The views in this presentation are those of the author and do not necessarily reflect those of the California Environmental Protection Agency or the its Office of Environmental Health Hazard Assessment

ENVIRONMENTAL HEALTH – CAUSAL PATHWAYS AND OPPORTUNITIES FOR INTERVENTION

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Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

California Breast Cancer Research Program 2016 Conference:
Joining Forces to Understand the Causes of Breast Cancer
San Francisco CA, February 29, 2016

Sources of response and variability in response in people

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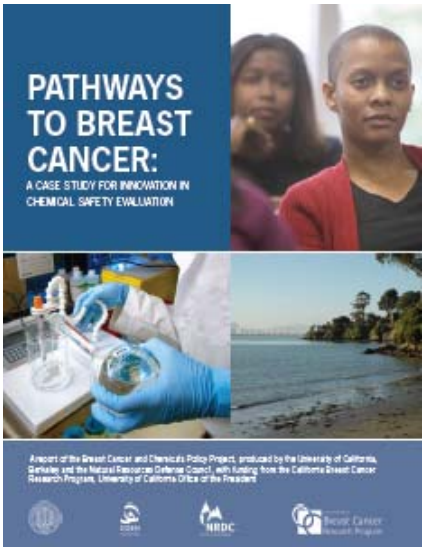
Breast Cancer and Chemicals Policy Project*

Expert Panel

- **Sarah Janssen**, UCSF & NRDC, now Kaiser
- **Meg Schwarzman**, UCB & UCSF
- Susan Braun, Commonweal
- Vince Cogliano, WHO IARC, now US EPA
- Shanaz Dairkee, California Pacific Medical Research Center (CPMRC)
- Suzanne Fenton, National Institute of Environmental Health Sciences (NIEHS)
- William Goodson, CPMRC
- Joe Guth, Science and Environmental Health Network
- Dale Johnson, UCB, Emiliem
- Jean Latimer, University of Pittsburgh
- Ron Melnick, NIEHS, now retired
- Rachel Morello-Frosch, UCB
- Ruthann Rudel, Silent Spring
- Gina Solomon, UCSF & NRDC, now CalEPA
- Carlos Sonnenschein, Tufts
- Lauren Zeise, CalEPA OEHHA



*Funded by CBCRP

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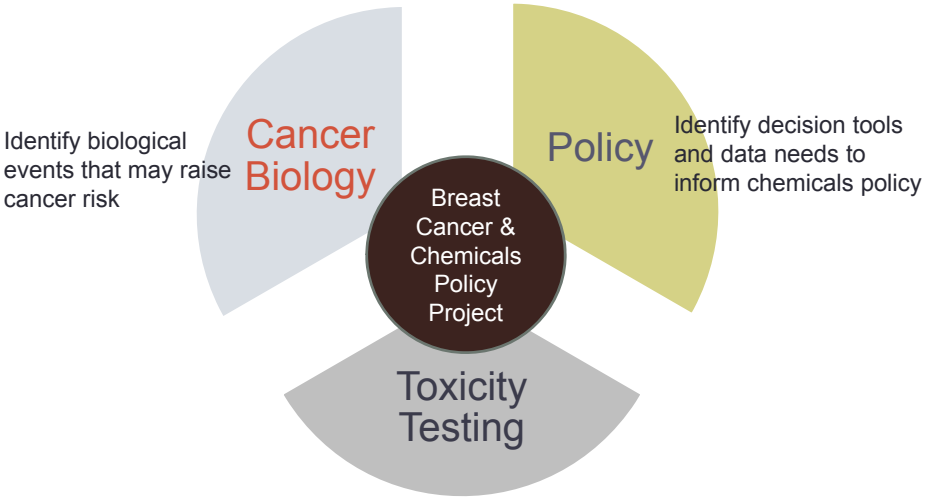
Panel Charge: Develop a conceptual strategy for screening chemicals for their potential to cause or contribute to breast cancer in humans.



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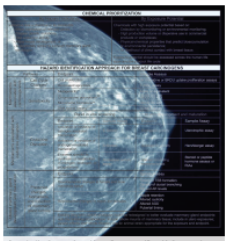
Review

Screening for Chemical Contributions to Breast Cancer Risk: A Case Study for Chemical Safety Evaluation

News | Science Selections

Identifying Potential Breast Carcinogens

A New Approach



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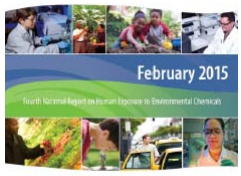
Context

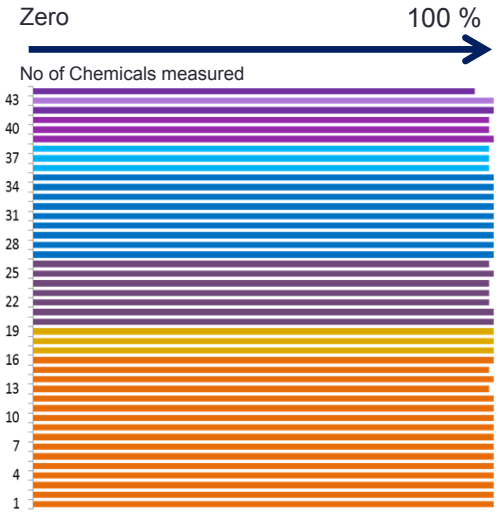
Learned from biomonitoring: Chemicals in wide use are measured in people

Federal NHANES



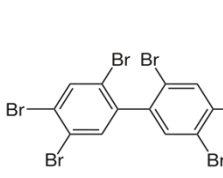
BIOMONITORING CALIFORNIA

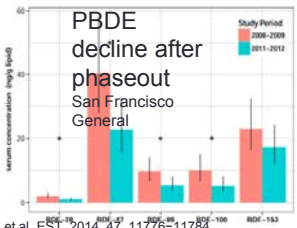
Chemicals in Women (Zota et al.)



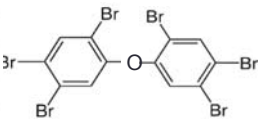
Intervention works, but watch for regrettable substitutions

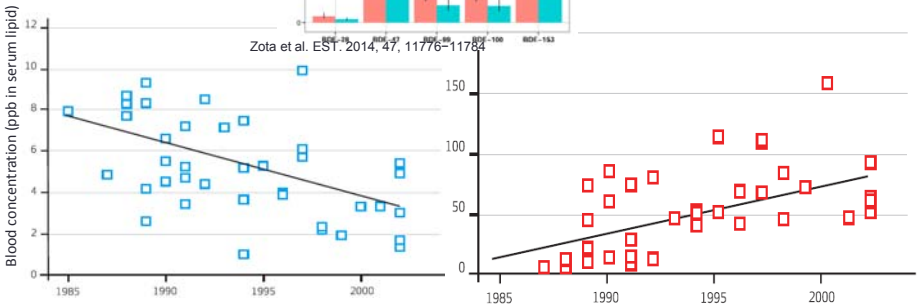
- PBBs: Phase out - 1974
- PBDEs: A substitute





Zota et al. EST. 2014, 47, 11776−11784





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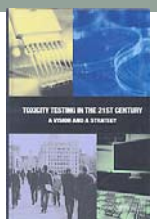
Focusing chemical exposure intervention efforts

…too many chemicals to test using standard methods

2007 New Testing Paradigm



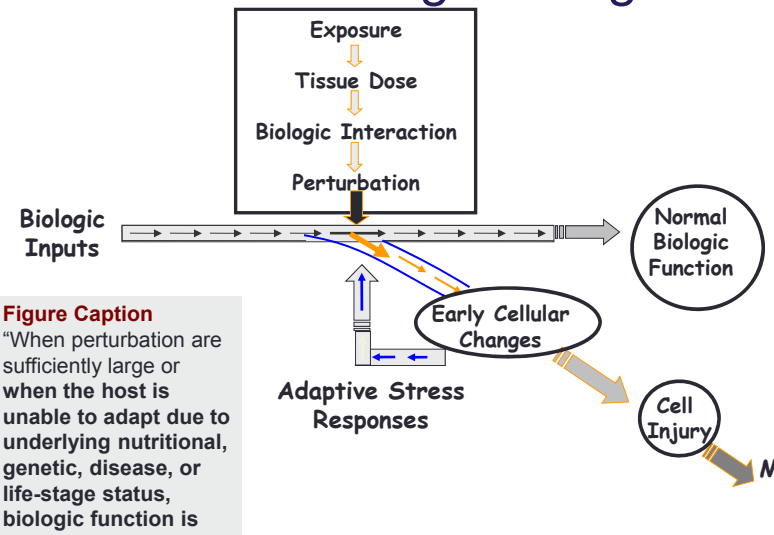


Figure Caption
"When perturbation are sufficiently large or **when the host is unable to adapt due to underlying nutritional, genetic, disease, or life-stage status, biologic function is compromised** resulting in toxicity and disease."

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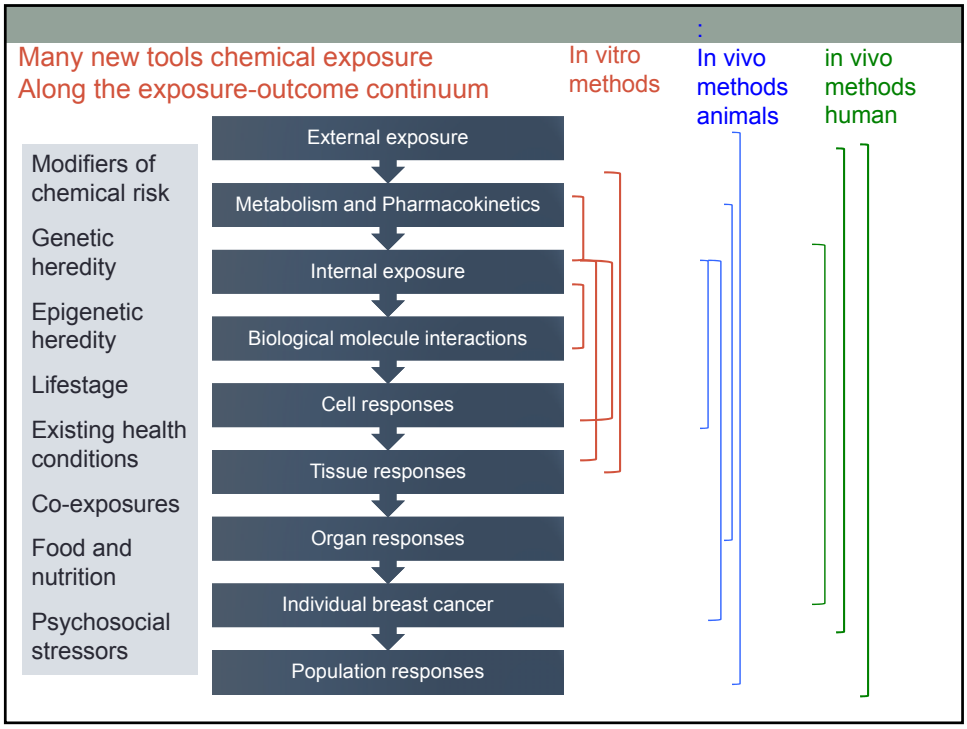
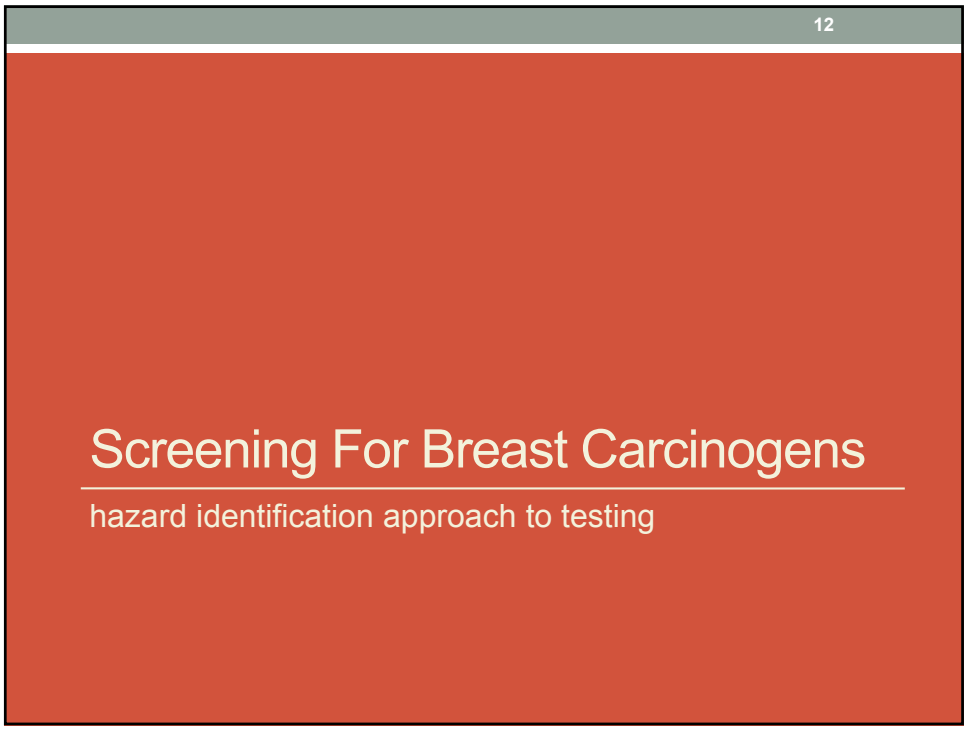

Many new tools chemical exposure
Along the exposure-outcome continuum
In vitro methods
In vivo methods animals
in vivo methods human
Modifiers of chemical risk
Genetic heredity
Epigenetic heredity
Lifestage
Existing health conditions
Co-exposures
Food and nutrition
Psychosocial stressors
External exposure
Metabolism and Pharmacokinetics
Internal exposure
Biological molecule interactions
Cell responses
Tissue responses
Organ responses
Individual breast cancer
Population responses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Screening For Breast Carcinogens
hazard identification approach to testing

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Steps to Develop the Approach

1. Identify toxicity "endpoints": Alterations in biological processes related to increased breast cancer risk
2. Identify toxicity assays: testing methods for detecting chemicals that affect these biological processes
3. Testing strategy: Propose an approach for prioritizing and testing chemicals for breast cancer causing potential
4. "Ground truth": "Virtual pilot" test by reviewing how well chemicals with well-known activity perform
5. Check coverage of current proposals: Compare endpoints with federal programs (ToxCast, Tox21 and EDSP)

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1. Identify toxicity "endpoints": Alterations in biological processes related to increased breast cancer risk

Cellular and molecular events	Tissue changes	Susceptibility factors
• Alterations in hormone levels, metabolism, receptors • Cell cycle changes • Changes in transcription, translation, epigenetic programming • Alteration in activity of growth hormones • Immune modulation • Inflammation • Oxidative stress • Genotoxicity • Limitless replication potential • Evasion of apoptosis • Autocrine growth	• Altered mammary gland development • Terminal end bud proliferation • Ductal hyperplasia • Atypical hyperplasia • Increased breast density/stromal hyperplasia • Adenomas • Carcinoma in situ • Tissue invasion • Sustained/enhanced angiogenesis	• Early onset of puberty • Increased lifetime duration of estrogen exposure (early menarche or late menopause) • Atypical function of metabolizing enzymes • Obesity

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- Identify toxicity assays: testing methods for detecting chemicals that affect the process alterations in Step 1

System	Alterations in biological processes, for example:					
	Cell cycle changes		Genotoxicity			
	Proliferation	Decreased apoptosis	Mutagenicity	Chromosome aberrations	Micronuclei formation	DNA strand breaks
in vitro						
in vivo animal						
in vivo human						

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- Testing strategy: Propose an approach for prioritizing and testing chemicals for breast cancer causing potential

Hazard Identification Approach for Breast Carcinogens					
Mechanisms		Endpoint		Sample Assays	
General carcinogenesis mechanisms	Cell cycle changes	Increased proliferation		3H-thymidine or BRDU uptake	
		Decreased apoptosis		TUNEL	
	Genotoxicity	Mutagenicity		Ames of equivalent	
		Chromosome aberrations		OECD TG 473	
		Micronuclei formation		OECD 487	
		DNA strand breaks		COMET assay	
Mechanisms associated with endocrine disruption	Endocrine Disruption	Rapid *in vitro* screening		In vivo development and maturation	
		Endpoint	Sample assay	Endpoint	Sample assay
		Estrogen mediated transcription change	E-Screen	Estrogenic activity	Uterotrophic assay
		Androgen mediated transcription change	A-Screen	Androgenic activity	Hershberger assay
		Steroidogenesis enzyme change	Aromatase activity assay	Altered circulating hormone levels	Hormone assays or RIAs
Altered Mammary Gland Development and Maturation	Precursor changes, biomarkers, tumors	Endpoint		Sample assays	
		Developmental changes in male and female tissue		Timing of TEB formation, density of ductal branching, ER & AR levels	
		Reproductive changes in ♀ or ♂		Nipple retention, altered cyclicity, AGD, pubertal timing	

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- "Ground truth": "Virtual pilot" test by reviewing how well chemicals with well-known activity perform

- 11 Well-studied chemicals and classified by IARC
  - 2 Known human breast carcinogens
  - 5 Some but less than sufficient evidence of breast cancer in humans (4 with animal evidence)
  - 3 No evidence of breast cancer in humans
    - 2 Animal mammary carcinogens
    - 1 Known human carcinogen (non-mammary tissue)
  - 1 Not known to cause cancer in animals
- Conducted literature search: Summarized assays results for endpoints in the HI Approach
  - Genotoxicity, endocrine disruption, altered mammary gland development,decreased apoptosis and autocrine growth

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- "Ground truth": "Virtual pilot" test by reviewing how well chemicals with well-known activity perform

CHEMICAL	CARCINOGENICITY FINDINGS[a]																						MECHANISMS																							
		HUMAN										ANIMAL										GENOTOXICITY ASSAYS						ENDOCRINE DISRUPTION ASSAYS										ALTERED MAMMARY GLAND DEVELOPMENT	EVIDENCE FOR OTHER MECH.										
		Breast			Other Sites							Mammary				Other Sites												Steroid synthesis		Estrogenic activity			Androgenic act.																
	IARC Group[b]	Cancer	Suggestive/limited	Some assoc. reported	Liver	Lung	Lymphoma	Reproductive tract	Skin	Kidney	Mouse	Rat	Hamster	Cat	Dog	Reproductive tract	Other endocrine org.	Liver	Lung	Skin	Mutagenicity in salmonella	Chromosome alterations	Micronuclei formation	DNA adduct formation	DNA strand breaks	Decreased DNA repair	Δ Steroidogenesis	Δ Aromatase activity	Estrogen transcription	E-Screen	Uterotrophic activity	A-Screen		Adocrine growth	Decreased apoptosis										
Known to Cause Breast Cancer in Women																																														
Diethylstilbestrol	1																				Ø	+	+	+	+	+	+	+	+	+	+	+	+	+										
HRT	1																				Ø	+	Ø	+	+	NI	NI	+	+	+	+	NI	NI	+	+									
Less Than Sufficient Human Evidence of Breast Cancer																																														
Ethylene oxide	1																				+	+	+	+	+	+	NI	NI	NI	NI	NI	NI	NI	NI	NI									
Tobacco smoke	1																				+	+	+	+	+	+	+	+	+	+	+	NI	NI	+	+									
MPA	2B																				Ø	Ø	Ø	Ø	NI	NI	Ø	+	Ø	+	+	+	NI	NI	+									
TCDD	1																				■	Ø	Ø	Ø	+	+	◆	◆	+	+	+	+	+	+									
DDT	2B																				Ø	■	Ø	+	+	Ø	◆	◆	◆	+	◆	●	+	●									
Animal Mammary Tumorigens																																														
Perfluorooctanoic acid	2B																				Ø	+	Ø	NI	Ø	NI	+	✚	+	+	+	NI	+	NI	+									
Vinyl chloride	1	NI																			+	+	+	+	+	+	NI	NI	NI	NI	NI	NI	NI	NI	NI									
Carcinogens with No Evidence of Breast Cancer																																														
Arsenic	1	No effect			●			●			No effect					/	/	/	/		■	◆	◆	+	◆	+	+	+	+	+	■	Ø	NI	+	Ø									
Chemicals Not Known to Cause Cancer																																														
Caprolactam	4	NI			NI						No effect					No effect					Ø	Ø	Ø	NI	Ø	Ø	NI	NI	NI	NI	NI	NI	NI	NI	NI									

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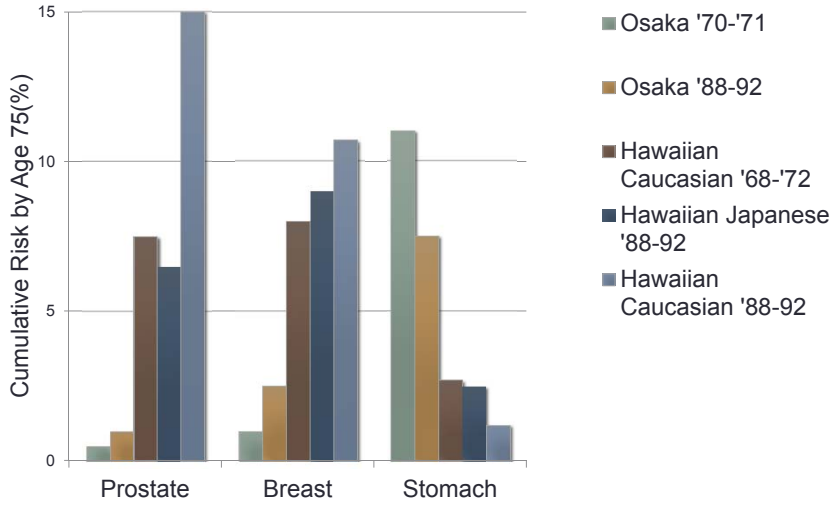
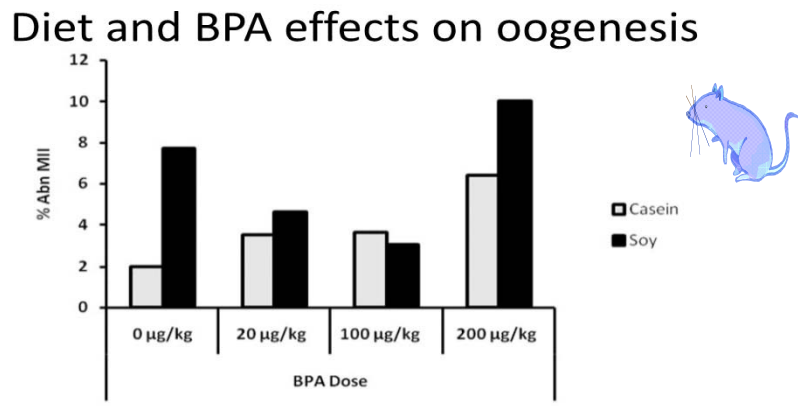
Concluding Remarks

Background: Immigration changes in cancer incidence



Background dependent dose-response

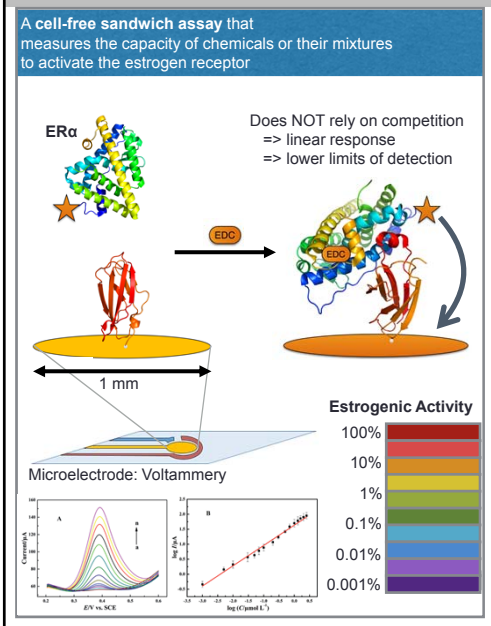


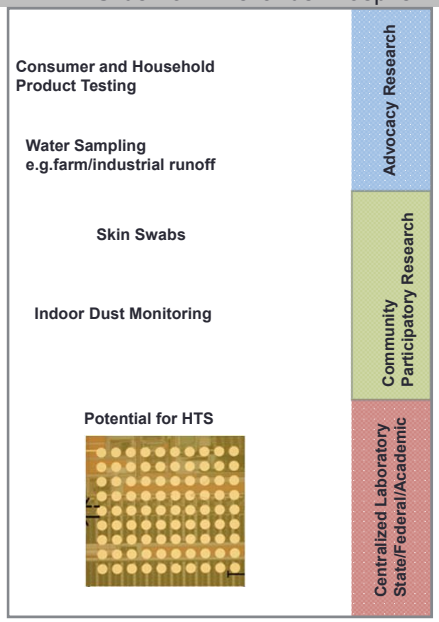
"Exogenous estrogens are a serious confounding variable in rodent studies designed to assess the effects of endocrine-disrupting chemicals, and, because estrogenic contaminants can be present in food, bedding materials, water, and caging materials, controlling for their presence is a daunting task." Muhlhauser et al. Biol Reprod 2009.

Kim Boekelheide slide, NRC Emerging Sciences Workshop, June 2012

Development of cost-efficient, simple readout Sensor of estrogenic activity

Slide from: Alexander Hoepker





Hazard Identification Approach to Breast Cancer

- Premise – Lack of relevant toxicity data can be addressed
- Chemicals can be screened for their their ability to alter processes involved in breast cancer
  - genotoxicity, cell cycle changes, endocrine disruption, altered mammary gland development
- Some endpoints may require a mammary tissue derived system (e.g., aromatase transcription)
  - Investigate where using breast tissue–derived cells and proteins would make existing assays more relevant to breast cancer.
- Specific gaps were identified in available tests (e.g., progesterone receptor binding)
- New assays suited to high throughput screening should be developed