

## Poster Abstracts

### Effect of Prepubertal Exposure to Environmental Contaminants in the Rat Mammary Gland

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**Background:** Over the course of one month, we had the opportunity to participate in the BCRL through “The Students and Scientists Environmental Research Scholarship” sponsored by *Prevention Is The Cure, Inc.*, a campaign of the Huntington Breast Cancer Action Coalition (HBCAC).

**Objective:** The aim of this internship was to learn how scientific research was conducted through studies on the effects of environment contaminants in the rat mammary gland.

**Work performed:** To accomplish our objective we studied the morphology and the effects of prepubertal exposure to Bisphenol A (BPA) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), two well known endocrine disruptors (ED) in mammary glands. For our experiment we used whole mounts (WM) and tissue sections of mammary glands, collected and prepared at a collaborating institution. Lactating rats were treated either with solvent (control groups), BPA or TCDD. The treatments were given as follows, either: a daily intragastric administration of BPA for 21 days; or TCDD when the pups were 14 and 17 days of age. Tissue was collected from female offspring of all groups at 50 days of age.

First, we learned about the mammary gland structures, by counting the number of terminal end buds (TEBs) from previously prepared WMs. Secondly, we learned how to recognize the cell division phases, counting cells that were in metaphase and anaphase, in 500 epithelial cells from hematoxylin and eosin stained sections. Ten slides per group were counted and the treatments were maintained blinded. Thirdly, to assess the interpersonal variation the individual results from both groups were counted by each of us and compared. Lastly, we learned how to use the student t-test to assess the statistical significances of the data.

**Conclusions:** Through this experience, we gained an understanding of the research process and the significance of environmental contaminants to human health. We understand that it is possible that early exposure to hormonally active environmental compounds may affect the architecture and cell division of the rat mammary gland during critical stages of development. These effects could facilitate the occurrence of mutations and eventually initiation of cancer.

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### Pubertal Exposure to High Fat Diet Alters Mouse Mammary Gland Development and Estrogen Responsiveness

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Increased obesity rates in the United States are associated with health risks. It is possible that obesity or increased adiposity in girls during puberty, an important period of breast development and a window of exposure sensitivity, may influence breast development and cancer risk. To investigate the impact of pubertal obesity on mammary gland development, obesity-susceptible C57BL/6 mice and obesity-resistant BALB/c mice were fed a high fat diet (HFD) (60% kcal fat) or control diet (CD) (12% kcal fat) from weaning (3 wks old) to 7 weeks of age. Only pubertal C57BL/6 mice fed the HFD had a significant increase in body weight ( $19.3 \text{ g} \pm 0.4$ ,  $n=7$ ) compared with CD fed mice ( $18.1 \text{ g} \pm 0.2$ ,  $n=8$ ,  $p<0.02$ ). Pubertal HFD-fed C57BL/6 mice had significantly reduced mammary growth as evidenced by reduced numbers of terminal end buds (TEBs), stunted ductal elongation, and reduced epithelial cell proliferation. Weight loss initiated by switching C57BL/6 mice from HFD to CD restored TEBs and ductal elongation. In contrast, BALB/c mice fed HFD had a modest weight gain (CD  $17.9 \text{ g} \pm 0.2$ ,  $n=7$  vs. HFD  $18.5 \pm 0.3$ ,  $n=8$ ), exhibited significantly increased mammary epithelial cell proliferation and increased susceptibility to carcinogen-induced mammary cancer. Importantly, HFD had no effect on the mammary glands of adult C57BL/6 or BALB/c mice, indicating that the effects of diet and adiposity were specific to the pubertal period of development. Pubertal HFD-fed C57BL/6 mice showed a decreased mammary gland response to estrogen (E) compared to CD-fed mice and decreased production of amphiregulin, an E-induced mammary growth factor. In summary, dietary fat and adiposity had a profound strain-specific effect on mammary gland development during puberty and also affected subsequent susceptibility to mammary cancer.

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### Measurement of Fat Mass: Concordance between Field Research Measures and Dual-Energy X-ray Absorptiometry.

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**Background:** There are several approaches to measure total fat mass (FM). Some measures are suitable to field research, such as skinfolds, circumferences and resistance from Bioelectrical Impedance Analysis (BIA), while others, including underwater weighing, MRI, and Dual-Energy X-ray Absorptiometry (DXA), require specialized equipment.

**Objective:** Test the concordance of field measures to estimate FM against the index measure of Dual-Energy X-ray Absorptiometry (DXA) in girls 7 to 12 years of age.

**Methods:** Equations developed by Slaughter et al., Goran et al., and Dezenberg et al. were used to calculate FM from skinfold measures and equations developed by Goran et al and Houtkooper et al. were used to calculate fat free mass from resistance. DXA scans and anthropometric measures were collected on 108 peripubertal female participants. Bland-Altman analysis was used to compare the FM as estimated from the field measures with the index measure from DXA. Sensitivity analysis was done to examine the effect of overweight status on the estimates.

**Results:** Age of the participants was 9.37 +/- 0.91 years (range, 7-12); 43.5% African American and the remainder white. The Slaughter, Goran and Dezenberg estimates of FM were all correlated to the DXA measurement at  $r > 0.97$ . The Goran skinfold equation showed the largest mean underestimation of FM at 2.62 kg +/- 1.66. The Dezenberg equation overestimated FM by 0.70 kg +/- 1.45. The difference between the Dezenberg estimate and the index measure was correlated to the mean of the measures at  $r = -0.24$   $p = 0.01$ .

The Goran and Houtkooper equation estimates of FM from BIA were both correlated with the DXA measure at  $r > 0.94$ . The Bland Altman plot for Houtkooper showed that the equation underestimated FM by 3.28 kg +/- 1.78 with correlation between mean and difference of  $r = 0.14$   $p = 0.13$ . The associations did not change when the heaviest 5% of girls were removed from the sample.

**Conclusions:** The Dezenberg and Houtkooper equations did not show systematic bias due to weight status, and the estimates of FM correlated highly with DXA. Dezenberg skinfold equation and the Houtkooper resistance equations were optimal for field measurement of FM in this group of peripubertal girls.

### Assessing breast development in The Jersey Girl Study: agreement between physician and mom assessment.

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Epidemiologic studies have identified early onset of puberty as a risk factor for breast cancer. The Jersey Girl Study is a cohort study in 9 and 10 year old healthy girls aiming to evaluate predictors of early onset of puberty in New Jersey girls. This ongoing study has currently recruited 135 girls through pediatric practices, media, and community recruitment efforts. Data collection includes completion of a brief phone questionnaire to assess eligibility and a self-administered questionnaire (both by mothers), three 24-hour recalls to assess girls' dietary intake, and an appointment during which body measurements (weight, height, body composition, body circumferences), as well as a morning urine sample and saliva sample are collected. In addition, mothers and study physicians independently assess girls' breast and pubic hair development at baseline using the same Tanner scale forms. Physician assessment is conducted by physical exam during the appointment. We conducted preliminary analyses to evaluate the agreement between physician and mom assessment among 118 girls who completed both. According to physician assessment, 54.9% of the 9 year-old girls (n=71) and 68.1% of the 10 year-old girls (n=47) had started breast development. There was substantial agreement between mom and physician assessment of pubertal development. For our 9 year-old girls, there was an 86% agreement between physicians and mom assessment of the onset of breast development (marked by reaching Tanner Stage 2), with a kappa of 0.7 (95% CI: 0.6-0.9). For 10 year-old girls, the agreement was 85%, with a kappa of 0.7 (95% CI: 0.4-0.9). Agreement was stronger for the assessment of the onset of pubic hair. Our preliminary results suggest that, although physician assessment of breast development is more accurate, for research purposes, Tanner stage assessment by mothers may be considered when physician assessment is not feasible, at least in an educated population similar to ours.

**Funding:** The Cancer Institute of New Jersey, CINJ Foundation, Komen Foundation Central and South Jersey Affiliate.

### Translating BCERC Findings into Usable Information: Four Messages for Dissemination

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**Background:** One key task of the Community Outreach and Translation Core (COTC) of the Breast Cancer and the Environment Research Centers (BCERC) is to develop public health messages translated from the published biology and/or epidemiology research from the BCERC. These messages are designed to educate young girls and women about the role(s) of specific environmental stressors in breast cancer and how to reduce exposures to those stressors.

**Objective:** Develop a communication campaign that will disseminate research from the BCERC biology projects, promote the BCERC web site and encourage behavior change.

**Work Performed:** Zero Breast Cancer contracted a social media company, OneWorld Communications (OWC), to create an ad template based on BCERC research that would encourage smart choices regarding exposure to environmental factors and healthy lifestyle to reduce the risk of breast cancer.

Each COTC selected one message based on published research from their biology project as follows: Bay Area, radiation; Michigan State, PFOA; Fox Chase, BPA; and Cincinnati, Windows of Susceptibility. The COTCs worked together to provide input for each message, ensure consistent presentation of the template, and create message content with a 9<sup>th</sup> grade maximum reading level.

**Results:** The key outcome of this collaborative project is the series of four professional, scientifically accurate messages created for the priority target audience of mothers with young daughters. This project also showcases the transdisciplinary nature of the BCERC as COTC members collaborated with each other and with biologists across the four center sites to create appropriate messages. Finally, this project has created messages that are ready to be tested with the priority target audience.

**Conclusions:** The COTC plans to obtain formal feedback on these messages at the annual meeting in November via a survey form that will be handed out during the poster session. If supplemental funding is awarded, the messages will be tested with the target audience. After final fine-tuning of messages based on feedback and message testing, the messages will be ready to launch a communication campaign related to the BCERC and breast cancer risk reduction.

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### Prenatal Exposure to Bisphenol A increases Dimethylbenzanthracene-induced mammary cancer in adult rats

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Mammary gland development is influenced by many hormones including estrogen. Although with less potency, some chemicals found in the environment have the ability to elicit similar responses as those caused by hormones. These chemicals are known as endocrine disruptors. One such a chemical is the plasticizer Bisphenol-A (BPA) which possesses estrogenic properties. Since exposure to estrogenic chemicals during critical periods of mammary gland development has been suggested to have the potential to influence the susceptibility to develop breast cancer, we investigated the ability of *in utero* exposure to BPA to alter the susceptibility of the mammary gland to carcinogenesis.

**Methods:** Pregnant Sprague-Dawley CD rats were fed phytoestrogen-free AIN-76A diet and gavaged from post-conception day 10 until birth with 1) sesame oil (control), 2) 25 µg/kg BW BPA (low BPA), or 3) 250 µg/kg BW BPA (high BPA). For tumorigenesis studies, female offspring were exposed to 30 mg dimethylbenzanthracene (DMBA)/kg body weight at 50 or 100 days of age.

**Results:** In rats exposed prenatally to BPA and at day 100 with DMBA as compared to DMBA at day 50, mammary tumor multiplicity was increased and latency (time to develop first tumor) was significantly decreased. Western blot analysis showed different effects of prenatal BPA exposure on estrogen receptor- $\alpha$  (ER- $\alpha$ ), steroid receptor co-activators (SRCs) 1–3, phospho-IGF receptor beta, EGF-receptor, phospho c-Raf, and 14-3-3  $\eta$  and sigma protein expressions between 50 and 100 day old rats that can account for differential mammary cancer susceptibility.

**Conclusions:** Prenatal exposure to BPA alters expression of steroid receptor and co-activator proteins, growth factor signaling proteins and susceptibility to chemically-induced mammary cancer in a rodent model.

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**Content Analysis of Breast Cancer Webpages**

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This research was supported by the Breast Cancer and the Environment Research Centers grant number U01 ES012800 from the National Institute of Environmental Health Sciences (NIEHS), and the National Cancer Institute (NCI), NIH, DHHS

**Background** The Internet is a popular source for Americans who are searching for health information, and its potential for relaying breast cancer information makes it important to understand the types and amount of content on breast cancer websites. Prior research has examined the content of breast cancer messages via various media sources, but there has been little work examining the content of breast cancer web pages. Research has found little content on prevention and environmental risks.

**Research Questions** The objective of this study is to analyze the content of popular breast cancer websites. Specifically, the research questions ask what content is present, and to what degree (coding for major emphasis of content, minor emphasis of content, or no content present). Seven breast cancer topic areas included are: environmental risk factors, prevention, detection, treatment, awareness, social support, and survivorship.

**Method** The most popular search engines (Yahoo, MSN, and Google) were used to search for the terms “breast cancer” and “breast cancer and environment.” The first 10 websites that appeared across these searches were analyzed. For these websites, each page that was linked by one click of the main webpage was coded (N=420). The individual web page was the unit of analysis. An ordinal coding scheme was employed to assess the level of coverage of the seven areas in each web page, and coding reliability was established.

**Results: Environmental risks:** 13% major emphasis, 36% minor emphasis, and about 50% no content. Most common environmental risk topics were chemicals in the environment (5% major emphasis, 7% minor emphasis, and 88% with no content) and hormones (2% major emphasis, 10% had a minor emphasis, and 88% with no content). **Awareness:** 16% major emphasis, 36% minor emphasis, and 47% no awareness content. **Prevention** 16% major emphasis, 45% minor emphasis, and 39% no content. **Detection:** 9% major emphasis, 43% minor emphasis, and 48% no content. **Treatment:** 22% major emphasis, 41% minor emphasis, and 38% no content. **Social support:** 2% major focus, 30% minor emphasis, 67% no content. **Survivorship:** 4% major focus, 34% minor emphasis, 62% no content.

**Conclusion** This study demonstrates that about half of the most searched websites provide at least some degree of information regarding environmental risks and risk reduction behaviors. Awareness, prevention, detection, and treatment were each present in approximately 50-60% of websites, while social support and survivorship topics were less frequently appearing topics.

**Father Absence, BMI and Pubertal Development in the Bay Area Breast Cancer and the Environment Research Center**

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**Background:** Early life events play an important role in the development of breast cancer.<sup>1,2</sup> The link between puberty and breast cancer<sup>3,4</sup> is of particular concern, given that the average age of pubertal onset among U.S. females is declining.<sup>5,6</sup> Hereditary factors, physical activity levels and BMI only partially explain this secular trend. Evidence suggests that environmental and behavioral factors also influence puberty.<sup>7-10</sup> Characteristics of the family environment, in particular father absence, have been established as determinants of pubertal timing.<sup>13-21</sup> Analogous animal studies confirm the important role of family composition.<sup>22-24</sup>

**Objective:** To prospectively examine effects of father absence at age 6-8 years on girls' BMI, and pubertal status two years later.

**Method:** In BABCERC, we assessed biologic father absence, breast and pubic hair development, and height and weight annually. The current investigation focuses on the effect of father absence at age 6-8 years on BMI and pubertal status outcomes 2 years later. The sample is comprised of 444 ethnically diverse girls and their primary caregivers. We treated onset of puberty as a time to event outcome. We used the girl's age at the exam in which she is first determined to have had puberty onset to estimate the age at pubertal onset. A multivariable Cox proportional hazards regression model was then used to analyze the effect of father's absence on age at onset of puberty (breast /pubic hair). In analyzing the effect of father absence we controlled for household income and ethnicity and we assumed BMI to be in the casual pathway for the effect of father's absence on onset of puberty.

**Preliminary Results:** At baseline, about 80% of participants lived with both a biologically-related father. Crude correlations between father absence at Year 1 and Tanner Stage at Years 2 and 3 showed significant associations between Year 1 father absence and Year 2 pubic hair development (p<.01) and Year 3 breast (p<.05) and pubic hair (p<.01) development. Results from Cox hazards regression analyses will better illuminate effects of father absence on pubertal development, while adjusting for family income, and allow us to test the mediating role of BMI and potential moderating role of ethnicity.

**Implications:** By incorporating BMI, ethnicity and breast and pubic hair measures into our models, we seek to extend previous research linking father absence to menarche. The current study has potentially significant clinical implications for girls' health in the short term and breast cancer in adulthood.

### **Prenatal exposure to BPA induces changes in genomic DNA methylation in rat mammary gland**

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Prenatal exposure to xenoestrogens may be responsible for the increased incidence of breast cancer observed over the last 50 years. The xenoestrogen bisphenol A (BPA) has been shown to leach from plastic containers and dental materials among other consumer products. Our laboratory has shown that fetal BPA exposure induces the development of preneoplastic and neoplastic lesions in the adult rat mammary gland. The mechanism underlying the origin and progression of neoplastic lesions after developmental exposure to xenoestrogens remains largely unknown. In this regard, the link between epigenetic changes and carcinogenesis is being investigated intensively. Perinatal exposure to various xenoestrogens has been suggested to induce trans-generational effects via germ line DNA methylation.

We postulate that prenatal exposure to BPA induces epigenetic changes through DNA methylation in the rat mammary glands which may be associated with tumor formation in adulthood. To explore this hypothesis, 30 Wistar-Furth rats were exposed to either 250µg BPA/kg BW/day or vehicle from gestational day 9 to postnatal day (PND) 1. The animals were sacrificed at PND4, PND21 and at first estrous after PND50, and the genomic DNA was isolated from the mammary glands. We compared the methylation status in approximately 58,000 sites throughout the genome between BPA- and vehicle-treated animals using the Nimblegen ChIP array. We observed that 1) as expected, a large part of the genome (89% of sites) was unaffected, 2) BPA-treated animals displayed statistically significant changes in methylation status in several chromosomal regions compared to control, 3) the total number of hypo- and hyper-methylation events was similar in all BPA-treated animals regardless of age, and 4) the majority of the methylation changes occurred on PND21 (pre-pubertal) and PND50-60 (puberty). Certain chromosomal regions including Chr1q4, Chr6q2.4, Chr7q3.4 and Chr8q3.2 were found to be especially rich in hypo- or hyper-methylated areas. Anti-apoptotic Bcl-2 and tumor-associated Wnt7b were among the genes with consistent changes in promoter methylation status on PND21 and PND50-60. These preliminary results may help understand the underlying developmental mechanisms that contribute to breast cancer initiation and progression in adulthood.

### **Prenatal exposure to bisphenol A promotes angiogenesis and alters estrogen receptor alpha-mediated responses in the mammary gland of cycling rats**

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Prenatal exposure to bisphenol A (BPA) disturbs mammary gland histoarchitecture and increases the carcinogenic susceptibility to chemical challenges administered long after BPA exposure. Our aim was to assess the effect of prenatal BPA exposure on mammary gland angiogenesis and steroid hormone signaling pathways in virgin cycling rats. Pregnant Wistar rats were exposed to either 25 or 250 µg/kg bw/day (25 and 250 BPA, respectively) or to vehicle. Female offspring (8-10 per group) were autopsied on postnatal day (PND) 50 or 110. Ovarian steroid serum levels, proportion of hyperplastic ducts, the expression of steroid receptors and their co-regulators in the mammary gland, and angiogenesis were evaluated. At PND 50, all BPA-treated animals had lower serum levels of progesterone (25 BPA 12.40±4.56 ng/ml; 250 BPA 12.91±4.52 ng/ml vs Control 25.04±7.89 ng/ml;  $p<0.05$ ), while estradiol levels remained unchanged (Control 21.59±2.86 pg/ml; 25 BPA 24.41±2.59 pg/ml; 250 BPA 29.14±2.28 pg/ml;  $p>0.05$ ). At PND 110, animals exposed to 25 BPA exhibited an increased frequency of hyperplastic ducts (Control 9.02±0.78%; 25 BPA 18.18±2.44%;  $p<0.01$ ). Additionally, samples from animals exposed to 250 BPA showed a trend toward increased presence of hyperplastic ducts (12.48±0.79%;  $p=0.06$ ). The higher dose of BPA increased estrogen receptor alpha and decreased Steroid Receptor Coactivator-3 expression at PND 50 and PND 110 ( $p<0.05$ ). Silencing Mediator of Retinoic Acid and Thyroid Hormone Receptor (SMRT) protein levels were similar among groups at PND 50, whereas at PND 110, animals exposed to 250 BPA showed a lower SMRT expression. Interestingly, in the control and 25 BPA groups, SMRT increased from PND 50 to PND 110. At PND 50, an increased vascular area associated with higher Vascular Endothelial Growth Factor (VEGF) expression was observed in the 250 BPA-treated rats. At PND 110, the vascular area was still increased, but VEGF expression was similar to that of control rats. The present results demonstrate that prenatal exposure to BPA alters the endocrine environment of the mammary gland and its angiogenic processes. Increased angiogenesis and altered steroid hormone signals could explain the higher frequency of pre-neoplastic lesions found later in life in treated rats. (This study was supported by grants from the Argentine National Agency for the Promotion of Science and Technology and from Universidad Nacional del Litoral).

**The Marin Women's Study Biorepository**

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**Funding Acknowledgement:** Centers for Disease Control & Prevention, Avon Foundation for Women

**Background:** In response to the high rates of breast cancer seen in Marin County, Marin County Health & Human Services initiated the Marin Women's Study (MWS) in 2006, with initial funding from the Centers for Disease Control (CDC) and two additional grants from the Avon Foundation. An important element of the study is the collection and storage of biospecimens for examination of hormonal, genetic, and environmental exposures in the context of a large-scale epidemiologic study.

**Objectives:** To design data and biospecimen collection that allows for the study of a broad set of research questions now and in the future. To develop a biospecimen collection and storage infrastructure that minimizes participant burden and study costs and maximizes yield.

Specific study questions currently being addressed by biorepository specimens include: 1. Are systemic hormone levels associated with the characteristics of reproductive patterns, including age at first birth? 2. Can existing models used for calculating breast cancer risk be improved by the addition of salivary hormone levels and/or genetic data obtained from saliva specimens collected in the MWS? 3. Is the protective value of parity, age at first live birth, and breastfeeding history modified by individual genetic differences?

**Methods/Work Performed:** To date over 14,000 women have enrolled in the MWS and over 85% have consented to providing a saliva specimen. Collaboration with a local research institute, the Buck Institute for Age Research, in Novato, CA, was key to the successful establishment of the biorepository.

Consenting participants are mailed a saliva collection kit, which they complete at home and mail directly to the Buck Institute for processing and storage. The specimens are processed by Buck Institute personnel with separation of supernatant available for steroid hormone analysis, and a cellular component from which DNA is isolated using Invitrogen's PureLink Genomic DNA kits. This has resulted in the extraction of substantial quantities of high-quality purified DNA, adding the potential of performing genetic analysis to the hormone testing already being done.

**Results/Conclusions:** Over 8,000 saliva specimens have been collected and over 2,000 samples have been assayed for levels of estradiol, testosterone, DHEA, and progesterone. Funding has been received for studies examining the association between genetic information (SNPs) and breast density and breast cancer, and additional studies are planned. The MWS has had significant success with simple saliva collection methodology done noninvasively at home, and has demonstrated that saliva collection is a cost-effective approach for gathering biospecimens in large-scale epidemiologic studies.

**Marin Women's Study**

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**Funding Acknowledgement:** Centers for Disease Control & Prevention, AVON Foundation

**Background:** Marin County has one of the highest rates of invasive breast cancer in the world yet, no research had been done to examine individual-level risk factors and breast cancer outcomes in these women. In 2005, the Marin County Department of Health and Human Services (MCDHHS) partnered with community members and received the first of three CDC grants to explore the County's excess incidence.

**Hypothesis/Objectives:** Research aimed to examine known and suspected breast cancer risk factors in Marin County residents, to increase our understanding of the etiology of breast cancer and the reasons for excess cancer rates in the County. An overarching aim was to develop a resource-efficient infrastructure for collecting and storing data locally.

**Methods/Work Performed:** The Marin Women's Study (MWS) was structured to ensure ongoing liaison with the local community through formation of the Community Involvement Group (CIG), and with researchers and providers through formation of a Steering Committee. The CIG represents 15 Marin County based organizations. This group provided input and feedback throughout the development of MWS including questionnaire development, biospecimen collection, website development and community campaign assistance. The Steering Committee guides the design, structure, and conduct of the study and comprises local oncologists, surgeons, researchers, and advocates.

MWS is a cross-sectional study of the Marin mammography population, with cohort follow-up on cancer diagnoses and deaths. Primary data collection includes risk factor data gathered by questionnaire distributed at the time of mammography and biospecimen data (saliva), collected via mail. Secondary data collection of radiology data, cancer registry data, death data, and additional personal risk factor information is linked using the San Francisco Mammography Registry.

A community campaign to ensure that women obtaining mammograms were aware that they would be invited to participate at the mammography appointment was developed in collaboration with the CIG.

14,000 women have completed a questionnaire. 8,000 women have given saliva samples, 2100 of which have been assayed for estradiol, testosterone, DHEA, and progesterone. Response rates varied by site and by method of distribution of the questionnaire and enrollment materials, but were over 80% at primary site.

**Conclusions/Next Steps:** Continued linkages are performed annually to cancer, death, and radiology data. Plans are currently underway to collect longitudinal risk factor and other biospecimen data that could be used to examine factors that we cannot examine using saliva, including environmental toxins. Analyses of factors such as reproductive history and hormone therapy utilization are underway.

### Role of Activating Protein-1 and Antisense Progesterone Receptor (PR) mRNA in the Regulation of PR Isoforms in Mouse Mammary Epithelial Cells

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The progesterone receptor (PR) is an important transcription factor involved in key stages of mammary gland development and breast cancer. We recently discovered that the mouse PR locus contains an antisense mRNA transcript (PR<sup>antisense</sup> mRNA) which overlaps with the beginning of the PR gene. This unusual arrangement may be important because antisense RNA potentially modulates gene expression through a variety of novel mechanisms including transcriptional interference, mRNA stability, and translational efficiency. Adding to this complexity, there are two PR isoforms (PRA and PRB), whose expression in the mouse mammary gland is temporally and spatially regulated during development. PRA expression predominates in pubertal and virgin adult mice and PRB expression is restricted primarily to the period of alveologenesis during mid-pregnancy. Since PR<sup>antisense</sup> mRNA overlaps with the promoters that are believed to control PRA and PRB expression through separate PRA and PRB mRNA transcripts, antisense transcription may influence the balance between these two PR isoforms. Superimposed on hormonal influences are important growth factor pathways, many of which converge on the Activating Protein-1 (AP-1) family of transcription factors, consisting of homologous or heterologous dimers between several jun and fos family members. The 5'-flanking region of the mouse PR gene contains several predicted AP-1 binding sites, suggesting that its expression may be regulated not just by steroid hormones (estrogen and progesterone), but also by growth regulatory pathways that signal through AP-1 and other transcription factors. Our hypothesis, therefore, is that a change in the composition of AP-1 subunits is, at least in part, responsible for the shift from PRA to PRB expression during pregnancy. To test this hypothesis, we examined the behavior of sense and antisense promoter constructs in response to co-expression of different AP-1 isoforms and found that AP-1 containing either c-jun or JunB weakly but significantly up-regulated sense as well as antisense PR promoter activity, but that JunD uniquely increased the activity of the PR antisense promoter. Presence or absence of steroid hormones had only minimal effects on PR transcription. Of interest, co-expression of PR<sup>antisense</sup> mRNA appeared to decrease PRA protein expression from a co-transfected PR cDNA, as assessed by indirect immunofluorescence analysis. On the other hand, co-expression of PR<sup>antisense</sup> mRNA lacked any reproducible effect on the various PR promoter-reporter plasmids themselves. These results suggest that PR<sup>antisense</sup> mRNA can indeed interfere with the efficiency of PRA protein expression, but it likely does so by a co-transcriptional or post-transcriptional mechanism.

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### Using Action Tendency Emotions as Persuasive Tools for Mothers to Encourage a Healthy Diet for their Preadolescent Daughters in a Theory of Planned Behavior Framework

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This research was supported by the Breast Cancer and the Environment Research Centers grant number U01 ES012800 from the National Institute of Environmental Health Sciences (NIEHS), and the National Cancer Institute (NCI), NIH, DHHS.

**Background:** About 17% of American preadolescent girls are overweight. These girls are more likely to become overweight women, and more likely to incur weight-related conditions in adulthood, such as breast cancer. The diet of preadolescent girls is highly influenced by their mothers. Using the theory of planned behavior as a framework, the action tendency emotions of anger, sadness, and guilt were explored to determine what sources elicit these emotions and whether these emotions predict mothers' intent and encouragement of a healthy diet in their preadolescent daughters.

**Hypotheses:** Mothers' attitude (H1), subjective norm (H2), perceived behavioral control (H3) of encouraging a healthy diet for their preadolescent daughter(s), will be positive predictors of intent (H4) and actual encouragement of a healthy diet in their preadolescent daughter(s). H5: Mothers-in-law and other mothers will evoke anger. H6: Own mothers will evoke guilt. RQ1: Will own mothers, mothers in law, and other mothers differ in evoking sadness? Anger (H7) and sadness (H8) will be positive predictors of intent and encouragement, whereas guilt (H9) will be a negative predictor of intent and encouragement of a healthy diet by mothers to their preadolescent daughter(s).

**Methods:** In a 2 X 3 between subjects design, participants (N = 132) who were mothers of preadolescent daughters read a message from one of three sources (own mother, mother-in-law, or another mother). One message focused on the reduced likelihood of having an overweight daughter if a mother encouraged her to eat healthily and the second focused on the improvement of a daughter's general well-being now and as an adult if a mother encouraged her to eat healthily. Participants returned a week later (N = 32) to report on their behaviors.

**Results:** The TPB components of attitude and perceived behavior control predicted intent, but none of the hypothesized variables predicted behavior. A negative emotional response mechanism was found to be the only predictor of encouraging behavior, showing that as negative emotions were evoked mothers were less likely to encourage their daughters to eat a healthy diet. Mothers-in-law and other mothers evoked anger more than own mothers, and sadness was evoked more by other mothers. The overweight message from another mother was most likely to result in actual encouragement.

**Conclusions:** Practitioners should avoid aggravating this population as undesired behaviors may result. Messages from other mothers about reducing daughter's future chances of obesity were powerful predictors of actual encouragement.

### BCERC Collaborative Partnerships: Perspectives of a Breast Cancer Advocacy Organization

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**Background:** The Request for Application (RFA) for the Breast Cancer and the Environment Research Centers (BCERC) designated the Community Outreach and Translation Core (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 aim of the COTC was to build and promote partnerships between the BCERC researchers and the breast cancer advocacy community.

The Breast Cancer Alliance of Greater Cincinnati (BCA) has served as a collaborative partner with the Cincinnati BCERC since 2003. After six years, we wanted to determine the success and challenges from the collaboration.

**Objectives:** Conduct an evaluation of BCA members via a survey, focus groups and interviews to determine the lessons learned from this partnership.

**Methods:** The BCA received a programmatic capacity building grant from the National Breast Cancer Coalition Fund to conduct this evaluation. We hired Evaluations Services Center (ESC) from the University of Cincinnati to complete this work.

We mailed a survey to BCA members in August 2009 and conducted focus groups and phone interviews in September 2009 with a small group of BCA advocates who were both involved and not involved with BCERC.

**Results:** We plan to share the results from the survey, focus groups and phone interviews.

Some of the themes to be presented include: the motivation to be involved with this research, having advocates involved in meaningful ways, the benefits to the advocates and to the research process, if expectations were met and recommendations for future involvement.

**Conclusions:** As more research opportunities will require the need for advocate and/or community participation, it is important to document the successes and challenges of the work that we have done within the BCERC. Results from this evaluation process can be shared with other breast cancer advocacy organizations.

**Funding Acknowledgement:** The evaluation was paid for from a 2009 Programmatic Capacity Building Grant from the National Breast Cancer Coalition Fund.

### Factors Influencing Retention and Participation of Girls in a Longitudinal Study

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**Background:** The Growing Up Female study is the epidemiology project within the Cincinnati Breast Cancer and the Environment Research Center. Study participants have been seen semiannually at one of two sites: Cincinnati Children's Hospital Medical Center ("Clinic-Based Girls") or their local schools ("School-Based Girls"). During the planning stages of future studies, it is important to reflect upon the efficacy of this study design as it relates to retention and participation of young females.

**Hypotheses:** The purpose of this study is to determine the impact of various factors on retention and participation. We analyzed the impact of site, socioeconomic status (SES), and race on the following outcomes: completed visits, phlebotomy rate, questionnaire return rate, and retention (drop-out rate). We hypothesized that site would be the most influential factor.

We also projected that School-Based Girls would be more likely to complete visits than Clinic-Based Girls, while Clinic-Based Girls would be more likely to complete phlebotomy, return questionnaires, and drop out.

**Methods:** Participation and retention data were obtained from study logs. Race and SES (defined by income) were obtained from the year one questionnaire. SAS and SPSS were used to perform the analyses.

**Results:** In bivariate analyses, site, SES, and race had statistically significant impacts on visit completion ( $p < 0.001$ ,  $p < 0.001$ , and  $p < 0.005$ , respectively). There were no significant predictors of phlebotomy rate. Race alone had a statistically significant impact on questionnaire return rate ( $p < 0.05$ ). Site alone had a statistically significant impact on drop-out rate ( $p < 0.05$ ). Multivariate analysis revealed that site and SES were both significant predictors of visit completion ( $p < 0.02$  and  $p < 0.002$ , respectively).

**Conclusions:** Our analyses revealed School-Based Girls and subjects with higher SES were more likely to complete visits, and Clinic-Based Girls were more likely to drop out. In addition, our analyses showed that SES had a greater impact than site on visit completion; race fell out of this analysis when site and SES were included in the model.

### Childhood Hair Product Use and Age at Menarche in a Racially Diverse Study Population

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**Background:** Recent studies suggest that some hair products may contain exogenous hormones and endocrine disruptors (EDCs). African-Americans are more likely to use hair products. EDCs in hair products could alter normal hormone functioning and increase the risk of early puberty and menarche.

**Objective:** To evaluate the relationship between childhood hair product use and age at menarche and to determine whether product use mediates the relationship between race/ethnicity and age at menarche.

**Methods:** We evaluated the association between hair product use and age at menarche among African-American, African-Caribbean, Hispanic, and white women. We analyzed data on a convenience sample of 301 women. Data was collected on hair oil, lotion, leave-in conditioner, perm, and other types of hair products used prior to age 13. We used logistic regression to estimate the association between product use and early menarche (<12 years), adjusting for sociodemographic characteristics.

**Results:** African-Americans were more likely to use hair products and reach menarche prior to age 12 compared to whites. Women reporting childhood hair oil use had 2.5 times the odds of reaching menarche <12 years (95% CI: 1.3-5.2), adjusting for race/ethnicity, recruitment site, and year of birth. After adjustment, hair perm use conferred 2.3 (95% CI: 1.1-5.0) times the odds of early menarche. Hair oil use partially mediated the association between race/ethnicity and age at menarche.

**Conclusions:** Childhood hair oil and perm use increased the risk of early menarche and oil use partially explained earlier menarche in African-Americans. Future studies should evaluate commonly used hair products for endocrine disrupting activity.

### Breastfeeding versus Formula-Feeding and the Girls' Pubertal Timing

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**Background:** Previous studies document associations between breastfeeding and physical development (Martin *et al.*, 2002). Others have found correlations between peri-natal nutrition, infant weight gain and age at menarche (Ong *et al.*, 2009). Ethnicity also influences age at menarche (Anderson *et al.*, 2003). This research focuses on the effect of breastfeeding on pubertal timing, and the influences of ethnicity and BMI on this relation. As early menarche indicates an increased risk of breast cancer (Macmahon *et al.*, 1982), this study is particularly important due to the apparent decline in female pubertal age in United States.

**Objective:** To examine the effect of breastfeeding/formula-feeding on girls' pubertal timing and BMI.

**Method:** The proposed study is based on a sample of 444 socio-economically and ethnically diverse young girls, ages 6-8 years, who are participants in the CYGNET study (PI-Lawrence Kushi<sup>1</sup>). Correlations will be examined between early post-natal nutrition (breastfeeding/formula-feeding), infants' duration of exposure to breastfeeding/formula-feeding and girls' pubertal timing, indicated by breast and pubic hair development (Tanner staging), at several time points in the study (baseline: 6-8y; follow-up #1: 7-9y; follow-up #2: 8-10y). Covariates such as race/ethnicity and peripubertal BMI will also be considered in terms of potential mediating, confounding and/or moderating relationships.

**Preliminary Observations:** In the cohort, 94% mothers had breastfed their daughters, while the other 6% girls were exclusively formula-fed. At follow-up #2 for the total cohort, pubertal onset has occurred in 37% (per Tanner breast staging) and 29% (per Tanner pubic staging) girls. Among the breast-fed girls, 36% (Tanner breast) and 27% (Tanner pubic) of girls had attained puberty. On the other hand, among the formula-fed girls, 48% (Tanner breast) and 35% (Tanner pubic) of girls had attained puberty. Furthermore, in girls who were breastfed for 3 months or more (88% of all breastfed girls), pubertal onset has occurred in 35% (Tanner breast) and 26% (Tanner pubic). In girls breastfed less than 3 months (12% of all breastfed girls), onset of puberty has occurred in 45% (Tanner breast) and 34% (Tanner pubic).

**Implications:** Post-natal nutrition is a potential modifiable risk factor for early puberty, and specific interventions can be targeted in this domain to promote health in the short-term, and potentially ameliorate risk for breast cancer later in life. As these data are further examined, the findings have potential to provide more insight into causal pathways and thereby help identify appropriate targets for preventive interventions of breast cancer.

### Maternal dioxin exposure combined with a diet high in fat increases mammary cancer incidence

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**BACKGROUND:** Epidemiological studies show that breast cancer risk correlates with total lifetime exposure to estrogens and that early life 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure or diets high in fat can also increase cancer risk.

**OBJECTIVES:** Because both TCDD and diet impact the estrogen pathway, we examined how TCDD and a high-fat diet (HFD) interact to alter breast cancer susceptibility.

**METHODS:** At 12.5 days post coitus, we exposed pregnant FVB/NJ female mice to 1 µg/kg of TCDD or vehicle and at parturition randomly assigned dams to a low-fat diet (LFD) or HFD (n = 27 dams). Female offspring were maintained on the same diets after weaning and exposed to 7,12-dimethylbenz[a]anthracene (DMBA) at post-natal days (PND) 35, 49 and 63 to initiate mammary tumors. A second cohort of females was treated identically until PND 35 or 49, when mammary gland morphology was examined, or at PND 50, when mammary gland mRNA expression was analyzed (n = 20 dams).

**RESULTS:** We found that maternal TCDD exposure doubles mammary tumor incidence only in mice fed HFD (p-value < 0.05). Among mice fed HFD, maternal TCDD exposure caused rapid mammary development with 1.9 fold increased *Cyp1b1* and decreased *Comt* (0.5 fold) expression in mammary tissue relative to vehicle exposed mice fed HFD (0.9- and 0.8- fold, respectively, p-value < 0.05). Mammary tumor *Cyp1b1* expression was further increased to 15.0 fold by maternal TCDD exposure in mice fed HFD compared to vehicle exposed mice fed HFD (3.7 fold, p-value < 0.05).

**CONCLUSIONS:** Our data suggest that HFD increases sensitivity to maternal TCDD exposure, resulting in increased breast cancer incidence, by changing estrogen metabolism capability. These results provide a mechanism to explain epidemiological data linking early life TCDD exposure and diets high in fat to increased risk for breast cancer in humans.

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### Measuring the Neighborhood Environment: Associations with Young Girls' Energy Intake and Expenditure

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**BACKGROUND:** Neighborhoods environments affect children's health outcomes. Observational methods used to assess neighborhoods can be categorized as indirect, intermediate, or direct. Direct methods are recognized as an accurate representation of current neighborhood conditions. **OBJECTIVE:** The authors investigated the associations of various neighborhood characteristics with young girls' diet and physical activity, using both direct and indirect methods of neighborhood assessment. **METHODS:** This study is based on a subset of participants in the Cohort Study of Young Girls' Nutrition, Environment, and Transitions (CYGNET). In-person street audits were conducted on 2,328 street segments within 215 girls' residential neighborhoods using a modified St. Louis Audit Tool. Exploratory factor analysis revealed five scales: "mixed residential and commercial," "food and retail," "recreation," "walkability," and "physical disorder." A Neighborhood Deprivation Index was also derived from census data. The authors investigated if these six neighborhood measures were associated with quartiles of total energy intake and expenditure (metabolic equivalent (MET) hours/week). **RESULTS:** After adjustment for demographic characteristics, there was an inverse association between prevalence of "food and retail" destinations and total energy intake (for a one quartile increase, OR=0.84, 95% CI 0.74, 0.96). Among Hispanic/Latina girls, positive associations were observed between the "recreation" and "walkability" scales with physical activity (for a one quartile increase in METs, OR=1.94, 95% CI 1.31, 2.88, for recreation; OR=1.71, 95% CI 1.11, 2.63, for walkability). There was also a strong inverse association between "physical disorder" and physical activity among African-American girls (OR=0.31, 95% CI 0.12, 0.80). **CONCLUSIONS:** Results suggest that neighborhood food and retail availability may influence children's energy intake differently from that of adults. There is also variation in neighborhoods' influences on children's physical activity behaviors, particularly for children of different racial/ethnic backgrounds.

### Changes in Tumor Suppressor and Oncogene Expression Induced in the Mammary Gland of Animals Exposed to BPA, BBP, and TCDD at different developmental stages.

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Xenobiotics, such as Bisphenol A (BPA), n-benzyl butyl phthalate (BBP) and 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), are endocrine disruptors that can affect the susceptibility of the mammary gland to carcinogenesis. This study investigates whether exposure to these compounds at different stages (prenatal (PN), prepubertal (PP)) and dose levels (low, high), affects the gene expression profile of oncogenes and tumor suppressor genes in the rat mammary gland at different ages. To examine the significance of biological functions represented by the genes differentially expressed between exposed and non-exposed rat mammary glands at 21, 35, 50 and 100 days we used GO annotations, that describe groups of genes in terms of their associated biological processes. Through GOstats tool provided by Bioconductor, conditional hypergeometric test statistics were performed to evaluate the overrepresentation of GO terms among statistically differentially expressed genes. No significant overlap of enriched GOs among these xenoestrogenic compounds was found. For further analyses, GO terms were combined according to their commonality of biological functions in a variety of groups related to oncogenes (Onc) and tumor suppressor (TS) genes. A list of 327 known and novel oncogenes was annotated using GeneRIF information from Gene Database of NCBI. A similar list of over 550 tumor suppressor genes was annotated. Rat homologues were taken and viewed as heat maps with TCDD having the largest effect on the expression of tumor suppressor genes.

Prenatal exposure to TCDD induced the highest amount of down-regulated and up-regulated genes at 100 days of age (Onc: 59 Upreg and 52 downreg.); whereas there were no changes in gene expression at 21 days of age. Neither BAP nor BBP treatments resulted in changes in gene expression at 100 days. However, both BBP and BPA showed the highest change in gene expression at 35 and 50 days for both oncogenes and tumor suppressor genes (BBP PN 35 days Onc: 15 upreg. and 28 downreg.; BBP PN 50 days Onc: 3 upreg. and 3 downreg./ BBP PN 35 days TS: 30 upreg. and 25 downreg.; BBP PN 50 days Onc: 4 upreg. and 6 downreg.) (BPA PN 35 days Onc: 8 upreg. and 9 downreg.; BPA PN 50 days Onc: 10 upreg. and 8 downreg./ BPA PN 35 days TS: 15 upreg. and 8 downreg.; BPA PN 50 days Onc: 3 upreg. and 10 downreg.).

Conclusions: 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.

Our data strongly indicate that each xenobiotic agent induces a set of specific changes in the mammary gland that may lead to different biological responses.

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### TGFβ mediates persistent gene expression changes in mouse mammary gland after low dose ionizing radiation exposure.

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Ionizing radiation (IR) is a known breast carcinogen that increases risk starting at doses above 0.5 Gy, which is encompassed in the range of diagnostic and therapeutic exposures. In addition to its carcinogenic action as a DNA mutagen, IR activates the cytokine TGFβ1 in the stroma of the mouse mammary gland, which then signals to alter extracellular composition and mediate epithelial cell fate. Microarray expression profiling has revealed distinct gene expression changes elicited by high or low doses of IR. We examined if an acute whole body low dose (0.1 Gy) would elicit a detectable gene expression program in the mammary gland. 10 week-old wildtype and *Tgfβ1* +/- Balb/c littermates were injected with an estrogen and progesterone mixture to synchronize estrus two days before irradiation. At 1 or 4 weeks post-radiation inguinal mammary glands were harvested for RNA extraction and genome-wide microarray profiling. Herein we report that an acute whole body dose of 0.1 Gy elicits persistent changes in hundreds of genes that are evident at least until 4 weeks after exposure, and that TGFβ1 levels modulate a distinct gene expression program in response to IR. Altered genes include those suggesting epithelial differentiation, stromal remodeling, and stress. Consistent with this role of TGFβ1 in gene expression changes, we have previously shown that acute low dose IR can increase frequency and shorten latency of tumors resulting from unirradiated *Trp53 null* mammary fragments transplanted into mammary glands of syngeneic wildtype Balb/c mice that were previously irradiated with 0.1-1.0 Gy, but only in *Tgfβ1* wild type hosts. Identical transplants into haplo-insufficient *Tgfβ1* +/- mice nullified the promotion (Nguyen et al. submitted). Together, these data suggest that IR, a known environmental carcinogen, has dual actions as a mutagen and significantly via the host. Furthermore, TGFβ1 is a key mediator of the mammary response to radiation exposure both in preneoplastic states and in tumorigenesis.

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**Bisphenol A: From public education to political advocacy.** Janet Nudelman<sup>a</sup>, Connie Engel<sup>a</sup>, Janet Gray<sup>a,b</sup>, Jeanne Rizzo<sup>a</sup>, *Breast Cancer Fund, San Francisco, CA;* <sup>b</sup>*Vassar College, Poughkeepsie, NY*

**Background:** Bisphenol A (BPA) is an endocrine disruptor that has been linked to increased risk for many diseases, including breast cancer. Of special concern is the growing scientific evidence in rodent models demonstrating that prenatal or neonatal exposures to environmentally significant levels of BPA predispose an animal to increased risk for mammary cancer.

**Objective:** Given the ubiquity of this chemical in our environment and in our bodies, the Breast Cancer Fund (BCF) has set as main objectives educating the public about the growing science around BPA's link to breast cancer, and leading a coalition of environmental health groups in advocating for public policy change that eliminates exposures to BPA, especially for our most vulnerable citizens.

**Work Performed:** Through scientific publications including the signature piece, *The State of the Evidence: The Link between the Environment and Breast Cancer*, which was published both as a white paper and as companion scientific and public policy pieces in a peer-reviewed journal, BCF has (1) translated the complex empirical data surrounding the broad topic of environmental links to breast cancer, including the data on BPA, for diverse audiences; and (2) generated scientifically driven breast cancer prevention policy and research agendas for the public and policymakers.

In particular, BCF has taken a lead role in efforts to educate Congressional representatives and their staff about the science surrounding BPA's links to breast cancer and other diseases, supporting the introduction by Rep. Markey (D-MA) and Sen. Feinstein (D-CA) of the Ban Poisonous Additives (BPA) Act of 2009, and leading a broad based coalition effort to secure passage of this a critical piece of legislation. . Concurrently, BCF has been a leader in collaborative efforts with environmental health and environmental justice organizations to affect chemical regulatory change at the state and federal levels.

**Results and Conclusions:** The Breast Cancer Fund is committed to supporting the identification and eradication of preventable causes of breast cancer, especially those that are present as pollutants in our every day life. BCF has been an innovator in public education on these issues and is a leader in the translation of science into public policy advocacy.

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**Lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters proliferation and gene expression of the rat mammary gland during development**

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**BACKGROUND:** 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a member of the chlorinated dioxins and one of the most potent environmental contaminants.

**OBJECTIVES:** This work studied cell proliferation and gene expression in mammary glands of 21, 35, 50 and 100 days old rats exposed to TCDD during lactation (prepubertal treatment).

**METHODOLOGY:** To study prepubertal exposure, on the 14<sup>th</sup> and 17<sup>th</sup> days post-delivery, lactating dams received 0, 6.67ng or 20.0ng TCDD/g BW, via gavage. When the female offspring reached 21, 35, 50 and 100 days of age, they were sacrificed and their mammary glands extracted for cell proliferation or gene expression analyses. Proliferation was analyzed in ten rats/group using 5-bromo-2'-deoxyuridine (BrdU). Another ten rats/group were processed for gene expression analysis using Agilent microarray platform. Differentially expressed genes (p<0.01) were determined by empirical Bayes moderated one sample t-test, and categorized accordingly to biological functions and canonical pathways. Important genes were validated using real time RT-PCR.

**RESULTS:** Prepubertal TCDD treatment reduced cell proliferation in the 35 and 50 day-old rats and increased it in the 100 day-old rat mammary glands, mainly in the terminal end buds. TCDD induced changes in the expression of genes with a wide range of biological functions, especially with the higher dose. The most activated canonical pathway was via aryl hydrocarbon receptor signaling. Cyp1a1 and Cyp1b1, important genes in estradiol biosynthesis and metabolism, were highly up regulated at different ages. Following this pathway, we found other enhanced genes (Hsd17b12 and Sod1), while others had no alteration (Comtd1 and Gstp1), indicating that this pathway was inducing the production of genotoxic metabolites. In addition, several of the dysregulated genes were related to protection against establishment of mutations (DNA damage repair, apoptosis, and tumor suppressor genes), among other groups of genes, such as, oncogenes, lipid metabolism and immunity.

**CONCLUSIONS:** TCDD induced changes in cell proliferation and gene expression of the rat mammary gland. The genomic changes observed indicate that the mammary cell has been submitted to genotoxic stimuli and DNA repair genes, such as tumor suppressor genes, are activated to maintain the genomic stability.

(This work was supported by grant U01 ES/CA 12771 from NIEHS and NCI)

### **The Sisters are Doing It for Themselves: Putting Knowledge to Use in Bayview Hunters Point**

Karen J. Pierce JD (*San Francisco Department of Public Health/Bayview Hunters Point Community Advocates*), Mary K. Anglin PhD, MPH (*University of Kentucky*), Gail Bishop (*The Sisters Network, San Francisco*), Barbara Cicerelli MPA (*San Francisco Department of Public Health, Breast and Cervical Cancer Services*), Alyson O'Daniel MA (*University of Kentucky*), and Maya Yoshida-Cervantes (*San Francisco State University*)

**Background:** The poster addresses the category of community outreach/engagement, and is the product of collaboration between community activists, public health practitioners, and academic researchers. We focus on Bayview Hunter's Point (BVHP) as a district within the City and County of San Francisco where the residents are predominantly African American and low income; practices of community activism are well-established; there is a long history of hazardous industry and environmental contamination; and elevated rates of breast cancer incidence and mortality have been documented.

**Objectives:** The objectives of this poster are to:

1. Present case studies based on the work of the Breast Cancer Town Hall Collaborative (1998-2004), the Sisters Network, San Francisco Chapter (founded 2001), and the Bayview Hunters Point Community Advocates (founded 1994);
2. Describe how their community-based projects have applied scientific information from epidemiologic and clinical research on breast cancer, as well as residents' experiential knowledge;
3. Demonstrate the significance of community-researcher-practitioner partnerships in efforts to address health inequalities.

**Methods:** Using ethnographic field notes, interviews, focus group data, audio-visual records, minutes and reports of meetings, and firsthand accounts, we provide empirically based descriptions of each case study.

**Results and Conclusions:** Scientific information about breast cancer, including research on environmental exposures, has been effectively utilized through advocacy networks, educational forums, and citizen efforts to change public policy. By combining locally derived information with the results of scientific research, community initiatives in BVHP contribute to the production of knowledge on the social determinants of health.

For support of the projects described in the poster, evaluation of the Community Town Hall Meetings on Breast Cancer, and qualitative research on breast cancer health inequalities in the San Francisco Bay Area, the authors acknowledge funding from: the California Pacific Medical Center Foundation, Friends of Faith, College of Arts and Sciences at the University of Kentucky, San Francisco Department of Public Health, Susan G. Komen Foundation, and The San Francisco Foundation.

### **Development of an Online Database of Laboratories for Measurement of Exposure Biomarkers (MEB-Lab)**

Susan M. Pinney<sup>1</sup>, Peter J. Embi<sup>2,3</sup>, Robert A. Witzke<sup>3</sup>, Emily Jones<sup>1</sup>, <sup>1</sup>University of Cincinnati College of Medicine, Dept. of Environmental Health, Cincinnati, OH, USA; <sup>2</sup>University of Cincinnati College of Medicine, Dept. of Internal Medicine, Cincinnati, OH, USA; <sup>3</sup>University of Cincinnati College of Medicine, Center for Health Informatics, Dept. of Public Health Sciences, Cincinnati, OH, USA.

**Background and Objective:** Environmental epidemiology is a rapidly expanding field, fostered by the ever-increasing capacity to measure internal exposure to environmental chemicals. The Breast Cancer and Environment Research Centers epidemiology studies have very successfully used the CDC Environmental Health Laboratory for their environmental biomarker measurements. However, these laboratories support many other studies, and are not always available to take on additional work or able to process samples quickly when preliminary data are needed for grant applications. We have designed and developed a Web-based database of laboratories capable of measuring exposure biomarkers for research studies (MEB-Lab database) and offer it as a resource to the community of environmental scientists.

**Methods:** Information to populate the database was initially gathered from the websites and other printed materials of relevant laboratories across the US. To augment this information, we also contacted by mail/email these laboratories asking for additional details. Data elements requested include the laboratory name, location, contact information, link to website, chemical biomarkers measured, analytic technique, limits of detection specific to the biologic media, and laboratory publications.

The backend of the system was developed by using a MySQL database instance, and the frontend interface was developed using HTML and PHP. After an iterative process of initial user needs assessment conducted with local domain experts, we designed the front-end interface to enable users of the system are to invoke simple standard queries to obtain information about laboratories that are capable of measuring a specified environmental biomarker or class of biomarkers, or to obtain information about the biomarkers measured by a specified laboratory.

**Results:** The MEB-Lab searchable online database currently contains detailed information as indicated above from about 44 laboratories capable of measuring exposure biomarkers. Each laboratory is linked to information about 400 environmental biomarkers within 16 chemical classes. Initial testing indicates that the system functions as intended. The database can be accessed through the University of Cincinnati Center for Environmental Health website [www.eh.uc.edu/ceg](http://www.eh.uc.edu/ceg).

**Conclusions:** The MEB-Labs online database is a first-of-its kind, openly accessible resource that addresses a need for the environmental epidemiology community. Ongoing work includes continued communication with and updating of laboratories' information and the addition of an online evaluation module that prompts users to provide feedback to help us modify and improve future versions of the system.

Support for this project provided by the National Institute of Environmental Health Sciences to the University of Cincinnati Center for Environmental Genetics (P30-ES06096).

### **The Long Island Teen Environmental Activists (LITEA)**

Rubab Rehman\*

**Background:** The Long Island Teen Environmental Activists (LITEA) is a community based, volunteer driven organization comprised of like-minded high school teenagers who wish to heighten the public's awareness of environmental pollutants and their effects on human health.

**Hypothesis/Objectives:** It is our goal to identify toxic triggers within our environment that mimic endocrine-disrupting cells and potentially contribute to breast cancer. We believe that if today's youth become advocates of environmental safety then it will compel the local community to take action.

**Work Performed:** At monthly meetings, students discuss methods on how to reduce their exposure to these deleterious contaminants through interactive methods such as quiz bowls and movie nights. This past March, students presented testimony in front of the Suffolk- County Legislature to secure the "first in the nation" ban on Bisphenol-A (BPA), known as the Toxin Free Toddlers and Babies Act. The chemical BPA, which is used to make certain plastics and canned food liners, has been linked to breast cancer. Furthermore, last year, members cleaned a portion of Heckscher State Park in celebration of Earth Day. **LITEA** initiated a school-wide recycling program, which will be implemented soon at Walt Whitman High School. This year, LITEA members intend to visit local middle schools to educate students on how many heavy metals have been identified as environmental estrogens. These metals are found in electronics that many teenagers use, such as cell phones, and may end up in landfills. LITEA members have certainly put their newfound knowledge to good use. They take a cautious approach when purchasing household products and are on the lookout for goods that may contain environmental estrogens by clearly reading ingredient labels. Some of them write articles about the information they have learned for international publications such as Teen Ink Magazine and Pakistan Post. Others aspire to pursue careers in environmental advocacy or environmental health.

**Conclusions:** LITEA members have realized that there is in fact no planet B, and it is up to us to actively make sure that our planet Earth will continue to be habitable for future generations. They feel it is their obligation to try and eradicate the illnesses that are plaguing the environment and take proactive measures to preserve it.

Rubab Rehman was supported by Huntington Breast Cancer Action Coalition scholarship.

### **An Unexpected Excess of Breast Cancer among Young U.S. Asian Women**

P. Reynolds, S. Hurley, D. Goldberg, R. Rull, T. Quach, J. Von Behren, *Northern California Cancer Center, Berkeley, CA*

The objective of this analysis was to compare the risk of breast cancer among young African American (AA) and Asian/Pacific Islander (API) women to white women, all of whom were born in California during the 1960s. Invasive breast cancer cases diagnosed in California 1988-2004 were identified from the California Cancer Registry (CCR). Probabilistic record linkage was used to link breast cancer cases to their California birth records. Population controls were selected from California birth records, frequency matched to cases 2:1 on year of birth. Race/ethnicity was abstracted from the birth certificates. Odds ratios (ORs) and 95% confidence intervals (95% CI) were estimated using unconditional logistic regression. Analysis was conducted on 3,799 cases of breast cancer and 8,789 controls. Our study population was very young (age range for cases = 22 to 44 years) and predominantly white (87%) but included substantial numbers of AA (n=1,207) and API (n=422) women. Compared to young white women, AA women (OR=1.6, 95% CI: 1.4 – 1.9) and API women (OR = 1.3, 95% CI: 1.1 – 1.6) had higher risks of breast cancer. Among API women, the risks were highest among women of Japanese descent (OR=1.5, 95% CI 1.1 – 2.1). Our finding of an elevated risk of breast cancer among young API women contradicts the lower incidence rates among women of this age group reported by national surveillance data. This may be due to the fact that a large proportion of API women in the U.S. are foreign-born while all the women in this study were California-born. Our findings suggest the need for further study of breast cancer risk among young API women and underscore the need to take into account nativity in such evaluations. Breast cancer disparities in this rapidly-growing population may serve to inform cancer control efforts.

### **Mammary Gland Carcinogens and Endocrine Disrupting Chemicals Should Be Disclosed on Personal Care and Cleaning Product Labels**

Zoe Schacht-Levine, *Great Neck Breast Cancer Coalition*,  
Aliyah Cohen, *Huntington Breast Cancer Action Coalition*,  
*Silent Spring Institute*

Collaborating with two Long Island breast cancer coalitions, Silent Spring Institute offered internships for two Long Island High School students. The Silent Spring Institute is developing recommendations to help consumers select products to reduce exposures to chemicals that can affect breast cancer risk, including mammary gland carcinogens and endocrine disruptors. The purpose of our summer research project was to determine if it is possible for an average consumer to follow the Institute's selection criteria for personal care and cleaning products relying only on ingredient labels.

After visiting two stores, one being conventional and one alternative, we analyzed over 300 product labels. Notes were taken regarding the price, which ingredients were on the criteria list, and eco-claims made by the manufacturer. We collected and analyzed the data in Excel, quantifying differences between the alternative and conventional products, and noting challenges we encountered in applying the criteria.

We found that not all products in conventional stores are less expensive than those found in alternative stores. Also, just because a product is advertised as alternative, it doesn't mean that the product meets the criteria. The same holds true for conventional products in that some products marketed towards the more conventional shoppers may meet the criteria and have a significantly lower exposure rate when compared to an "alternative" product. These and additional comparisons are important to consumer education and may lead to limiting exposure to harmful chemicals.

We found it very challenging for the average consumer to follow the criteria list. Ingredient labels tended to present misleading eco-claims. Manufacturers also neglected to disclose all ingredients because there are no regulating laws. As a result, consumers are left uncertain of the product's contents. It took about five minutes to collect data from each label, while the average consumer typically does not have time to research each purchase.

The following federal policies should be implemented and enforced to assist consumers: 1) disclosure of all ingredients on product labels, 2) labels must identify ingredients as carcinogenic or endocrine disruptors, and 3) manufacturers may not falsely market products as "eco- safe" or "natural".

If producers of personal care and cleaning products did not neglect to disclose all ingredients to the consumer and carried out safety tests for their products, the health of the general public would improve as exposure rates to mammary gland carcinogens and endocrine disrupting chemicals diminish.

### **Effects of Progesterone on Inflammatory Processes in the Mammary Gland**

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Progesterone (P) is a steroid hormone that promotes cell proliferation and development of the normal mammary gland. However, long term treatment with P, either alone or in combination with estrogen (E), promotes the development of mammary cancer in rodents and humans. The latter is evidenced by the increased risk of breast cancer in women receiving postmenopausal hormonal therapy that includes progestin. We recently showed that exposure to physiological levels of P causes an inflammatory response in the mammary glands of mice involving the production of inflammatory cytokines and chemokines, and resulting in the recruitment of leukocytes to the mammary peri-epithelial stroma. The role of hormones, P in particular, in mediating an inflammatory response in the mammary gland has not been previously recognized. Leukocyte populations including macrophages and eosinophils are known to be crucial for the tissue remodeling that takes place in normal mammary gland development. These same inflammatory processes that recruit leukocytes can promote tumorigenesis in the mammary gland and other tissues. The ability of P to mediate an inflammatory response in the mammary gland presents one plausible mechanism underlying the promotional effects of P in mammary tumorigenesis.

In order to investigate the role of P in recruiting specific types of leukocytes in the mammary gland, female adult BALB/c mice were ovariectomized and injected either with vehicle (C), E alone, P alone, or the combination of both E and P. Fixed sections of mammary glands from the various treatment groups were stained for leukocyte cell types that have previously been implicated in mammary gland development: macrophages were stained for F4/80 and eosinophils were stained with Vital New Red. Three animals received each treatment, and between 25 and 150 total structures evaluated for each animal. P treatment increased the levels of macrophages (duct ends, 7-fold,  $p < 0.05$ ) and eosinophils (duct ends, 59-fold,  $p < 0.01$ ) observed relative to C and E, especially in the stroma near duct ends and small ducts, as well as near alveolar and lobular structures that develop after hormone treatment. Treatment with E+P suppressed, but did not eliminate, leukocyte recruitment.

In summary, P treatment produced profound alterations in leukocyte populations in the peri-epithelial stroma of adult virgin mammary glands. The recruitment of macrophages and eosinophils, previously known to be involved in mammary ductal development, is consistent with P induction of inflammatory processes having a crucial role in mammary development.

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### Personal Care Product Use and Endocrine Disrupting Chemicals in Urban Minority Children

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**Background:** Concern about the effects of endocrine disruptors on early child development has increased as information about widespread use and biological activity becomes available. Urinary phthalate concentrations have been associated with personal care product (PCP) use. Parabens are often contained in PCPs; but associations with urinary paraben concentrations have not been examined.

**Methods:** In a cohort study of Hispanic and Black, New York City children ages 6 to 8 years old, parents/guardians were interviewed in-person about their child's environmental exposures and neighborhood characteristics. Child urine samples were analyzed by the CDC for phthalate and phenol metabolites; these were summed on a molar basis and then expressed as ug/L based on MEP, MEHP or propyl paraben. Biomarkers were compared across groups based on frequency of PCP use over the past year (<1, 1-4, or 5-7 days/week). Models were adjusted for age, race/ethnicity, gender, body size, SES, residence type and creatinine. Analyses include data from 406 girls and 102 boys.

**Results:** Products with the highest frequency of use over the past year were body lotion, shampoo, and chapstick (Mean: 5.5, 2.9, 2.6, days/week, respectively). Perfume use was significantly associated with higher monoethyl phthalate concentrations ( $p < 0.0001$ ; 147.1, 171.2, 257.8 ug/L by frequency category of use, respectively). Additionally, more frequent use of lotion was associated with higher paraben concentrations ( $p = 0.01$ ; 61.7, 108.8, and 116.4 ug/L by frequency category of use, respectively). Shampoo use was associated with low molecular weight phthalates ( $p < 0.01$ ). Product use and biomarker levels were also examined in relation to the children's neighborhood environment.

**Conclusion:** Phthalates and parabens have been widely detected in children and our findings indicate that PCPs may be sources of exposure. Therefore, reducing children's PCP use should be considered as a method for decreasing their exposure to these chemicals.

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**Disclaimer:** The findings and conclusions in this presentation are those of the authors and do not necessarily represent the official position of the CDC.

### Phenols and Breast Cancer Risk among Residents of Long Island, NY

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It is unknown whether the phenols that are frequently used in personal care products increase breast cancer risk. Prior laboratory studies have shown that some of these chemicals have estrogenic properties. We investigated the association between breast cancer risk and urinary concentrations of parabens, triclosan, and benzophenone-3 (BP-3) among women living in Long Island, NY from 1996-1997.

**Methods:** The Long Island Breast Cancer Study Project is a population-based case-control study with 1508 newly diagnosed cases and 1556 controls. Comprehensive risk factor information was collected through in-person interviews. A random sample (400 cases and 400 controls) was selected for analysis of urinary phenol compounds by solid phase extraction coupled to high performance liquid chromatography-isotope dilution tandem mass spectrometry. Creatinine-corrected biomarker concentrations were quantiled according to the control distribution (tertiles or non-detect/<median/≥median). Odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression. All models were adjusted for age (at diagnosis for cases or at identification for controls). A wide range of known and suspected breast cancer risk factors was assessed as potential confounders; none changed the estimates of effect by more than 10%.

**Results:** Phenol concentrations measured in urine collected over a decade ago from these women were similar in magnitude to recent US National Health and Nutrition Examination Survey measurements; however absolute differences between population medians suggest that paraben concentrations may have been slightly higher while BP-3 and triclosan concentrations may have been slightly lower. Women with the highest methyparaben concentrations were at increased risk of breast cancer compared to those with the lowest concentrations ( $OR_{age-adj} = 1.50$ , 95% CI=1.07-2.12,  $p_{trend} = 0.02$ ). Similarly, higher triclosan concentrations were associated with increased breast cancer risk ( $OR_{age-adj} = 1.49$ , 95% CI=1.06-2.10,  $p_{trend} = 0.02$ ). No association between breast cancer risk and BP-3 was observed. Results for quantiles based on uncorrected biomarker concentrations were similar.

**Discussion:** Few modifiable breast cancer risk factors are known. The chemicals examined in this study are found in commonly used personal care products. Should these findings be confirmed, reduced use of such phenol-containing products could potentially lead to decreased breast cancer risk.

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### **Alterations in mammary gland function and reproductive capacity in CD-1 mice exposed perinatally to bisphenol-A**

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Bisphenol A (BPA) is used in the manufacture of polycarbonate plastics and epoxy resins; thus, exposure to BPA occurs through the use of many consumer products, dental and medical materials. Pups born to mothers exposed to BPA during gestation present a plethora of health effects that appear long after exposure has ended. Our studies showed that female mice perinatally exposed to low doses of BPA have altered mammary gland (MG) development at all ages. We also observed alterations in reproductive tract, hypothalamus, gonadotropin levels and estrous cyclicity in the exposed offspring. We hypothesize that the first generation (F1) of female mice perinatally exposed to BPA (*BPA daughters*) will develop abnormal MGs during pregnancy and lactation, and their reproductive success will be compromised.

Pregnant CD-1 mice were exposed to 50% DMSO (vehicle-control), 25ng, 250ng or 25µg BPA/kg body weight/day via an osmotic pump. The *BPA daughters* were exposed from the end of embryonic day 8 until postnatal day (PND) 16. At two months of age all F1 mice were mated. MGs were collected 1) on the day of delivery, or 2) on lactation day 15 (N=15-20/treatment). In a cross-fostering experiment, pups born to unexposed dams and nursed by the 250ng *BPA daughters* gained significantly more weight (11.45±0.2g) than pups of *BPA daughters* nursed by unexposed dams (10.56±0.37g). Similar results were observed when the 250ng *BPA daughters* were mated at 6 months of age and allowed to nurse their own pups. The MGs of all *BPA daughters* were studied and subtle changes in their development were observed which suggests the *BPA daughters'* MG development and function is altered during pregnancy and lactation.

Additionally, *BPA daughters* were housed with fertile males for a period of 8 months. *BPA daughters* exposed perinatally to 25ng or 25µg BPA, and DES (positive controls) (N=18-22/treatment), had significantly lower cumulative number of pups compared to unexposed control. *BPA daughters* exposed to 25µg BPA also had significantly fewer pregnancies compared to controls. Furthermore, 40% of *BPA daughters* exposed to 25µg BPA delivered less than 4 times, and 65% had less than 6 deliveries. These preliminary results indicate that the reproductive outcome may be altered in mice exposed perinatally to BPA.

### **Mechanisms of Perfluorooctanoic acid stimulation of pubertal mammary gland in C57Bl/6 mice**

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Perfluorooctanoic acid (PFOA) is a synthetic, widely used perfluorinated carboxylic acid and persistent environmental pollutant. PFOA is an agonist of peroxisome proliferator activated receptor  $\alpha$  (PPAR $\alpha$ ) and PFOA exposure causes hepatocellular hypertrophy, tumorigenesis and developmental toxicity in rodents, depending on the presence of PPAR $\alpha$ . Our recent study revealed a stimulatory effect of peripubertal PFOA exposure (5 mg/kg) on mammary gland development in C57Bl/6 mice. The present study was designed to examine the underlying mechanism(s). The presence of ovaries was required for PFOA exposure (5 mg/kg) to stimulate mammary gland development and PFOA exposure significantly increased serum progesterone levels (0.52-1.87ng/mL in control group; 1.91-5.15ng/mL in PFOA treated group) in ovary intact C57Bl/6 mice (5-10 mice/treatment group). No significant effect of PFOA on serum estradiol level (18.93-59.47pg/mL) or sex hormone binding proteins was detected. However, PFOA treatment resulted in increased mammary gland levels of estrogen receptor  $\alpha$  (ER $\alpha$ ), amphiregulin (Areg), a downstream target of ER action, and IGF-1, the latter both potent mammary gland growth factors. PFOA exposure significantly decreased the levels of liver metabolic enzymes related to steroid hormone metabolism/excretion, and significantly increased the levels of enzymes involved in steroid hormone synthesis in ovary. Importantly, PFOA mammary gland stimulation was also obtained in PPAR $\alpha$  knockout C57Bl/6 pubertal mice. These results indicate that PFOA likely stimulates mammary gland development by modifying the systemic hormonal milieu through its effects on the ovary and liver and is independent of the expression of PPAR $\alpha$ .

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