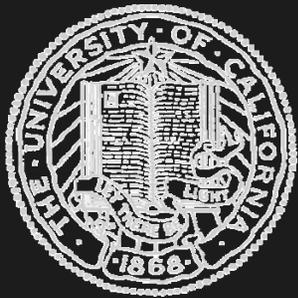


An Approach to Hazard Identification: Breast Cancer and Chemicals Policy Project



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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?

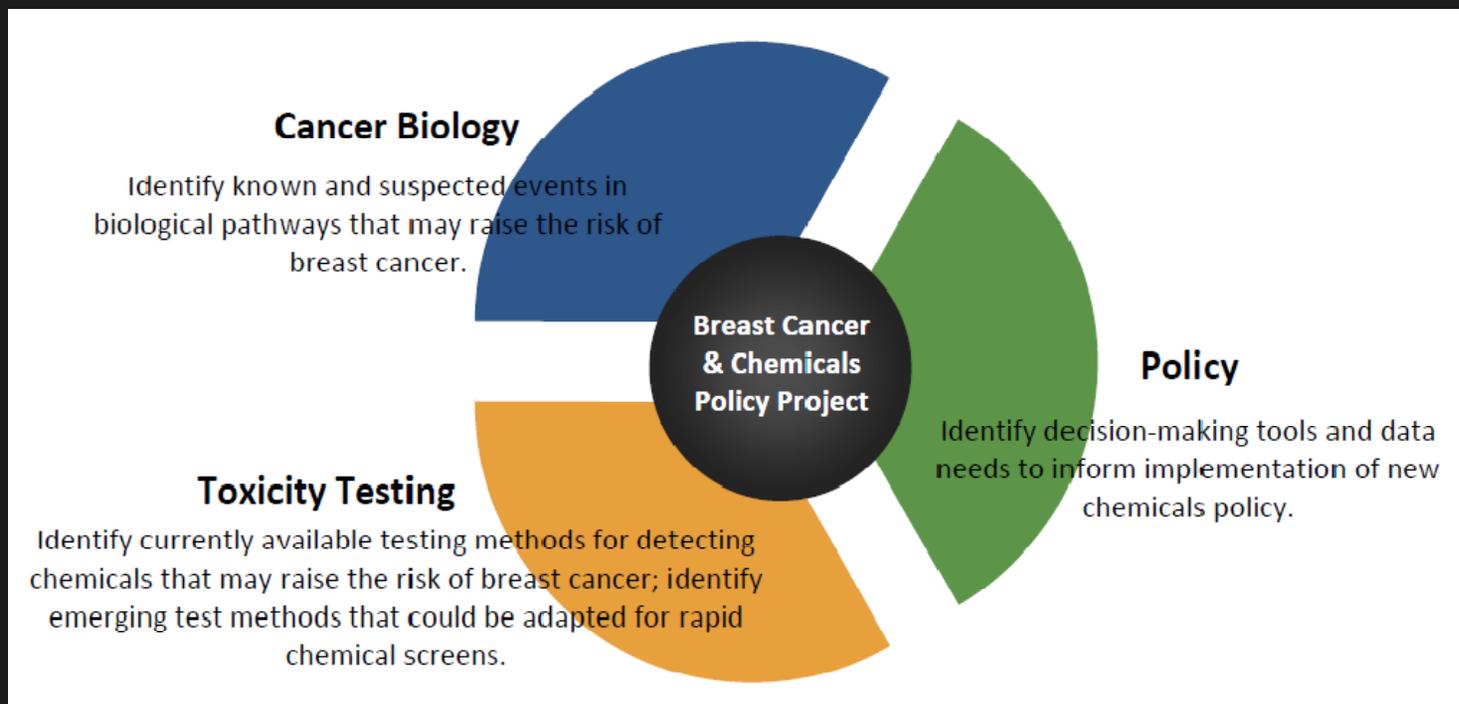
Why Breast Cancer?

- 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

Selection of disease pathways

Susceptibility and Risk Factors

- ▶ Exposure to Known Breast Carcinogens (e.g., radiation, DES, HRT)
- ▶ Obesity
- ▶ Altered Timing of Breast Development
- ▶ Alterations in Cyclicality
- ▶ Early Menarche or Late Menopause
- ▶ Lactational changes
- ▶ Immune Modulation

Detected “upstream”

Mechanisms

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

Detected

“downstream”

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)

Detectable Events Affecting Breast Cancer Risk

| Model System | Molecular Mechanisms | | | Phenotypic Indicators | | |
|------------------------|----------------------|--------------|------------------|-----------------------|-------------------|-----------|
| | Gene Expression | Genotoxicity | Steroid Hormones | Pathological Markers | TEB Proliferation | Carcinoma |
| <i>In Silico</i> | | | | | | |
| <i>In Vitro</i> | | | | | | |
| <i>In Vivo</i> | | | | | | |
| <i>Epidemiological</i> | | | | | | |

Step 3: Toxicity Testing Methods

(Sample 2)

| Detectable Events Affecting Breast Cancer Risk | | | | | | |
|--|------------------------|----------------------|----------------------|----------------------|---------------------|----------------------|
| | Susceptibility Factors | | | Biological Programs | | |
| Model System | Altered Cyclicity | Metabolic Factors | Estrogen Exposure | Immune Modulation | Oxidative Stress | Apoptosis Evasion |
| <i>In Silico</i> | | | | | | |
| <i>In Vitro</i> | | | | | | |
| <i>In Vivo</i> | | | | | | |
| <i>Epidemiological</i> | | | | | | |

etc...

| Molecular Events Potentially Affecting Breast Cancer Development | | | | | | | | |
|---|---|---|--|---|---|---|--|--|
| Model System for Evaluating Effects | Alterations in Gene Expression | Cell cycle changes (proliferation; programmed cell death) | Genotoxicity | Development | Steroid hormones (Estrogen, Androgen, Progesterone) | Melatonin and circadian rhythms | Peptide Hormones (Growth hormones) | Metabolism Transporters |
| in silico | databases on gene expression microarrays (based on cell lines); | databases based on tissue arrays | models based on Ames test; other databases; Databases on CGH | databases on sNPs | QSAR; steroid receptor activity (agonist/antagonist); altered metabolism; transport protein interaction | database arrays of genes involved in circadian rhythms? | databases to be id'd by Dale J. | databases to be id'd by Dal J. |
| in vitro | RT-PCR (targeted gene expressions - ER, PR); microarray (pathway arrays); Western blots/proteomics. Examples of genes: EGF-receptor phosphorylation, p53 expression; estrogen metabolizing genes; E2 regulated genes; alternative RNA splicing; Aromatase promo | Ki67; cyclins, BrdU, (IHC, flow cytometry); apoptosis; | | | Changes in activity of enzymes involved in steroid metabolism, and which should specify aromatase, 5-alpha reductase, and other p450 enzymes. | | Caseins | Estrogen and adrenal metabolism genes (liver); Transporter assay kits |
| bacterial | | | Ames test | | | | | |
| Primary cell culture/ extended explants | BrCa1 homozygous/heterozygous carriers - could look for many endpoints in primary culture - e.g. p53 mutations, RT-PCR (targeted gene expressions - ER, PR); microarray (pathway arrays); Western blots/proteomics. Examples of genes: EGF-receptor phosphorylation | Ki67; cyclins, BrdU, (IHC, flow cytometry); apoptosis; | microsatellite instability; LOH, GWAS; sNP, CGH; chromosomal aberrations, translocations, strand breaks, aneuploidy, Spectral karyotyping (SKY); DNA adducts (agents or adducts of estrogen); MLA (autosomal assay for chromosomal changes - mouse lymphoma ce | | ELISA; Receptor binding assays; Receptor levels; Receptor isoforms; Receptor translocation (IHC, Flow); E-Screen, A-screen; | | ELISA; Receptor binding assays; Receptor levels; Receptor isoforms; Receptor translocation (IHC, Flow); | |
| mammalian cell lines | RT-PCR (targeted gene expressions - ER, PR); microarray (pathway arrays); Western blots/proteomics. Examples of genes: EGF-receptor phosphorylation, p53 expression; estrogen metabolizing genes; E2 regulated genes; alternative RNA splicing; aromatase promo | Ki67; cyclins, BrdU, (IHC, flow cytometry); apoptosis; | microsatellite instability; LOH, GWAS; sNP, CGH; chromosomal aberrations, translocations, strand breaks, aneuploidy, Spectral karyotyping (SKY); DNA adducts (agents or adducts of estrogen); | | ELISA; Receptor binding assays; Receptor levels; Receptor isoforms; Receptor translocation (IHC, Flow); E-Screen, A-screen; transcriptional activation (in presence or absence of natural ligand) | | ELISA; Receptor binding assays; Receptor levels; Receptor isoforms; Receptor translocation (IHC, Flow); | |
| organ cultures | DNA Methylation patterns for epigenetic changes (methylation based arrays); MLA; methylation based sequencing (\$\$); | Ki67; cyclins, BrdU, (IHC, flow cytometry); apoptosis; | microsatellite instability; LOH, GWAS; sNP, CGH; chromosomal aberrations, translocations, strand breaks, aneuploidy, Spectral karyotyping (SKY); DNA adducts (agents or adducts of estrogen); | | Epithelial/stromal changes (hyperplasia, hypertrophy, morphological changes, other perturbations) | | | |
| in vivo | | | | | Changes in activity of enzymes involved in steroid metabolism, and which should specify aromatase, | | | |
| Whole animal (1- or 2 generation studies) also includes genetically modified breast cancer models | time points: pnd4 (culling); weaning; study endpoint 60 or 90d; carcinogen-induced steroidogenesis; mammary specific gene expression changes - correlate with body burden) | Ki67; cyclins, BrdU, (IHC, flow cytometry); apoptosis; | microsatellite instability; LOH, GWAS; sNP, CGH; chromosomal aberrations, translocations, strand breaks, aneuploidy, Spectral karyotyping (SKY); DNA adducts (agents or adducts of estrogen); | Transgenic animal models - PPAR-KO; knockout models: IGF-1, PRL KO, ERKO, PRKO, p53, wnt KO | Uterotrophic assay (separate study design); leiomyoma models; uterine wt; whole mount of mammary gland, tissue changes and developmental staging (precocious or abnl development); altered branching patterns; circulating hormone levels; Epithelial/stromal c | Ferrets for melatonin | Altered branching patterns; whole mount of mammary gland and developmental staging (precocious or abnl development); Dog models of mammary cancer? | Estrogen and adrenal metabolism genes (liver); Transporter assay kits; analytic assays to assess levels of parent compound and metabolites in tissue; (Naive and carcinogen-induced model); strain specific transport and metabolism; N11KO model |
| Human epidemiology | longitudinal exposure data (NCS, Danish Natl birth cohort, BCERC, Ag Health study, Nurses Health study, EPIC, Million Women study, UK) | | HLA based somatic mutation assays (flow cytometry) Exposure and DNA adducts; micronuclei; DNA repair | | Potential biomarkers: hormonal levels, Steroid Receptor isoforms; | Occupational and other studies of breast ca rates with disrupted circadian rhythms. (e.g. Nurses Study) | Measure EGF-R, HER2/neu | |

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

Step 4. Hazard Identification Approach: Animal-based Screening Methods

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

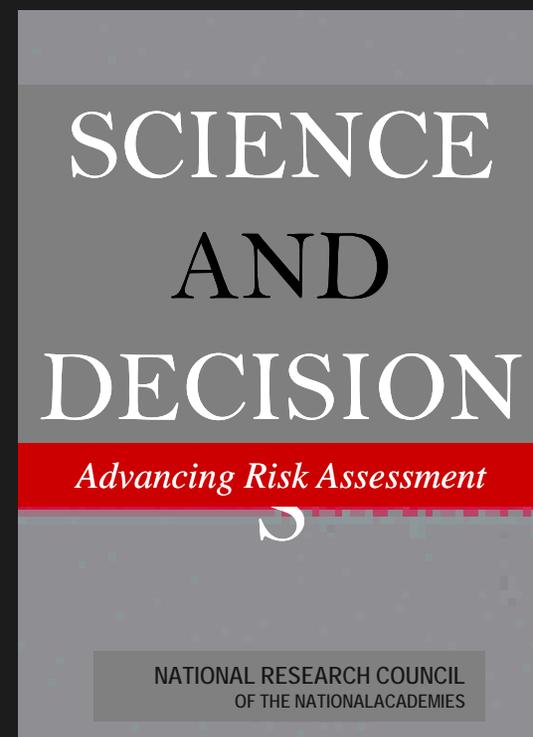
- ▶ Estrogenic activity (e.g., Uterotrophic assay)
- ▶ Androgenic activity (e.g., Hershberger assay)
- ▶ Altered mammary gland development (both sexes), e.g.,
 - terminal end bud formation
 - ductal branching)
- ▶ Reproductive changes in males and females, e.g.,
 - AGD
 - nipple retention
 - altered 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

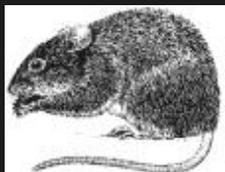
- Toxicity testing methods
- Understanding biological pathways
- Application of science in decisions



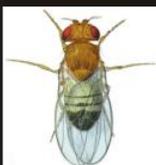
Chemical Testing Capacity



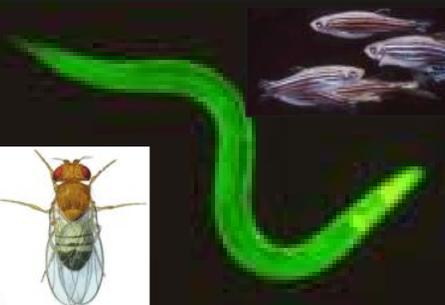
1-3/yr



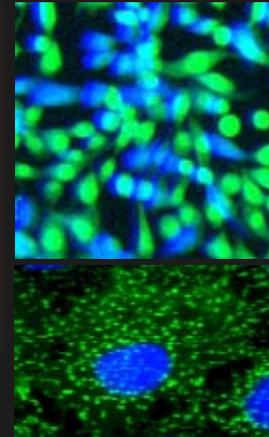
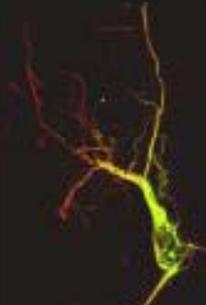
10's/yr



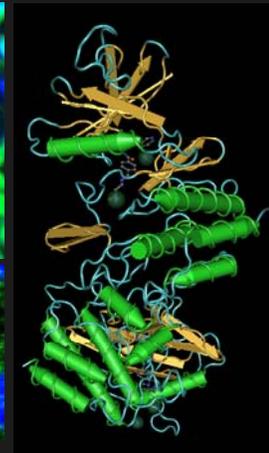
100's/yr



10,000's/day



100,000's/day



High Throughput &
Molecular mechanisms

Source: National Research Council, *Toxicity Testing in the 21st Century*, 2007

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