Pathways to Breast Cancer
A Case Study for Innovation in Chemical Safety Evaluation

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Natural Resources Defense Council
Core Question

As new chemicals policies develop toxicity testing requirements, what body of toxicity data—obtained using existing methods—could best identify chemicals that may increase the risk of breast cancer?
Breast Cancer & Chemicals Policy

Project Structure

Science Policy
Identify decision-making tools and data needs to inform implementation of new chemicals policy.

Cancer Biology
Identify known and suspected events in biological pathways that may raise the risk of breast cancer.

Toxicity Testing
Identify currently available testing methods for detecting chemicals that may raise the risk of breast cancer; identify emerging test methods that could be adapted for rapid chemical screens.

Breast Cancer & Chemicals Policy Project
Expert Panel

- Susan Braun, MA Commonweal
- Vincent James Cogliano, PhD WHO International Agency for Research on Cancer
- Shanaz Dairkee *, PhD California Pacific Medical Center Research Institute
- Suzanne Fenton, PhD National Institute of Environmental Health Sciences
- William H. Goodson III, MD California Pacific Medical Center Research Institute
- Joe Guth *, PhD, JD Science and Environmental Health Network
- Dale Johnson, PharmD, PhD University California Berkeley & Emiliem
- Jean Latimer, PhD School of Medicine University of Pittsburgh
- Ron Melnick, PhD National Institute of Environmental Health Sciences
- Rachel Morello-Frosch, PhD, MPH University of California Berkeley
- Ruthann A. Rudel, MS Silent Spring Institute
- Gina Solomon*, MD, MPH University of California San Francisco & Natural Resources Defense Council
- Carlos Sonnenschein, MD Tufts University School of Medicine
- Lauren Zeise*, PhD Cal/EPA Office of Environmental Health Hazard Assessment
Context: Information Needs

Methods for using existing data and current test methods in chemical decision-making.

New tools for:
- Understanding biological pathways
- Toxicity testing methods
- Application of science in decisions
High Throughput & Molecular mechanisms

European Union Affecting Global Change


RoHS: Restriction on Hazardous Substances (2006)

Context: New Chemicals Policy in the U.S.

Federal Toxic Substances Control Act reform
- House and Senate versions, 2010
- Will require chemical testing

California EPA Green Chemistry Initiative
- Ingredient Disclosure (SB 928 pending)
- Create an Online Toxics Clearinghouse (SB 509)
- Accelerate the Quest for Safer Products (AB 1879)

Other U.S. State Policies
- Regulation of specific product categories
- Identify and prioritize chemicals of concern
- Eliminate categories of hazards (e.g., known carcinogens)
Why Focus on Breast Cancer?

- Most common invasive cancer and a leading cause of cancer death in women.
- Affects one in eight U.S. women
- Most breast cancer is not caused by inherited genes
- Increasing recognition that environmental exposures contribute to the development of disease
- Over 200 chemicals have been associated with mammary cancer in laboratory animals.
- Most standard toxicity testing methods do not regularly evaluate potential chemical effects on the breast
Breast Cancer & Chemicals Policy
Project Goals

1. **Develop an approach for identifying chemicals** that may contribute to the development or progression of breast cancer based on their ability to affect key biological processes;

2. **Identify research needs** and suggest new methods to improve existing tests and inform a shift toward rapid chemical screening;

3. **Pilot a model process** that can be applied to other disease endpoints, with the ultimate goal of producing a comprehensive approach for identifying hazardous chemicals.
Steps of the Breast Cancer and Chemicals Policy Project

An interdisciplinary panel with expertise in breast cancer biology, toxicology, epidemiology, risk assessment, chemicals policy, community advocacy to:

1. Identify toxicity “endpoints”: alterations to biological processes resulting in an increased risk of breast cancer.
2. Identify toxicity testing methods capable of screening chemicals for their impact on biological processes relevant to breast cancer.
4. Conduct a “virtual” pilot test to validate the proposed Hazard Identification Approach by investigating how several well-studied chemicals would “perform” if tested.
Step 1. Identify Events in Biological Processes Associated with Breast Cancer

Premises:

In identifying chemicals likely to increase the risk of breast cancer, we should investigate chemicals that:

- Are associated with general carcinogenic mechanisms
- Increase estrogenic or other proliferative effects on breast tissue by any mechanism (e.g. altered hormone metabolism, early puberty)
- Interfere with development of the mammary gland

The impact of such substances is determined by two kinds of vulnerabilities:

- Population susceptibility factors (e.g. genetic polymorphisms, obesity, other exposures, occupation)
- Timing of exposure (developmental stage)
### Step 1. Events in Biological Processes Associated with Breast Cancer

#### Cellular Events

<table>
<thead>
<tr>
<th>Alterations in hormone levels, metabolism or receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changes in gene transcription &amp; translation</td>
</tr>
<tr>
<td>Cell cycle changes</td>
</tr>
<tr>
<td>Peptide hormones (growth hormones)</td>
</tr>
<tr>
<td>Genotoxicity</td>
</tr>
<tr>
<td>Oxidative stress</td>
</tr>
<tr>
<td>Immune modulation</td>
</tr>
<tr>
<td>Limitless replication potential</td>
</tr>
<tr>
<td>Evasion of apoptosis</td>
</tr>
</tbody>
</table>

#### Tissue Changes

<table>
<thead>
<tr>
<th>Breast density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue invasion</td>
</tr>
<tr>
<td>Sustained angiogenesis</td>
</tr>
<tr>
<td>Self-sufficiency in growth</td>
</tr>
<tr>
<td>TEB proliferation</td>
</tr>
<tr>
<td>Altered mammary gland development</td>
</tr>
<tr>
<td>Ductal hyperplasia</td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
</tr>
</tbody>
</table>

#### Susceptibility Factors

| Obesity |
| Early onset of breast development |
| Alterations in cyclicity |
| Genetic polymorphisms in metabolizing enzymes |
| Duration of lifetime estrogen exposure |
# Step 2: Identify test methods

(Estimated reading time: 2 minutes)

Detectable Events Affecting Breast Cancer Risk

<table>
<thead>
<tr>
<th>Model System</th>
<th>Molecular Mechanisms</th>
<th>Phenotypic Indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gene Expression</td>
<td>Genotoxicity</td>
</tr>
<tr>
<td>In Silico</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In Vitro</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In Vivo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidemiological</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: [http://coeh.berkeley.edu/greenchemistry/cbcrpdocs/docs/matrix.pdf](http://coeh.berkeley.edu/greenchemistry/cbcrpdocs/docs/matrix.pdf)
### Step 2: Identify Test Methods (Sample 2)

<table>
<thead>
<tr>
<th>Model System</th>
<th>Susceptibility Factors</th>
<th>Biological Programs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Altered Cyclicity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Metabolic Factors</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estrogen Exposure</td>
<td></td>
</tr>
<tr>
<td>In Silico</td>
<td></td>
<td>Immune Modulation</td>
</tr>
<tr>
<td>In Vitro</td>
<td></td>
<td>Oxidative Stress</td>
</tr>
<tr>
<td>In Vivo</td>
<td></td>
<td>Apoptosis Evasion</td>
</tr>
<tr>
<td>Epidemiological</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Detectable Events Affecting Breast Cancer Risk

etc...

[http://coeh.berkeley.edu/greenchemistry/cbcrpdocs/matrix.pdf](http://coeh.berkeley.edu/greenchemistry/cbcrpdocs/matrix.pdf)
### Step 3. Hazard Identification Approach:
**Chemical Prioritization**

Chemicals, their metabolites and degradation products should be prioritized for testing based on the following parameters:

<table>
<thead>
<tr>
<th>Chemical Prioritization</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hazard indicators</strong></td>
</tr>
<tr>
<td>including structural similarities to other mammary gland carcinogens, or indicators that a chemical or its possible metabolite have endocrine activity, alter breast development or gene expression, or create genetic mutations.</td>
</tr>
<tr>
<td><strong>Exposure potential</strong></td>
</tr>
<tr>
<td>predicted by physical-chemical properties that indicate potential for bioaccumulation, persistence in the environment, or result in exposure to breast tissue. Also those identified by biomonitoring, environmental monitoring, or other proxy measures such as high production volume or dispersive use in consumer products or workplaces. Exposure potential should be assessed across the entire life-cycle.</td>
</tr>
</tbody>
</table>
### Step 3. Hazard Identification Approach: Rapid Screening Methods

#### Hazard Identification Approach

**Rapid (in vitro) screening**

<table>
<thead>
<tr>
<th>Genotoxicity</th>
<th>Endocrine disruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutagenicity (e.g., Ames or equivalent)</td>
<td>Activation or inhibition of:</td>
</tr>
<tr>
<td>Chromosome aberrations (e.g., OECD TG 473)</td>
<td>Estrogen-mediated transcription (e.g., E-screen)</td>
</tr>
<tr>
<td>Micronuclei formation (e.g., OECD TG 487)</td>
<td>Androgen-mediated transcription (e.g., A-screen)</td>
</tr>
<tr>
<td>DNA strand breaks (e.g., COMET assay)</td>
<td>Enzymes specific to synthesis or metabolism of estrogen, androgen or progesterone (e.g., aromatase activity assay)</td>
</tr>
</tbody>
</table>

**Cell cycle changes**

- Cell division (e.g., $^3$H thymidine proliferation assay)
- Altered apoptosis (e.g., TUNNEL assay)
Step 3. Hazard Identification Approach: \textit{in vivo} studies

### Hazard Identification Approach

**Animal studies (in vivo): development and maturation**

<table>
<thead>
<tr>
<th>Genotoxicity in breast epithelial cells</th>
<th>Cell cycle changes in breast epithelial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutagenicity</td>
<td>Cell proliferation</td>
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<tr>
<td>Chromosome aberrations</td>
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**Induction of mammary gland tumors, biomarkers, or precursor changes**

For example, long term cancer bioassays redesigned to evaluate mammary gland effects; include \textit{in utero} exposure; use appropriate animal strain; and assess multiple life stages.

**Endocrine disruption**

- Estrogenic activity (e.g., Uterotrophic assay)
- Androgenic activity (e.g., Hershberger assay)
- Developmental changes in female and male mammary gland tissue (e.g., TEB formation, ductal branching, ER and AR levels)
- Reproductive changes in males and females (e.g., AGD, nipple retention, altered cyclicity, pubertal timing)
- Altered circulating hormone levels (e.g., steroid or peptide hormones)

*Assessed in OECD extended one generation (draft) or NTP enhanced Reproductive...*
Breast Cancer & Chemicals Policy Recommendations

Chemical toxicity testing—and the public policies that require it—can serve as a critical tool in breast cancer prevention, providing a practical basis for reducing potentially harmful exposures.

1. Chemicals should be tested now for possible impact on breast cancer risk and include the following endpoints:
   - Genotoxicity
   - Cell cycle changes
   - Endocrine disruption (e.g., estrogenicity)
   - Altered mammary gland development
Breast Cancer & Chemicals Policy Recommendations

Chemical toxicity testing—and the public policies that require it—can serve as a critical tool in breast cancer prevention, providing a practical basis for reducing potentially harmful exposures.

2. To accurately evaluate the potential of a chemical to raise the risk of breast cancer, toxicity tests must be designed and conducted to include considerations of

   – *timing of exposure* and

   – *underlying susceptibility factors.*
Breast Cancer & Chemicals Policy Recommendations

Chemical toxicity testing—and the public policies that require it—can serve as a critical tool in breast cancer prevention, providing a practical basis for reducing potentially harmful exposures.

3. Research needs:

- Further elucidation of biological pathways
- Adaptation of current testing methods to be more relevant to breast cancer
- Development and validation of new toxicity tests – HTS screening
Tests on the Horizon

Panel recommended an approach, not specific tests
• The field of toxicity testing is rapidly evolving
• Best practices can evolve with emerging tests

High throughput screens are under development
• Promise of testing thousands of chemicals
• Potential to address metabolic differences by testing many possible metabolites

Medium throughput screens using human breast tissue
• Methods currently applied in research could be adapted for toxicity testing to replace some animal studies (e.g., for mammary gland development effects)
Breast Cancer & Chemicals Policy Recommendations

Chemical toxicity testing—and the public policies that require it—can serve as a critical tool in breast cancer prevention, providing a practical basis for reducing potentially harmful exposures.

4. Process used by Panel could be applied to other disease endpoints to develop a comprehensive approach to identify chemicals that pose a risk to human health.
Final report: http://coeh.berkeley.edu/greenchemistry/cbcrp.htm

http://www.cbcrp.org/
Next steps

- **Scientific publications**
  - "Virtual" pilot screen of chemical compounds
  - Utility of *in silico* (QSAR) methods in evaluating mammary carcinogens (SOT abstract, 2010)
  - Science policy commentary
  - Need for improved toxicity testing to incorporate mammary gland endpoints