Predicting Toxicity: Silent Spring Institute's High-throughput Screens for Chemicals Related to Breast Cancer

Margaret Kripke, Cancer Prevention and Research Institute of Texas
Ruthann Rudel, Silent Spring Institute
Paul Yaswen, Lawrence Berkeley Labs

Collaborative on Health and the Environment
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What do established breast carcinogens teach us about how chemicals might increase risk?
How might chemicals increase breast cancer risk?

- Damaging DNA
- Ionizing radiation
- Promoting tumor growth
- Disrupting development -> vulnerability
- HRT

www.sileentspring.org
FIG. 1. Estimated relative risk of breast cancer, with 90% confidence limits, by exposure status and radiation dose, with fitted linear dose response for exposed subjects with dose estimates. All ages combined.
How might chemicals increase breast cancer risk?

- Damaging DNA
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- Promoting tumor growth
- Disrupting development -> vulnerability
- DES

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Age-adjusted annual incidence rates for invasive breast cancer at Kaiser Permanente Northwest

Prevention is powerful!

Breast cancer incidence dropped among older women after study showed risks of HRT.

Subsequent economic analysis by Roth et al. 2014

The WHI scenario resulted in:
- 4.3 million fewer cHT users
- 126,000 fewer breast cancer cases
- expenditure savings of $35.2 billion


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How might chemicals increase breast cancer risk?

- Damaging DNA
  - Ionizing radiation
- Promoting tumor growth
  - HRT
- Disrupting development -> vulnerability
  - DES
Diethylstilbestrol (DES)

Prescribed to pregnant women in 1940s-60s

60+ years to develop human evidence of breast cancer link

Hoover et al, 2011
Breast cancer risk factors

- Family history
- Ionizing radiation
- Reproductive history – menarche, menopause, births
- Overweight after menopause
- Pharmaceutical hormones: HRT, DES
- Alcohol
- Lack of physical exercise
- Tobacco smoke
- Shift work
We compiled 216 rodent mammary carcinogens

www.silentspring.org/sciencereview
Rudel et al. 2007
Similarities between established risk factors and potential breast carcinogens

- reproductive factors
- Rx hormones
- alcohol
- ionizing radiation
- (tobacco smoke)

environmental EDCs
solvents
genotoxicants (esp DSB-inducing)
PAHs
Animal and human studies—generally consistent

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Human Breast Cancer</th>
<th>Rodent Mammary Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT (E + P)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HRT (E)</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Oral Contraceptives (E + P)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DES</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Griseofulvin, Furosamide, Metronidazole</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Indomethacin, Nitrofurantoin</td>
<td>(-)</td>
<td>+</td>
</tr>
<tr>
<td>Ionizing radiation</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alcohol</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Heterocyclic amines (meat)</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Sleep disruption</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>PAH</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Solvents</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>DDE (adult exposure)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DDT (early life exposure)</td>
<td>(+)</td>
<td>Not tested</td>
</tr>
<tr>
<td>PCBs (general population)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PCBs (polymorphism)</td>
<td>(+)</td>
<td>Not tested</td>
</tr>
<tr>
<td>Dioxin (early life exposure)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

Rudel et al. 2014. Environmental Health Perspectives
What does chemical safety testing have to do with breast cancer?

Goals

– chemicals evaluated for safety
– tests relevant to breast cancer
Approach: By studying biological mechanisms of agents that increase breast cancer risk, induce rodent mammary tumors, or alter cancer susceptibility, we can learn to predict risks from chemicals that we can’t study directly.
Revolution in toxicology – high throughput screening

Robots

96-, 384-, 1536 Well Plates

Chemical Exposure

Cell Population

Pathway

Target Biology (e.g., Estrogen Receptor)
Halifax Project – Redefining Carcinogens

Acquired Hallmark Phenotypes

- Genomic instability
- Sustained Proliferative Signaling
- Insensitivity to Anti-growth Signals
- Resistance to Cell Death
- Replicative Immortality
- Deregulated Metabolism
- Tumor Promoting Inflammation
- Evading Immune Destruction

Disruptive Environmental Chemicals

- Tumor Microenvironment
- Angiogenesis
- Intravasation
- Blood Vessel
- Extravasation
- Tissue Invasion and Metastasis
- Metastases

Goodson et al. 2015. Carcinogenesis
### Events in biological processes potentially associated with breast cancer

#### Cellular & Molecular Events
- Alterations in hormone levels, metabolism or receptors
- Changes in gene transcription & translation
- Cell cycle changes
- Peptide hormones (growth hormones)
- Genotoxicity
- Oxidative stress
- Immune modulation
- Limitless replication potential
- Evasion of apoptosis
- Self-sufficiency in growth

#### Tissue Changes
- Breast density
- Tissue invasion
- Sustained angiogenesis
- TEB proliferation
- Altered mammary gland development
- Ductal hyperplasia
- Atypical hyperplasia

#### Susceptibility Factors
- Obesity
- Early onset of breast development
- Alterations in cyclicity
- Genetic polymorphisms in metabolizing enzymes
- Duration of lifetime estrogen exposure

Schwarzman et al. 2015. Environmental Health Perspectives
<table>
<thead>
<tr>
<th>Breast cancer-related endpoints in EPA’s ToxCast</th>
<th>Schwarzman et al. 2015, EHP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Steroid hormones</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Covered</strong></td>
<td><strong>Some gaps</strong></td>
</tr>
<tr>
<td>ERa; AR; estrogen metabolism; steroid</td>
<td>ERb</td>
</tr>
<tr>
<td>intermediates</td>
<td>Progesterone receptor</td>
</tr>
<tr>
<td></td>
<td>Aromatase</td>
</tr>
<tr>
<td><strong>Other endocrine (molecular)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Covered</strong></td>
<td><strong>Some gaps</strong></td>
</tr>
<tr>
<td>Thyroid receptor, AhR, PPAR, ROR; glucocorticoid</td>
<td>ERR</td>
</tr>
<tr>
<td></td>
<td>No assays</td>
</tr>
<tr>
<td></td>
<td>Other thyroid endpoints;</td>
</tr>
<tr>
<td></td>
<td>Her2; prolactin</td>
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<tr>
<td><strong>Carcinogenesis</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Covered</strong></td>
<td><strong>Some gaps</strong></td>
</tr>
<tr>
<td>Inflammation, xenobiotic metabolizing enzymes</td>
<td>Genotoxicity</td>
</tr>
<tr>
<td>cellular stress; other cancer hallmarks</td>
<td></td>
</tr>
<tr>
<td><strong>Mammary gland development &amp; other organism-level endocrine effects</strong></td>
<td>We don’t know how to study these in vitro!</td>
</tr>
<tr>
<td></td>
<td>MG morphology; hormone receptors in developing MG; reproductive development; circulating hormone levels</td>
</tr>
</tbody>
</table>
Cancer Prevention Science

Biological mechanism + Human exposure = Basis for action

Strength of evidence, not “proof”

Educate, Regulate, Reformulate
THANK YOU

Collaborators
• Janet Ackerman, Julie Brody, Silent Spring Institute
• Chris Vulpe, UC-Berkeley, now U Florida
• Paul Yaswen, Lawrence Berkeley Labs
• Megan Schwarzman, UC Berkeley Center for Green Chemistry
• Keith Houck and others at US EPA, NCCT
• Ray Tice and others at US National Toxicology Program

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• Avon Foundation for Women
Thank you!
Visit www.silentspring.org

p.s. We’re hiring!

- Data Science and Informatics
- Chemistry, Biochemistry and Biomonitoring
- Molecular Biology and Toxicology