An Approach to Hazard Identification:
Breast Cancer and Chemicals Policy Project

Megan Schwarzman, MD, MPH
Center for Occupational and Environmental Health
School of Public Health
University of California, Berkeley
Breast Cancer & Chemicals Policy Project

Core Question
As governments identify chemicals of concern, what body of toxicity data could we obtain –using existing methods– to best identify chemicals that may increase the risk of breast cancer?

Why Breast Cancer?
- Most common invasive cancer in women
- Second leading cause of death from cancer
- Most breast cancer is not caused by inherited genes (10-25%)
- More than 200 chemical compounds cause mammary gland tumors in animals in at least one study
- Most standard toxicity testing methods do not regularly evaluate potential chemical effects on the breast
Breast Cancer & Chemicals Policy Project
Structure and Goals

Develop an approach to chemical hazard identification based on currently available methods for detecting chemicals that may raise the risk of breast cancer;

Pilot a project model applicable to other disease endpoints, with the ultimate goal of producing a comprehensive approach to chemical hazard identification;

Identify data gaps and research needs to improve chemical decision-making, including informing a shift toward rapid screening methods.
Steps in the Breast Cancer & Chemicals Policy Project

1. Convened an expert panel with expertise in breast cancer biology, toxicology, epidemiology, risk assessment, chemicals policy, community advocacy
2. Identified biological pathways relevant to the development or progression of breast cancer
3. Identified test methods for detecting chemicals that could act via these pathways to raise the risk of breast cancer
4. Developed a hazard identification approach, considering:
   - How to prioritize chemicals for testing
   - Currently validated tests
   - Emerging methods and assays used in research
Step 2. Identify Biological Pathways Associated with Breast Cancer

Premise: 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 2. Identify Biological Pathways Associated with Breast Cancer

**Susceptibility and Risk Factors**
- Exposure to Known Breast Carcinogens (e.g., radiation, DES, HRT)
- Obesity
- Altered Timing of Breast Development
- Alterations in Cyclicity
- Early Menarche or Late Menopause
- Lactational changes
- Immune Modulation

**Mechanisms**
- Genotoxicity
- Steroid Hormone
- Cell Cycle Changes, e.g. reduced apoptosis
- Melatonin and Circadian Rhythms
- Peptide Hormones (Growth Hormones)
- Metabolism Transporters

**Phenotypic Tissue Level Observations**
- Atypical Hyperplasia
- Adenoma
- Ductal Carcinoma in Situ
- Carcinoma
- Terminal End Proliferation
- Abnormal Breast Development
- Ductal Hyperplasia
- Pathological Markers

**Observation of Cancer Hallmarks**
- Sustained Angiogenesis
- Limitless Replication Potential
- Tissue Invasion/Metastasis
- Insensitivity to Anti-Growth Signals
- Self-Sufficiency in Growth Signals
### Step 3: Toxicity Testing Methods

*(Sample 1)*

<table>
<thead>
<tr>
<th>Model System</th>
<th>Molecular Mechanisms</th>
<th>Phenotypic Indicators</th>
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<tbody>
<tr>
<td></td>
<td>Gene Expression</td>
<td>Genotoxicity</td>
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<td><strong>In Silico</strong></td>
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<td><strong>Epidemiological</strong></td>
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Step 3: Toxicity Testing Methods  
(Sample 2)

<table>
<thead>
<tr>
<th>Detectable Events Affecting Breast Cancer Risk</th>
<th>Susceptibility Factors</th>
<th>Biological Programs</th>
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<td>Model System</td>
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<td>Estrogen Exposure</td>
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<td>Immune Modulation</td>
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<td>Oxidative Stress</td>
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etc...
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<th>Model System for Evaluating Effects</th>
<th>Gene Expression Alterations</th>
<th>Cell Cycle Changes (proliferation; programmed cell death)</th>
<th>Genotoxicity</th>
<th>Development</th>
<th>Steroid Hormones (Estrogen, Androgen, Progesterone)</th>
<th>Melatonin and Circadian Rhythms</th>
<th>Peptide Hormones (Growth Hormones)</th>
<th>Metabolism/Transporters</th>
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<tr>
<td><strong>in silico</strong></td>
<td>Databases on gene expression microarrays (based on cell lines); RT-PCR (targeted gene expressions - ER, PR); microarray (pathway arrays); Western blots/proteomics. Examples of genes: EGF-receptor phosphorylation; p53; estrogen metabolizing genes; E2 regulated genes; alternative RNA splicing; aromatase promo</td>
<td>Cell cycle changes (programmed cell death)</td>
<td>Databases based on Ames test; other databases; Databases on CGH</td>
<td>Databases on SNPs</td>
<td>QSAR; steroid receptor activity (agonist/antagonist); altered metabolism; transport protein interaction</td>
<td>Database arrays of genes involved in circadian rhythms?</td>
<td>Databases to be id’d by Cali J</td>
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<td><strong>in vitro</strong></td>
<td><strong>bacterial</strong></td>
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<td><strong>Ames Test</strong></td>
<td><strong>Microsatellite Instability; LOH, GWA; sNP; CGH; chromosomal aberrations; translocations; strand breaks; aneuploidy; Spectral karyotyping (SKY); DNA adducts (agents or adducts of estrogen); MRA (autosomal assay for chromosomal changes - mouse lymphoma 20G)</strong></td>
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<td><strong>mammalian cell lines</strong></td>
<td><strong>DNA methylation patterns for epigenetic changes (methylated based arrays); MRA: methylated based sequencing</strong></td>
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<td><strong>in vivo</strong></td>
<td><strong>Whole animal (1- or 2 generation studies) also includes genetically modified breast cancer models</strong></td>
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<td><strong>Human epidemiology longitudinal exposure studies (NCS, Danish, Natl birth cohort, BGCRC, Ag Health study, Nurses Health study, EPIC, Million Women study, UK)</strong></td>
<td>Buccal cell and buccal coat cells as DNA sources; Homogeneous populations - New Zealand/Finland/Sweden (potential to look for breast cancer); DNA methylation patterns for epigenetic changes</td>
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<td><em>Transgenic animal models - PPAR-KO; knockout models - IGF-1, PRL-KO, ERKO, PRKO, p53, wt KO</em></td>
<td><em>Western blots/proteomics. Examples of genes: EGF-receptor phosphorylation; p53; estrogen metabolizing genes; E2 regulated genes; alternative RNA splicing; aromatase promo</em></td>
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Step 4. Hazard Identification Approach

Chemical Selection:
Prioritize chemicals for testing by

- Hazard Indicators
- Exposure Potential

Chemical Testing

1. General Mechanisms of Carcinogenesis
   - Genotoxicity
   - Cell Cycle Changes

2. Endocrine Disruption

3. Altered Mammary Gland Development and Sexual Maturation

4. Induction of Mammary Gland Tumors, Precursor Changes or Their Biomarkers
Step 4. Hazard Identification Approach: Chemical Selection

**Chemical Selection: Prioritize for testing based on**

**Hazard Indicators**

- Chemicals, possible metabolites, or degradation products that may have:
  - Endocrine activity
  - Genotoxic properties
  - Structural similarities to other mammary gland carcinogens (e.g., epoxides);
  - Physical-chemical properties, or QSAR or other computational modeling indicating:
    - Potential to form active metabolites
    - Genotoxicity potential
    - Potential to reach breast tissue after exposure
    - Long biological half-life in humans

**Exposure Potential**

- Chemicals or degradation products:
  - Observed in:
    - Biomonitoring studies (e.g., NHANES)
    - Environmental monitoring
  - Physical-chemical properties indicating
    - Potential to bioaccumulate
    - Persist in the environment
  - With proxy measures indicating high exposure, e.g.:
    - High production volume
    - Dispersive use in consumer products or workplaces
  - Should consider
    - Chemical’s entire life-cycle
    - Potential exposures at different life stages, (e.g., prenatal, menopause)
Step 4. Hazard Identification Approach: Rapid Screening Methods

**Rapid (in vitro) Screening**

### Genotoxicity
- Mutagenicity (e.g., Ames or equivalent)
- Chromosome aberrations
- Micronuclei formation
- DNA strand breaks (e.g., COMET assay)

### Cell Cycle Changes
- Cell division
- Altered apoptosis (e.g., TUNNEL assay)

### Endocrine Disruption
Activation or inhibition of:
- Estrogen-mediated transcription
- Androgen-mediated transcription
- Enzymes specific to synthesis or metabolism of estrogen, androgen or progesterone

**Animal Studies**

**In Breast Epithelial Cells:**

- **Genotoxicity**
  - Mutagenicity
  - Chromosome aberrations
  - Micronuclei formation
  - DNA strand breaks

- **Cell Cycle Changes**
  - Cell proliferation
  - Decreased apoptosis

**Induction of Mammary Gland Tumors, Precursor Changes, or Their Markers**

- e.g., long term cancer bioassays that include in utero exposure; use appropriate animal strain for mammary site; and assess multiple life stages

**Endocrine Disruption**

- Estrogenic activity (e.g., Uterotrophic assay)
- Androgenic activity (e.g., Hershberger assay)
- Altered mammary gland development (both sexes), e.g.,
  - terminal end bud formation
  - ductal branching
- 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)
Information Needs

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

Better information and new tools

- Toxicity testing methods
- Understanding biological pathways
- Application of science in decisions

Toxicity Testing for Assessment of Environmental Agents

Interim Report

TOXICITY TESTING IN THE 21ST CENTURY A VISION AND A STRATEGY

Advancing Risk Assessment

SCIENCE AND DECISION

NATIONAL RESEARCH COUNCIL OF THE NATIONAL ACADEMIES
Chemical Testing Capacity

<table>
<thead>
<tr>
<th>1-3/yr</th>
<th>10's/yr</th>
<th>100's/yr</th>
<th>10,000's/day</th>
<th>100,000's/day</th>
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High Throughput & Molecular mechanisms

Other Diseases that May be Detected by Hazard Identification Approach

Cancer:
- Ovarian
- Uterine
- Prostate
- Testicular

Reproductive:
**Female**
- Precocious puberty
- Infertility
- Endometriosis
- Fibroids
- Early menopause

**Male**
- Precocious puberty
- Infertility
- Hypospadias
- Undescended testes
- Gynecomastia
- Brain feminization