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Symposium: A California Roadmap for Identifying Chemicals that Affect Breast Cancer Risk
American Academy for the Advancement of Science
2010 Annual Meeting, San Diego, California
The Breast Cancer & Chemicals Policy Project

- Project scope and contributors
- Scientific context
- Project work
- Project findings
GOALS

1. **Develop an approach to chemical hazard identification** based on currently available methods for detecting chemicals that may raise the risk of breast cancer; the approach should generate toxicity information relevant to a variety of users of chemical information.

2. **Identify data gaps and research needs** to improve chemical decision-making, including informing a shift toward rapid screening methods performed without laboratory animals.

3. **Pilot a project model** that could be applied to other disease endpoints, with the ultimate goal of producing a comprehensive approach to chemical hazard identification.
Expert Panel

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Panel chairs  * member of core
New Toxicity Testing Paradigm
NAS, 2007

Biologic Inputs

Exposure
Tissue Dose
Biologic Interaction
Perturbation

Adaptive Stress Responses

Early Cellular Changes

Normal Biologic Function

Cell Injury

Morbidity and Mortality
Risk for a given chemical is determined by:

- Other endogenous and xenobiotic chemical exposures
- Biological susceptibility

“...the current framework tends to place undue focus on “complete” carcinogens, ignoring contributions to ongoing carcinogenesis processes and the multifactorial nature of cancer.”
Expert Panel Focus

• Methods to identify chemicals that may increase breast cancer risk

• Starting point - Panel considered
  – Known and suspected disease mechanisms
    ✷ associated with breast cancer biology
    ✷ associated with known breast cancer risk factors
  – Potential toxic chemical contributions via these mechanisms

• Identify general approach to screening for chemicals that increase breast cancer risk, considering
  – Emerging methods
  – Breast cancer research assays
  – Current validated tests
General Approach

- Informs Importance of Mechanisms and Tests
  - 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

- Linked to “Upstream” Tests
  - Mechanisms
    - Genotoxicity
    - Steroid Hormone
    - Cell Cycle Changes, e.g., reduced apoptosis
    - Melatonin and Circadian Rhythms
    - Peptide Hormones (Growth Hormones)
    - Metabolism Transporters

- Linked to “Downstream” Tests
  - 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
### Expert Panel’s Toxicity Testing Matrix: e.g., Mechanisms and Phenotypic Indicators

#### Detectable Events Affecting Breast Cancer Risk

<table>
<thead>
<tr>
<th>Model System</th>
<th>Molecular Mechanisms, e.g.,</th>
<th>Phenotypic Indicators, e.g.,</th>
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<tr>
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<td>Gene Expression</td>
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<td>Epidemiological</td>
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Study Throughput and Cost

10’s/year

100’s/year

10,000’s/day

100,000’s/day

1-3/year

High Throughput Molecular mechanism

High Cost per study
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 mammary gland development

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 (e.g., developmental stage, menopause)
General Approach to Testing for Breast Cancer Risk Factors

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
## Priorities for Chemicals to Test

### 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)
Examples of Possible In Vitro Tests

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
# Examples of In Vivo Tests

## 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)
Tests on the Horizon

• Panel recommended an approach, not specific tests
  – Validated tests are available for major pathways
  – The field of toxicity testing is rapidly evolving
  – Best practices can evolve with emerging tests

• High throughput screens are under development
  – Promise of large coverage of chemicals
  – Promise of addressing metabolic differences through broad coverage of possible metabolites

• Medium throughput, human breast tissue platforms are being applied in research
  – If adapted to toxicity testing could replace certain animal studies (e.g., for mammary gland development)
“icity” Overlap and Lack of Specificity: Carcinogens causing other cancers potentially identified

♀ Breast
- Morbidity risk 14%
- Mortality risk 3%

♀ Uterine
- Morbidity risk 0.8%
- Mortality risk 0.3%

♀ Ovarian
- Morbidity risk 1.5%
- Mortality risk 1.1%

♂ Prostate
- Morbidity risk 16%
- Mortality risk 3%

♂ Testicular
- Morbidity risk 0.4%
- Mortality risk 0.02%

♂ Benign prostate hyperplasia
- > 80% Morbidity
Other Diseases Potentially Modulated by Pathways in Test Sets

♀ Fibroid Risk
- of getting 70-80%
- hysterectomy 5-12%
♀ Uterine polyps
♀ Endometriosis
♀ Precocious puberty
♀ Masculinization
♀ Premature reproductive senescence
♀ Infertility

♂ Hypospadias
- 1% of male birth
♂ Undescended testis
- 3% full term births
- 30% premature births
♂ Brain feminization
♂ Gynecomastia
♂ Precocious puberty
♂ Infertility
Adaptive Approach to Chemical Testing: Testing could be applied in sequences

First Round of Testing

Results

Second Round of Testing

Results

More Testing

More Testing

decision point

No action needed. No further testing.

Act to eliminate or reduce exposure. No further testing.