

RESEARCHING THE ENVIRONMENT AND WOMEN'S HEALTH

Identifying Likely Breast Carcinogens Using Complementary Mechanistic Approaches

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CHE

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www.silentspring.org

Cancer Prevention Science

**Biological
mechanism**



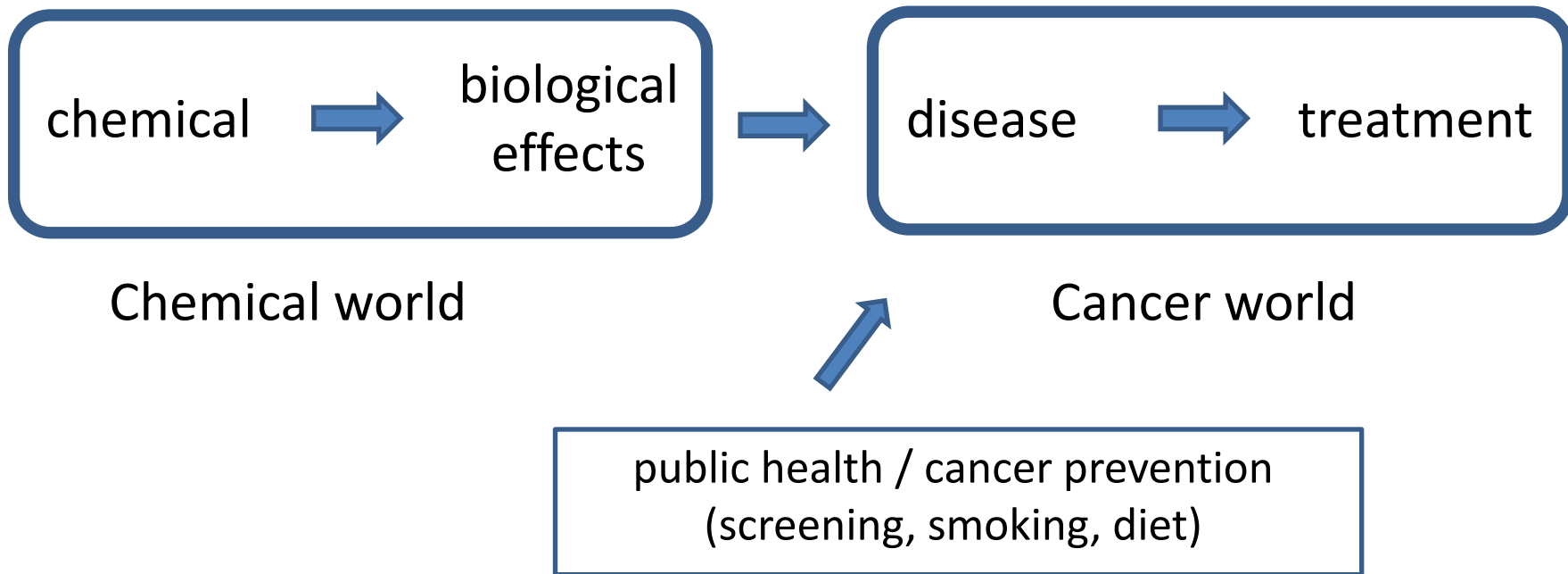
**Human
exposure**

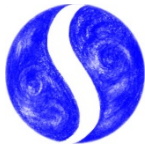
**Basis for
action**

Slide #4

**Strength of
evidence,
not “proof”**

**Educate
Regulate
Reformulate**





Silent Spring Institute

A shift in perspective leads to new questions

Two Questions

- What chemicals might cause breast cancer?
- What biological processes are important to include in chemical screening programs?

Three Approaches

- Pathways to breast cancer based on epidemiology
- Expert Panel - BC-related mechanisms for hazard screening
- Biological activities of mammary carcinogens in ToxCast

Breast cancer risk factors

- Family history

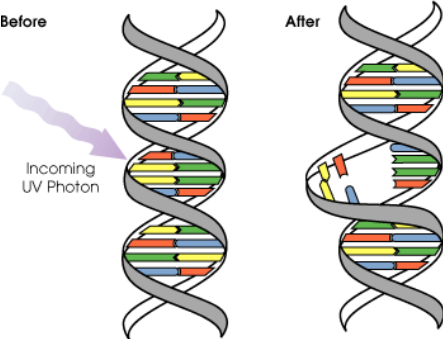
Carcinogens / Hormones

- Ionizing radiation
- Reproductive history – menarche, menopause, births
- Overweight after menopause
- Pharmaceutical hormones: HRT, DES
- Alcohol
- Lack of physical exercise
- Tobacco smoke
- Shift work

How might chemicals increase breast cancer risk?

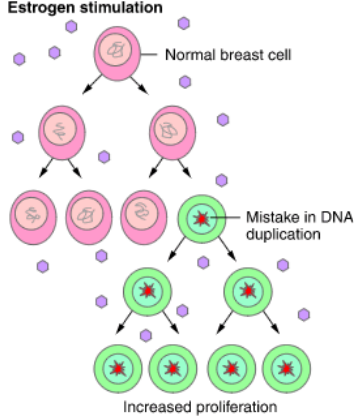
- Damaging DNA

Ionizing radiation



- Promoting tumor growth

HRT



- Disrupting development -> vulnerability

DES



Ionizing radiation and breast cancer

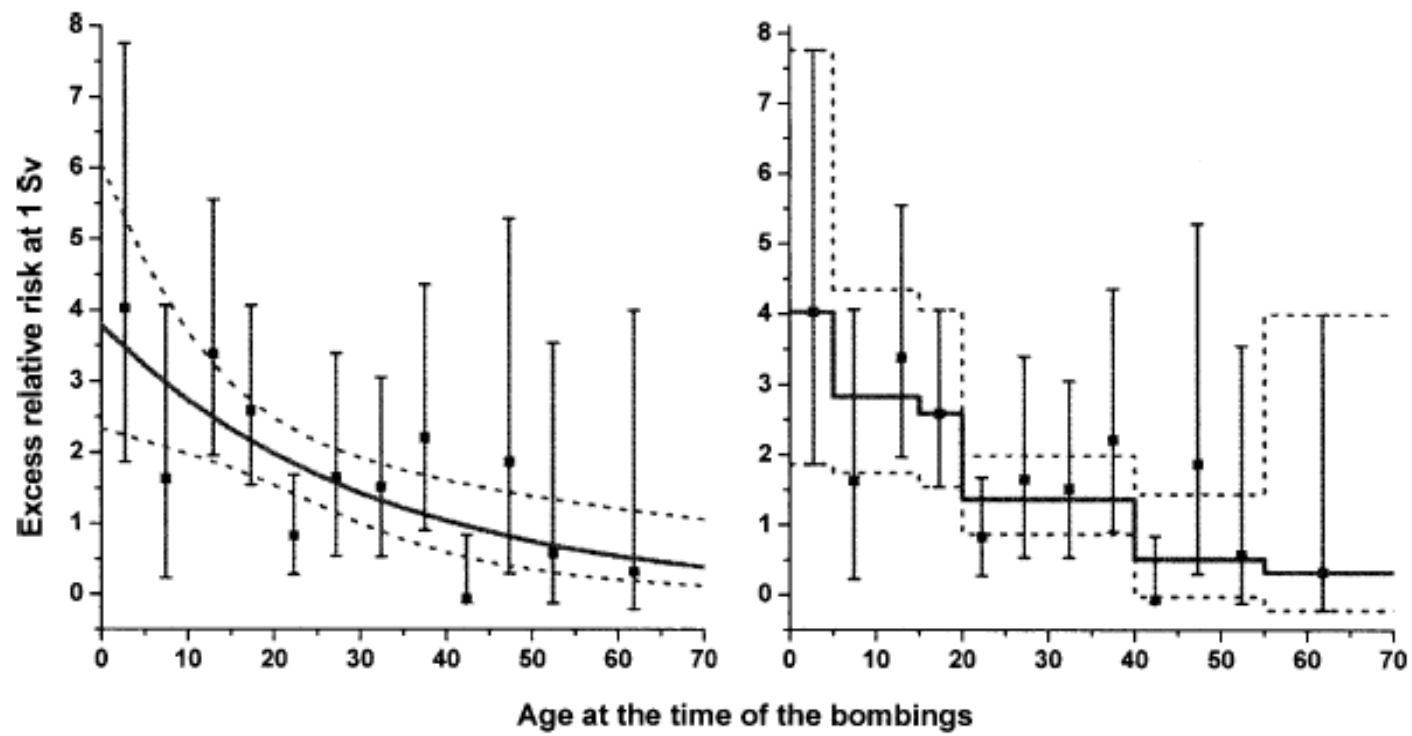
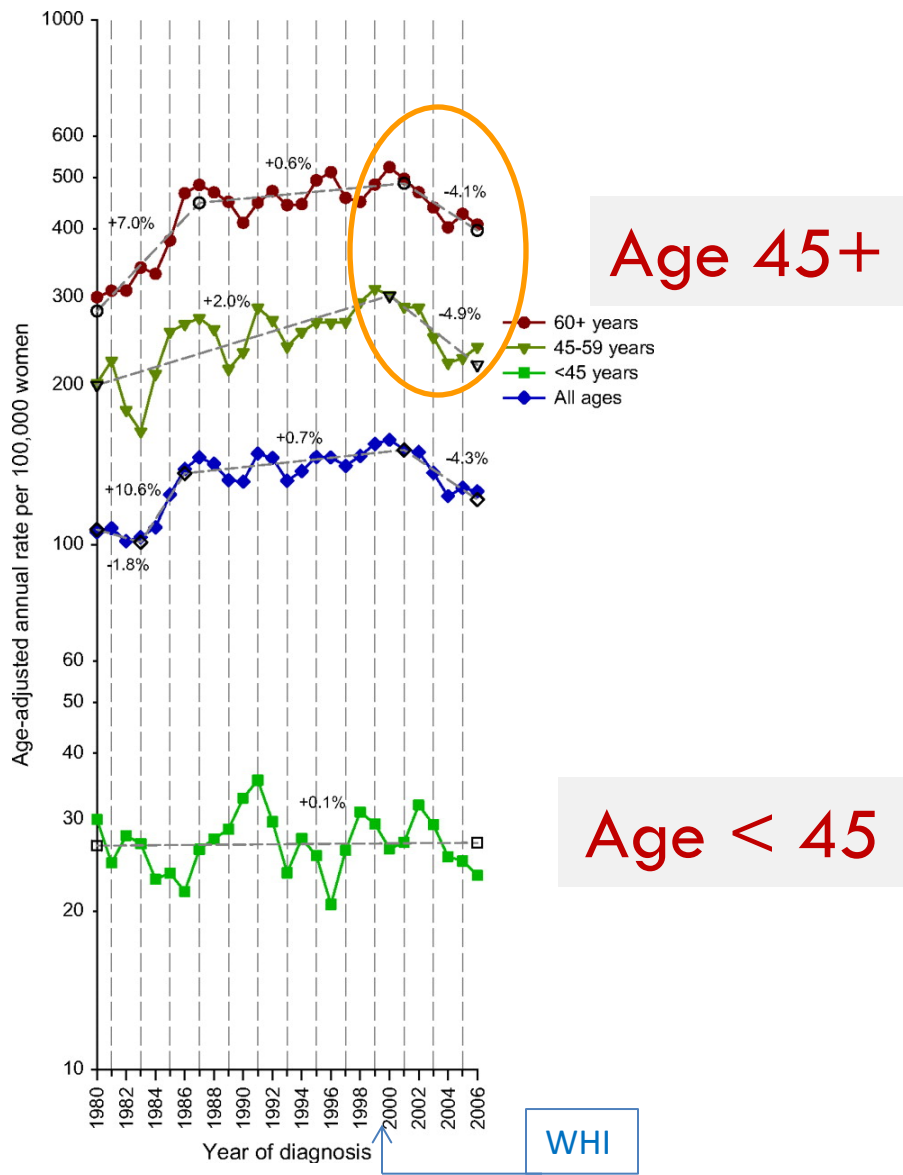


FIG. 3. Estimated excess relative risk per Sv with 90% confidence limits, by 5-year intervals of age ATB, e . The panels show a fitted exponential function on the left, $ERR_{1Sv} = a \times \beta^{e-25}$, and an isotonic regression on the right constrained only to be monotone non-decreasing in e .



Breast cancer incidence dropped when older women went off HRT

- 126,000 fewer breast cancer cases by 2012
- expenditure savings of \$35.2 billion.


Roth et al. 2014

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

Glass, A. G. et al. *J. Natl. Cancer Inst.* 2007 99:1152-1161

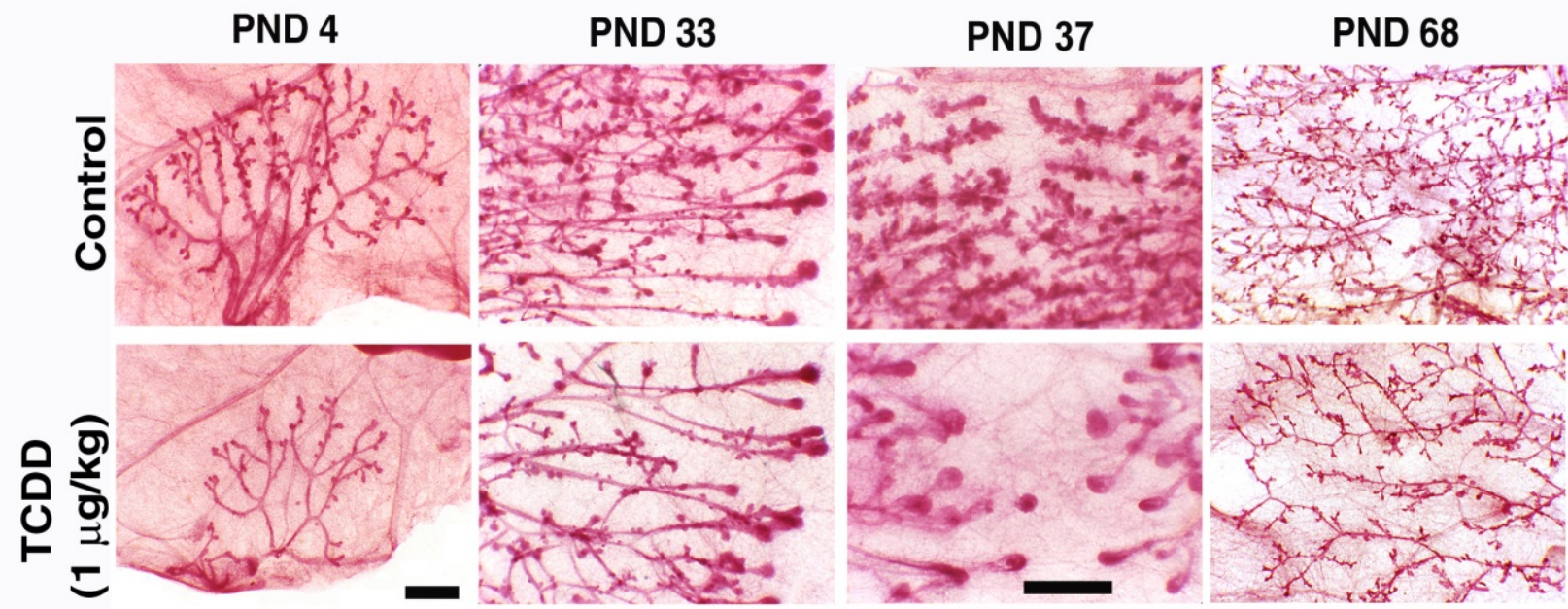
Diethylstilbestrol (DES)

Prescribed to pregnant women in 1940s-60s



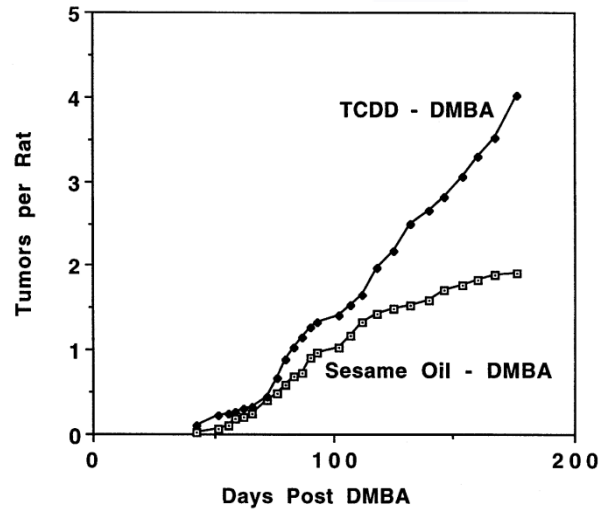
60+ years to develop human evidence of breast cancer link





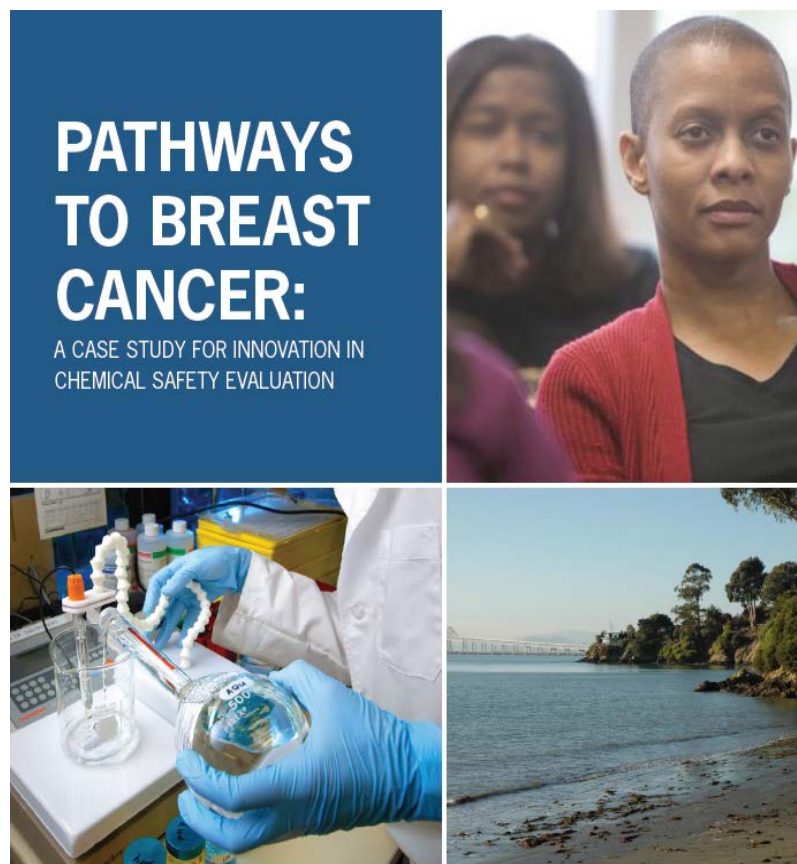
In utero exposure to TCDD alters MG development and susceptibility to carcinogens

Fenton et al. 2002 Tox Sci;
Brown et al. 1998, Carcinogenesis;
La Merrill et al. 2010, EHP



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

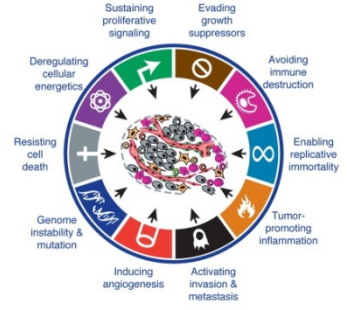
Megan R. Schwarzman,¹ Janet M. Ackerman,² Shanaz H. Dairkee,³ Suzanne E. Fenton,⁴ Dale Johnson,⁵ Kathleen M. Navarro,⁶ Gwendolyn Osborne,¹ Ruthann A. Rudel,² Gina M. Solomon,⁷ Lauren Zeise,⁸ and Sarah Janssen^{9,10}



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



ToxCast HTS Assay Overview

(>1100 Assay Endpoints/Readouts)

Assay Source

ACEA
 Appredica
 Attagene
 BioSeek
 CellzDirect
 NCGC/Tox21
 NHEERL MESC
 NHEERL NeuroTox
 NHEERL Zebrafish
 Novascreen
 Odyssey Thera

Organism

Human
 Rat
 Mouse
 Zebrafish
 Sheep
 wild boar
 Rabbit
 Cattle
 Guinea pig

Biological Process Target

cell Proliferation
 cell death
 mitochondrial depolarization
 protein stabilization
 oxidative phosphorylation
 regulation of TF activity
 regulation of gene expression
 receptor activity
 receptor binding

Detection Technology

RT-CES
 Fluorescence
 ELISA
 Alamar Blue Reduction
 Microscopy
 Reporter gene
 Spectrophotometry
 Radioactivity
 HPLC
 TR-FRET
 Fluorescence Polarization
 Luminescence
 Protein-fragment
 Complementation

Readout Type

Single
 Multiplexed
 Multiparametric

Tissue

Lung	Breast
Liver	Vascular
Skin	Kidney
Cervix	Testis
Uterus	Brain
Intestinal	Spleen
Bladder	Ovary
Pancreas	Prostate
Bone	

Target Family

Response Element
 Transporter
 Cytokines
 Kinase
 Nuclear Receptor
 CYP
 Cholinesterase
 Phosphatase
 Protease
 Metabolism
 GPCR
 Ion Channel

Assay Design Type

viability reporter
 morphology reporter
 conformation reporter
 enzyme reporter
 membrane potential reporter
 binding reporter
 inducible reporter

Cell Format

Cell line (6)
 Primary Cell (3)
 Cell-Free
 Cell-Based

Breast cancer-related endpoints in ToxCast

Steroid hormones

Schwarzman et al. 2015, *EHP*

Some coverage

ER α ; AR; estrogen metabolism; steroid intermediates

Gaps

ER β
Progesterone receptor
Aromatase

Other endocrine (molecular)

Some Coverage

Thyroid receptor, AhR, ROR; glucocorticoid

Gaps

ERR, PPAR

No assays

Other thyroid endpoints; Her2; prolactin

Carcinogenesis

Some coverage

Inflammation, xenobiotic metabolizing enzymes, cellular stress; other cancer hallmarks

Gaps

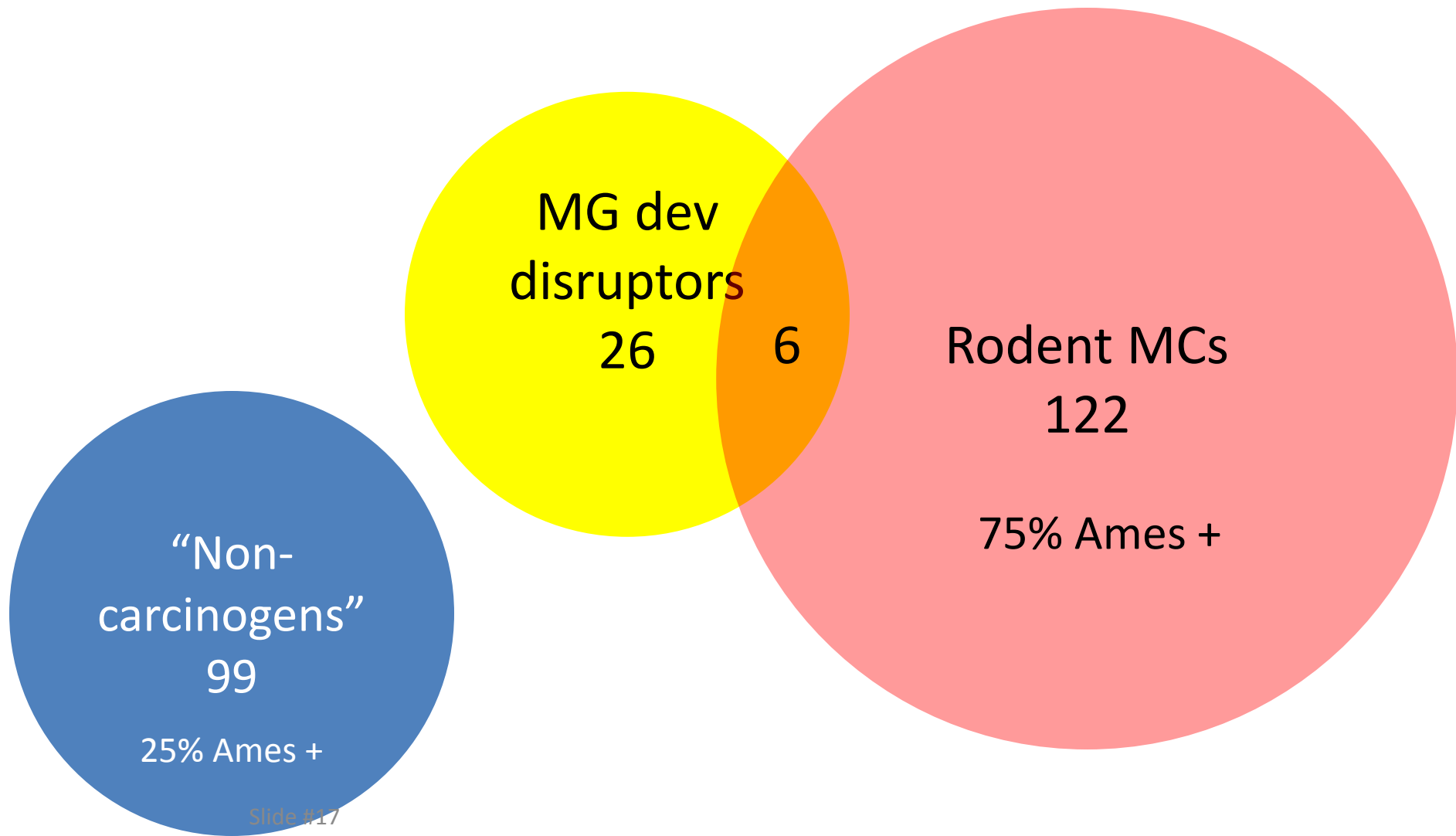
Genotoxicity

Mammary gland development & other organism-level endocrine effects

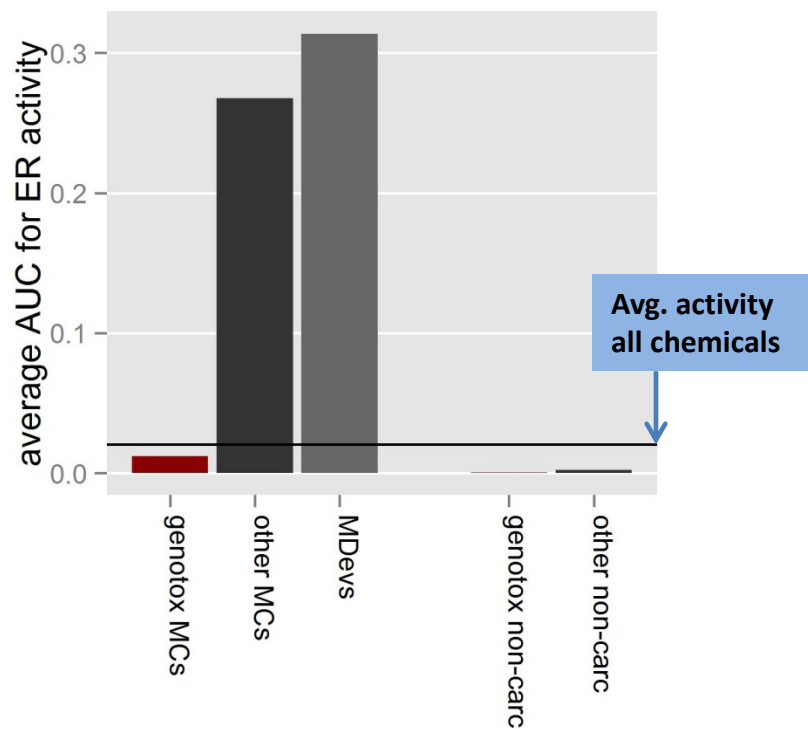
We don't know how to study these in vitro!

MG morphology; hormone receptors in developing MG; reproductive development; circulating hormone levels; altered susceptibility to carcinogen

Which ToxCast pathways “light up” for mammary gland carcinogens?



Estrogen activity is enriched for non-genotoxic MCs and mammary gland developmental toxicants



	Mammary gland targets			NTP non-carcinogens	
Tumors	+	+	NA	-	-
Genotoxicity	+	-	NA	+	-



p.s. We're hiring post-docs!

- **Endocrinology and Reproductive Bio**
- **Computational Toxicology**
- **Data Science/Informatics**