT exposures of women who gave birth from 1978 to 1985 and recorded the length of breastfeeding. The second study accumulated a similar data set, but involved more than 200 children from Tlahualilo, Mexico, where DDT exposure was higher.

Their results showed an association between exposure and weaning time, he said. “In the North Carolina study, about 750 of the 780 children were breast feeders. The women with the least DDE exposure breastfed on an average of 33 weeks, the ones with the most exposure for about seven weeks, and we had a fairly smooth dose-response curve in between,” he said.

“There was more recent use of DDT in Mexico, where they applied it to cotton fields, and there were high levels of DDE in breast milk,” he said. “We had 231 women enrolled and we followed 196 through weaning. What we saw was very much the same thing we saw in North Carolina: The women with the lowest DDE levels in breast milk breastfed for a long time, and the women with the highest didn’t breastfeed as long.”

In addition, he said, his research group followed up with the female children enrolled in the North Carolina study to determine whether the chemicals had an effect on the timing of puberty. This is a significant question, he noted, because numerous studies suggest early onset of puberty may be related to increased breast cancer risk later in life. “We were able to contact 302 girls from the North Carolina cohort in 1993–97 — remember they were born in 1978-82. We sent them and their parents the self-administered, line-drawing version of the Tanner scale,” he said. The Tanner scale is a measure of the various levels of pubertal development. “What we observed was a non-statistically significant trend for the earliest stages of both breast and pubic hair development to occur about six months earlier in the 41 girls who had the highest prenatal exposure to DDE.” He noted that the girls' development then slowed, so that later Tanner stages, including the timing of the first menstrual period, were similar to the other girls in the study.

Since, a third study of Chinese textile workers has found a correlation between high DDE exposure and an early first menstrual period, Rogan related. “Menarche was one year younger in the highest exposure." However, he said, the results of a study of Akwesasne Mohawk girls aged 10–17 indicated that DDE was unrelated to age at menarche.

Research inconsistencies have also surfaced for questions of whether DDE exposure is related to differences in child growth, another suspected factor in increased breast cancer risk, he said. Although the North Carolina showed that 14-year-old boys of high-DDE-exposed mothers were much taller and heavier than other like-aged boys, the same did not hold true for the girls, he said. However, Rogan noted, other studies have drawn a parallel between high prenatal DDE exposure and high body mass index among African-American girls.

Although DDT/DDE levels in the United States are continuing to decline since the ban on the pesticide’s use, he said the research should continue, especially since the chemical is still being used in other parts of the world, including South Africa, to control malaria-

causing mosquitoes inside homes, he said. “If women have to live in the house where you are doing indoor residual spraying for malaria, then you will have breast milk that is higher than in Mexico, higher than in North Carolina, and higher than in Chinese textile workers.”

Summary of Dr. Rogan’s presentation at the November 2006 BCERC annual meeting: DDE Associations with Some Hormonal Risk Factors for Breast Cancer: Adolescent Weight Gain, Puberty, and Duration of Lactation.

"A window of opportunity"

Why does an early, full-term pregnancy protect many women from breast cancer?

While current research studies have not yet provided all of the details of breast-cancer development in women, they have shown that an early first-term pregnancy does offer women considerable and lifelong protection against acquiring the disease. “This may be a window of opportunity for us to understand one way to prevent cancer,” said Jose Russo, M.D., of the Fox Chase Cancer Center in Philadelphia. He presented “The Role of Stem Cells in Breast Differentiation and Cancer Prevention” at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC.

His research group has demonstrated that the lifetime protective effect associated with an early pregnancy (before the age of 24) results because mammary gland cells have progressed to their final developmental stage, which involves full differentiation of the gland's ductwork. He explained how these changes occur. As a girl ages, the ductwork within her breast grows, divides and forms club-shaped, terminal end buds. At puberty, the end buds branch out and form structures called alveolar buds, and these, in turn, generate clusters of ductwork, known as lobules.

“The first lobule structure to appear is called lobule type 1,” he said. “With sexual maturity, these undifferentiated lobules type 1 start to become more complex, and we call these lobules type 2. If pregnancy takes place, they go on to lobules type 3. If pregnancy does not occur, they never go on to lobules type 3.” The type 3 lobules revert to types 2 and 1 following pregnancy, he said, and later in life, lobules type 1 again predominate in all women, regardless of whether they were ever pregnant. He also noted that although these lobules are present in both postmenopausal parous women (those who have had a full-term pregnancy) and nulliparous women (those who have never given birth), only the parous women experienced the cancer-protective effects.

Because of this disparity in cancer incidence between the two, Russo’s research group considered the possibility that the lobules type 1 in postmenopausal women were not as similar as they appeared and perhaps had unequal cancer susceptibility.

They hypothesized that the lobules type 1 in nulliparous women never went through the process of differentiation, and therefore retained a large number of cells that could be more easily co-opted by carcinogens and ultimately become cancerous. Russo calls these carcinogen targets “stem cells 1.” Parous women, conversely, have “stem cells 2,” cells that have previously undergone complete differentiation into lobules type 2 and 3, and are therefore resistant to carcinogens.

Russo and his research team analyzed the cells from parous and nulliparous women, and indeed discovered differences. “When we compared the parous and nulliparous breasts, 185 genes were expressed in parous women that were not expressed in nulliparous women, and 39 genes were expressed in nulliparous women that were not expressed in

parous women." Some of the genes present only in parous women had such potentially cancer-fighting functions as immune modulation, DNA repair, programmed cell death, chromatin remodeling and transcription, he noted.

Armed with the now-apparent variation in the so-called "genomic signatures" of parous and nulliparous women, Russo's research team turned its attention to rodents, which similarly show a clear connection between cancer prevention and early pregnancy/mammary gland differentiation. The animals are also an excellent experimental model, he said, because researchers can obtain the same cancer-preventive effect by treating virgin rats with the pregnancy-associated placental hormone called human chorionic gonadotropin (hCG). "When we gave 100 IU (international units, a dosage measure) of hCG-conferred parous protection to nulliparous individuals, we saw the same activation of genes present in them as we saw in parous individuals. The hCG induced inhibition of cell proliferation in primary tumors as well as in the metastatic lesions to the lymph node tissue," he said.

Through these studies, Russo and his group have concluded that both parity and the hormones of pregnancy — even without conception — can moderate the pattern of mammary development and the mammary gland's genomic signature, and yield lifelong protection against breast cancer.

Based on these results, Russo is expanding his breast-cancer work to consider hCG as a possible preventative agent. He is now also exploring additional research paths, including ways of measuring how healthy women respond to the hCG.

Summary of Dr. Russo's presentation at the November 2006 BCERC annual meeting: The Role of Stem Cell in Breast Differentiation and Cancer Prevention

Are lighted nights a factor in increased breast cancer risk?

Something as mundane as electric light just may have an impact on breast cancer incidence, according to Richard Stevens Ph.D., a cancer epidemiologist, at the University of Connecticut Health Center. He presented "Could Circadian Disruption Be Playing a Role in the Worldwide Increase in Breast Cancer?" at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC**.**

Breast cancer rates are not only currently higher in the U.S., Western Europe, and other industrialized nations than they are in developing countries, but they have experienced a sharp rise in the past few decades, he said. "In the early 1980s, I was thinking about what changes are associated with industrialization, and it occurred to me that there was an increasing use of electricity to light the night. In fact, it's kind of a hallmark of modern life," he said. "The question then became, Could that have any real biological consequences? It has only been in the last 15-20 years that a growing number of psychologists, biologists and epidemiologists have started to say, yes, it could have large effects on health and well-being."

The shift to a lighted existence has been quite profound, Stevens asserted. Until about 120 years ago, humans existed under sunlit days and dark nights. "Now we have electricity, and while there are many wonderful benefits of electricity, it has also made a lot of changes to our lives: We have shift work, late-night reading or TV, dimly lit bedrooms during sleep, short sleep duration, bright bathroom light during the night, night glow over the cities, and day work inside buildings. It's a cumulative effect where we have dim days inside buildings and light at night." This disrupts the normal, internal, daily rhythm — the approximately 24-hour circadian rhythm — of biochemical, physiological and behavioral patterns that occurs in humans and in nearly every other organism on Earth, he said.

The circadian disruption occurs as the result of a drop in level of the sleep-associated hormone melatonin circulating in the body, Stevens explained. The reduced melatonin can, in turn, reset the brain's internal clock pacemaker (a nerve center called the suprachiasmatic nuclei) and throw off the natural circadian rhythm.

"The reproductive physiologists have been very interested in melatonin particularly in strongly seasonally breeding mammals, where there's no question that melatonin has a strong impact on the sex hormones." While some research has linked melatonin to breast cancer incidence in humans, he acknowledged that scientists do not currently know the extent to which melatonin is important in human reproduction. However, he said, growing evidence from experimental systems now suggests that melatonin dramatically inhibits breast cancer in rats and also slows the growth of certain subclones (perfectly copied DNA fragments) of human breast cancer cell lines.

He cited several animal and human studies, including some of his own, that demonstrated a possible connection between life under constant light and carcinogenesis. On the human side, he said, "There have now been eight to 10 studies of shift-working women, and they have all been remarkably consistent: Shift-working women seem to be at higher risk for breast cancer. In

addition, studies of blind women in the United States have shown that, as anticipated, they have a lower risk for breast cancer." While those studies provide some clues, they do not necessarily prove the link between increased risk and light exposure, he noted, because other, unknown factors may be playing a role.

Animal studies, on the other hand, have had more conclusive results, he said. In one (Blask, D.E., et al. *Cancer Research*, 2005), researchers transplanted human tumors into lab rats, called nude rats, subjected the rats to different light conditions, and found that increased light correlated with increased tumor growth, he described. "The researchers next enrolled 12 young women, and drew blood at 2 a.m. in the dark and again at 3:30 a.m. after 1.5 hours of bright light," he said. "They infused the rats with one of these two types of blood for one hour in the early morning to determine the effect of exposure of young women to light at night on the growth of human breast cancer in a nude-rat breast. This presented the closest one can ethically get to a direct test of the light-at-night hypothesis on human tumor growth." Their results were striking, he said. "The blood taken during the dark stopped the growth of the human tumor in the nude rat. The blood taken from the young women after they had been exposed to an hour and a half of bright light had no effect, and the tumors went on to grow."

Other promising research includes the recent discovery of a new photoreceptor, called melanopsin, that may explain how the suprachiasmatic nuclei deep within the brain can register external light, and additional studies on biological-clock genes, Stevens said. He anticipates that these ongoing studies, and other research projects that they inspire, will fuel overall knowledge of human circadian rhythms and also provide much-needed information about the potential connections between alterations in those rhythms and the escalating incidence of breast cancer in women around the world.

Summary of Dr. Stevens's presentation at the November 2006 BCERC annual meeting: Could Circadian Disruption Be Playing a Role in the Worldwide Increase in Breast Cancer?

Changes in epigenetics, not just DNA code, affect cells

"All of the cells in our body have exactly the same type of DNA. While the DNA code remains the same, it is the epigenetic code that determines which genes are expressed and determines what tissues are actually developed. In other words, epigenetics is the study of heritable changes in gene expression that occur without a change in DNA sequence…. What you will be hearing about today are the severe changes in epigenetics that take place in a cancer cell," said Thea Tlsty, Ph.D., who presented the opening keynote address, "Re-Programming the Epigenome: Molecular Mechanisms for Responding to Environmental Exposures," at the 2006 Third Annual Early Environmental Exposures Meeting of the BCERC. Tlsty is Professor of Pathology at the University of California at San Francisco Department of Pathology and Director of the Program in Cell Cycling and Signaling in the UCSF Comprehensive Cancer Center.

Tlsty is interested in how aberrant epigenetic changes disrupt the genes that would normally suppress tumor development. Some of the major players in the gene-disruption process are DNA methylation and the overabundance of protein complexes known as polychrome repressor complexes, or PRCs.

In the first of the two, methyl groups (molecules made of carbon and hydrogen) attach to certain parts of the DNA, including areas known as gene promoters that can turn genes on or off. Research indicates that many tumor cells show increased DNA methylation that shuts down certain genes. In the second, the PRCs restrict the expression of homeotic genes, which are critical in a cell's development and help determine how, when and where tissues develop. One of the complexes, known as PRC2, has enzyme activity that ultimately is responsible for silencing these homeotic genes. When the genes are silent, the cells cannot differentiate. Research demonstrates that the level of PRCs, like that of DNA methylation, is increased in cancer cells compared to normal cells.

Epigenetic research is on the rise, she said. "This is a very active area, and studies have shown that nutritional factors, environmental factors and even behavioral factors can affect the epigenetic program." She gave several examples, including one mouse experiment in which a change in diet resulted in a difference in the animal's outward appearance. "In mice, we have a gene that encodes for yellow coat color. If that gene is methylated, that yellow color promoter is off and the coat becomes brown. This is the interesting part: If you feed the mouse a diet that is rich in methyl donors, you can increase the frequency of methylation, and if you have increasing methylation, the coat color becomes brown," she said. "This is a really striking example of how a nutrient can affect physical characteristics in an animal."

A major question regarding the development of cancer, however, was whether it was possible to detect alterations in the epigenetic profile in the very early stages of the disease. Tlsty explained, "As we all know, breast cancer originates in the epithelial cells that line the milk ducts, both the ducts as well as the alveoli." The epithelial cells include stem cells, which allow the renewal or regeneration of gland tissue and are therefore able

to divide rapidly. These stem cells, she said, are usually considered the cells most at risk for forming cancer, which is uncontrolled cell growth.

"It has been understood for some time now that there is a pathway that controls this self-renewal process, and that pathway is itself controlled through a cell-cycle regulator, called p16," she said. Scientists also know that a loss of p16, which is a protein, allows tumors to form. "Her studies of human breast tissue have found two populations of cells: one (the majority) that grows for a while and then arrests, and another rare subpopulation that continues growing for many, many more months in culture. The subpopulation cells have some really striking characteristics: They're resistant to apoptosis; they're not able to differentiate (become specific tissues); they have extended proliferation, and they acquire genomic changes that are seen in early events in cancer." The subpopulation cells are also the ones that target p16 for silencing.

Tlsty and her research group took a closer look at the role of p16. "We wanted to know if there was just a sporadic change in p16 in these cells or if there was a program that was constantly turned on. To our delight and surprise, we found that there was a program, or a non-random signature of methylation changes, that were turned on in these rare cells." Not only did they find that the methylation changes were in regulatory regions of genes involved in cell replication, but they also discovered that certain groups of genes were preferentially hypermethylated and therefore shut down. One of those genes was HOXA9.

HOXA9 has several roles in the mammary gland, and is vital for lactation. Without it, a female cannot produce milk, Tlsty said. In the rare cell subpopulation, HOXA9 is silenced, although they were able to turn it back on if they added a demethylating agent. For the next step, she and her research group examined 100 breast tumors, and counted 93 that had absolutely no HOXA9 expression. Of those 93, about half of the HOXA9 was missing because the cells were epigenetically remodeled. She concluded, "So this very commonly inactivated tumor suppressor gene, p16, turns out to have a function that was totally unanticipated. It actually turns on epigenetic remodeling."

She added that her research group has identified markers that are expressed only on this subpopulation of cells, and may ultimately prove important in early cancer detection and possibly preventative options.

Summary of Dr. Tlsty's presentation at the November 2006 BCERC Scientific Symposium: Re-programming the Epigenome: Molecular Mechanisms for Responding to Environmental Exposures