



December 10, 2009

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**Report to Funders: Mammary Gland Evaluation and Risk Assessment Workshop,  
November 16-17, 2009**

Dear Mhel, Elaine, Sally, Linda, Les, Claudia, John, and Julia:

Thank you so much for your support of the Mammary Gland Evaluation and Risk Assessment Workshop, which took place November 16-17, 2009, in Oakland, CA. We are extremely pleased with the progress made at this meeting towards standardizing methods for evaluating rodent mammary gland whole mounts and highlighting research that suggests altered mammary gland development is a sensitive indicator of early life or *in utero* endocrine disruptor exposure.

This letter summarizes the workshop and includes consensus statements that were generated there. We are preparing two journal articles for publication as meeting reports, and these will provide additional detail and a permanent record of the meeting. This letter report serves as an interim record of the meeting and may be used as a basis for supporting enhanced evaluation of the mammary gland in the Organization for Economic Cooperation and Development (OECD)



Extended One Generation Reproductive Toxicity Study draft guidelines<sup>1</sup> or in the EPA Endocrine Disruptor Screening Program (EDSP) male and female pubertal protocols.<sup>2</sup>

*Background:*

- A small number of government and academic research laboratories have reported over the past 10 years or so that *in utero* or early life exposure to endocrine disrupting chemicals (EDCs) and other environmental agents affects the development of the mammary gland – particularly, the timing and extent of growth and branching. These assessments of mammary gland development are typically observed using a technique known as “mammary gland whole mounts,” but mammary gland is not always assessed in standard chemical testing protocols and has never been assessed in those studies using whole mounts.
- It has been demonstrated that mammary gland development and tumor types are etiologically similar between rodents and humans. The particular name that is given to a certain epithelial structure or tumor type may vary slightly between species. In addition, some studies have shown that early life EDC exposure that alters mammary gland development can affect the number of tumors that develop following carcinogen exposure, or can induce abnormalities in tissue structure that may lead to spontaneous tumors (e.g., beaded ducts, ductal hyperplasia). Other studies have shown that early EDC exposures that induce morphological delays in mammary gland development have also been associated with functional effects on lactation when those animals reproduce.
- Few standard protocols for evaluating chemical toxicity include *in utero* or early life chemical exposure. Of the standard protocols that include *in utero* or early life dosing, mammary gland endpoints are infrequently assessed. In standard toxicity tests, the most common evaluations of mammary gland include pup weight as an indicator of lactational function in the mother, macroscopic observation for visible tumors, and histopathology of mammary tissue, in many cases conducted in a limited fashion and only where macroscopic changes are noted. There are no standard procedures for evaluating mammary gland whole mounts, which can demonstrate changes in the pace of development and the morphology/structure of the mammary gland. Thus, standard testing protocols continue to provide limited information on mammary gland effects, and data from whole mounts are often not considered in regulatory chemical risk assessments, in part because it is not a standardized endpoint. However, the US National Toxicology Program has begun routinely collecting whole mounts in their early life exposure studies.
- In some cases, the effects on mammary gland development are observed at lower exposures than any other endpoint that has been reported. Examples of these effects were discussed by meeting attendees for chlorotriazine metabolites, bisphenol A, dietary components. These findings raise concern that altered mammary gland development may

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<sup>1</sup> EPA is currently considering comments on the draft OECD guidelines for this test. Some evaluation of mammary glands is currently being considered for inclusion in these test guidelines, but there is also discussion about removing this requirement. In addition, evaluation of mammary gland whole mounts is not included. Comments should be sent to Don Bergfelt, US EPA, [Bergfelt.Don@epa.gov](mailto:Bergfelt.Don@epa.gov) and Christina Augustyniak, US EPA [Augustyniak.Christine@epamail.epa.gov](mailto:Augustyniak.Christine@epamail.epa.gov) as soon as possible.

<sup>2</sup> The EDSP pubertal protocols do not include mammary gland assessment. Comments on the EDSP protocols should be directed to Gary Timm, US EPA [Timm.Gary@epamail.epa.gov](mailto:Timm.Gary@epamail.epa.gov).



be a sensitive indicator of early EDC exposure, and that standard testing protocols have limited ability to detect effects on mammary gland development, function, and susceptibility to tumors.

- We organized this Mammary Gland Evaluation and Risk Assessment Workshop in order to bring mammary gland biologists, toxicologists, epidemiologists, risk assessors, and regulators together to learn from each other and to identify areas of agreement re: the significance of these effects on mammary gland development.

#### *Meeting Summary Day One – Round Robin Laboratories:*

In order to improve reliability and standardization of mammary gland whole mount assessments, directors and staff of seven laboratories from around the world (see attached) participated in a round robin evaluation of a set of mammary gland whole mounts from mice and rats treated *in utero* with vehicle and two different chemical treatments. Whole mounts were prepared from control and exposed mammary glands of female offspring at post-natal days 4, 21, and 45. The glands were evaluated independently by each laboratory for alterations in development - particularly, the timing and extent of growth and branching. The above-referred scientists, along with six other experts acting as observers, met to review their cumulative assessments and generate standardized guidelines for conducting the whole mount assessments of mammary glands. These methods and conclusions will be forthcoming as a scientific publication, and are available in the interim in the document: “Mammary Gland Whole Mount Round Robin Summary.”

This group of scientists unanimously agreed on the following statements:

- Mammary gland whole mount preparations should be routinely incorporated into test protocols that include *in utero* or early life exposures in order to determine potential effects of chemicals on early life mammary gland development. Examples of protocols where this endpoint will likely be informative include the EPA Endocrine Disruptor Screening Program male and female pubertal protocols, and the extended one-generation reproductive toxicity assay under consideration by the EPA and OECD.
- The use of rodent mammary gland whole mounts will enhance the understanding of developmental effects, particularly during the pubertal period, of test compound when exposure occurred during prenatal or other early life period. Assessing effects by utilizing tissue sections alone is essentially a “needle in the haystack” approach, whereas the analysis of whole mounts allows for the detection of important developmental changes in the mammary gland that may not be identifiable in tissue sections. However, in aged rats, complementing the whole mount analysis with the histological approach may be necessary due to the thickness of the gland. Use of the data from histological analyses (collected from adult rodents) in combination with early life developmental end points in whole mounts should be informative of early life perturbations. These may lead to, or be correlated with, later life effects, particularly mammary cancer risk or impaired lactation.



- We concur that the alterations we observed in the mammary glands evaluated in the round robin are developmentally abnormal and are of concern. These changes could suggest:
- An increased potential to develop intraductal hyperplasia, ductal bridging, or other preneoplastic conditions often present by 90 d of age.
  - Altered potential for spontaneous tumor development.
  - Altered susceptibility to chemical carcinogens or another exogenous insult.
  - Potential for functional abnormality (inadequate lactation).
  - Indication that endocrine disruption has occurred (other tissues should be evaluated for effects).

*Meeting Summary Day Two – Full Group Meeting:*

About 75 mammary gland biologists, toxicologists, pathologists, epidemiologists, risk assessors, and regulators met on the second day to hear a set of talks on this topic and have a discussion (see attached workshop schedule, meeting announcement/goals, and list of attendees). Among the talks were presentations focused on the similarities in mammary gland development and carcinogenesis between rodents and humans and a presentation demonstrating that standard toxicity testing conducted to comply with regulatory requirements has limited assessment of potential treatment-related effects on mammary gland function or susceptibility to carcinogens.

An overwhelming majority of the ~75 scientists agreed with the following statements:

- Given what we know about human, rat, and mouse mammary tissue developmental progression, the rat and mouse are adequate surrogates for human breast development.
- For some EDCs (includes pharmaceuticals and dietary constituents), *in utero* or early life exposure causes changes in mammary gland development in the rodent (e.g., altered branching, extent of growth, relative proportion of morphological structures such as terminal end buds, lobules, and terminal ducts). In some cases, these developmental changes occur at doses lower than other endpoints of endocrine disruption (e.g., atrazine, bisphenol A, 17 $\beta$ -estradiol, fatty acids).
- These changes in rodent mammary gland development following EDC exposure (includes pharmaceuticals and dietary constituents) are abnormal. This abnormal development raises concern about effects in humans. Further, these developmental effects can be interpreted to be adverse because: 1) altered developmental endpoints such as structural abnormalities or growth alterations are typically considered adverse; 2) the mammary gland developmental effects observed after certain chemical exposures result in altered breast function (e.g., lactation) and/or susceptibility to carcinogens in rodents.

Please let us know if you have further questions or need additional information.

Sincerely,

Ruthann Rudel  
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## **Mammary Gland Evaluation and Risk Assessment Workshop Round Robin Meeting, November 16, 2009**

### **Round Robin Participants:**

- Sue Fenton, National Institute of Environmental Health Sciences, RTP, NC
- Warren Foster, McMaster University, Ontario, Canada
- Sandra Haslam, Michigan State University, Lansing, MI
- Leena Hilakivi-Clarke and Anni Warri, Georgetown University, Washington D.C.
- Monica Munoz-de-Toro and Laura Kass, University of Litoral, Santa Fe, Argentina
- Jose and Irma Russo, Fox Chase Cancer Center, Philadelphia, PA
- Ana Soto and Carlos Sonnenschein, Tufts University, Boston, MA

### **Round Robin Meeting Observers:**

- Julie Boberg, Technical University of Denmark, Copenhagen, Denmark
- Daniel Desaulnier, Health Canada, Ottawa, Ontario, Canada
- Susan Euling, US Environmental Protection Agency, NCEA, Washington, DC
- John Latendresse, National Center for Toxicological Research, Jefferson, AR
- Ruthann Rudel, Silent Spring Institute, Newton, MA
- Jason Stanko, Fenton Laboratory, National Institute of Environmental Health Sciences, RTP, NC



## Workshop Schedule

Monday, November 16, 2009

<b>1:00-5:30 PM</b> <b>Chart Room</b>	<b>Mammary gland whole mount round robin</b> participants meet to discuss findings and propose standardized methods (Limited to round robin participants).
<b>5:30-9:30 PM</b> <b>Spinnaker Ball Room</b>	<i>Dinner for all workshop participants</i> (5:30-7:00 reception; 7:00 dinner) Linda Birnbaum, NIEHS Director, to address group during the reception.

Tuesday, November 17, 2009

<b>7:30-8:15 AM</b>	<i>Registration and continental breakfast – Spinnaker Ball Room</i>
<b>8:15-8:30 AM</b>	<b>Welcome</b>
<b>8:30-10:00 AM Experimental Models</b>	
Dan Medina Baylor College of Medicine	Overview of mammary gland development and carcinogenesis in rodent and human, with emphasis on species similarities and differences.
Jose Russo Fox Chase Cancer Center	Effects of developmental exposure to EDCs on mammary gland in rodent models, with emphasis on physiological changes affecting tumor incidence and strengths of models.
Michael Gould University of Wisconsin	Experimental models for evaluating altered mammary gland tumor susceptibility in rodents. Genetic variation in susceptibility and carcinogen challenge models.
<b>10:00-10:15 AM</b>	<i>Break</i>
<b>10:15-11:45 AM Human Studies</b>	
Barbara Cohn Child Health & Development Studies, Berkeley, CA	Human studies of early life exposures and breast cancer risk.
Walter Rogan NIEHS	Human studies evaluating effects of chemical exposure on lactation.
Frank Biro, Cincinnati Children's Hosp., and Larry Kushi, Kaiser Permanente	Human studies of factors affecting pubertal timing in girls.
<b>11:45 AM-12:15 PM</b>	<i>Facilitated Q&amp;A discussion period with morning speakers</i>
<b>12:15-1:15 PM</b>	<i>Lunch</i>
<b>1:15-2:45 PM Implications for Toxicology</b>	
Sue Makris US EPA	Mammary gland endpoints in standard experimental toxicology studies.
Sue Fenton US EPA	Developmental exposure and effects on mammary gland morphology: Challenges in evaluation and interpretation.
Ruthann Rudel Silent Spring Institute	Key issues and data needs for considering mammary gland effects in chemical risk assessment.
<b>2:45-3:00 PM</b>	<i>Break</i>
<b>3:00-5:30 PM</b>	<i>Facilitated discussion – Improving experimental models for evaluating mammary gland effects of developmental EDC exposure and maximizing research translation.</i>



# Mammary Gland Evaluation and Risk Assessment Workshop

November 16-17, 2009

Waterfront Plaza Hotel, Oakland, CA

Following the National Academy of Science's publication, *Toxicology in the 21<sup>st</sup> Century*, everyone in the toxicology field seems to be talking about how we will transform our biology-based science into the molecular and mechanism-based science of the future, but these are not our only challenges. We also need to understand the implications of specific chemically-induced biological changes for human health, and translate biological observations to risk assessment for many classes of compounds that we are exposed to every day.

This Mammary Gland Evaluation and Risk Assessment workshop will facilitate progress in evaluating the effects of early life chemical exposure on mammary gland development, function, and carcinogenesis in animal models and translating those effects to human health risk.

## Conference Goals:

- Improve research translation
  - Inform risk assessors and screening and testing entities about mammary gland development in animal models and humans, as well as potential implications of disruptions to this development on functional effects such as cancer, lactation, and puberty.
  - Inform mammary gland biologists and toxicologists working in this area about study design considerations that would facilitate use of their data to evaluate chemical risk.
- Develop and publish standardized protocols for evaluating effects of early life exposures on mammary gland development.
- Recommend mammary gland endpoints to be included in toxicology study designs.
- Identify and prioritize data gaps and future research needs.

The proposed conference represents a novel program of translational research between basic scientists studying effects of chemical exposures on mammary gland development; toxicologists, risk assessors, and regulators who consider these studies in developing chemicals testing protocols, human exposure guidelines and environmental regulations; and epidemiologists studying relevant endpoints in humans (lactation, puberty, breast cancer).

This workshop is supported by the US Environmental Protection Agency, the National Institute of Environmental Health Sciences, and the California Breast Cancer Research Program; and is being planned and coordinated by Dr. Suzanne Fenton (US EPA), in collaboration with Ruthann Rudel (Silent Spring Institute). Attendance at the workshop is limited to invited participants and a small number of registered observers.

**Please contact Laura Perovich (617-332-4288 x215 or [perovich@silentspring.org](mailto:perovich@silentspring.org)) at Silent Spring Institute for additional information.**



**Attendees: Mammary Gland Evaluation and Risk Assessment Workshop  
November 17, 2009**

<b>Name</b>	<b>Affiliation</b>
Mary Helen Barcellos-Hoff	New York University
Janice Barlow	Zero Breast Cancer
Don Bergfelt	Environmental Protection Agency
Linda Birnbaum	National Institute of Environmental Health Sciences
Frank Biro	Cincinnati Children's Hospital Medical Center
Julie Boberg	Technical University of Denmark
Julia Brody	Silent Spring Institute
John Budroe	Office of Environmental Health Hazard Assessment
Robert Cardiff	University of California Davis
Barbara Cohn	Child Health and Development Studies
Gwen Collman	National Institute of Environmental Health Sciences
Daniel Desaulnier	Health Canada
Caroline Dilworth	National Institute of Environmental Health Sciences
Jim Donald	Office of Environmental Health Hazard Assessment
Elizabeth Doyle	Environmental Protection Agency, Office of Water
Susan Euling	Environmental Protection Agency National Center for Environmental Assessment
Sue Fenton	National Institute of Environmental Health Sciences
Laurence Fitzgerald	California Breast Cancer Research Program
James M. Ford	California Breast Cancer Research Program advisory council
Warren Foster	McMaster University
Michael Gould	University of Wisconsin
Janet Gray	Vassar College, Breast Cancer Fund
Sandra Haslam	Michigan State University
Ann Hernick	Breast Cancer Alliance of Greater Cincinnati
Leena Hilakivi-Clarke	Georgetown University
Russell Hovey	University of California Davis
Laura Kass	University of Litoral, Santa Fe, Argentina
Marion Kavanaugh-Lynch	California Breast Cancer Research Program
Susan Knadle	Office of Environmental Health Hazard Assessment
Andreas Kortenkamp	School of Pharmacy, University of London
Larry Kushi	Kaiser Permanente
John Latendresse	National Center for Toxicological Research
Betina Lew	University of Rochester Medical Center
Anna Lowit	Environmental Protection Agency
Amal Mahfouz	Environmental Protection Agency, Office of Water
Susan Makris	Environmental Protection Agency
Elizabeth Maull	National Institute of Environmental Health Sciences
Katherine McKenzie	California Breast Cancer Research Program
Daniel Medina	Baylor College of Medicine
Rekha Mehta	Health Canada
Wynne Miller	Environmental Protection Agency, Office of Ground Water and Drinking Water
Monica Munoz-de-Toro	University of Litoral, Santa Fe, Argentina
Elizabeth Padilla-Banks	National Institute of Environmental Health Sciences
Laura Perovich	Silent Spring Institute
Susan Pinney	University of Cincinnati



Svetlana Popova	California Department of Public Health, Cancer Detection Section
Ahmed Raafat	National Institutes of Health
Leslie Reinlib	National Institute of Environmental Health Sciences
Jeanne Rizzo	Breast Cancer Fund
Gertraud Robinson	National Institute of Diabetes and Digestive and Kidney Diseases
Walter Rogan	National Institute of Environmental Health Sciences
Paul Rowsell	Health Canada
Ruthann Rudel	Silent Spring Institute
Jose Russo	Fox Chase Cancer Center
Irma Russo	Fox Chase Cancer Center
Martha Sandy	Office of Environmental Health Hazard Assessment
Meg Schwarzman	University of California Berkley
Pam Shubat	Minnesota Health Department
Suzanne Snedeker	Cornell University Program on Breast Cancer and Environmental Risk Factors
Carlos Sonnenschein	Tufts University
Ana Soto	Tufts University
Jason Stanko	National Institute of Environmental Health Sciences
Claudia Thompson	National Institute of Environmental Health Sciences
Catherine Thomsen	California Breast Cancer Research Program
Beth Vorderstrasse	Washington State University
Anni Warri	Georgetown University/University of Turku (Finland)
Terri Wood	New Jersey Medical School Cancer Center
Lily Wu	Office of Environmental Health Hazard Assessment
Mary Alice Yund	California Breast Cancer Research Program advisory council