



SILENT SPRING INSTITUTE



RESEARCHING THE ENVIRONMENT AND WOMEN'S HEALTH

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

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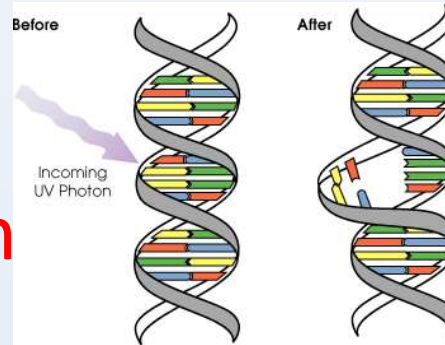
Collaborative on Health and the Environment
November 12, 2015

What do established breast carcinogens teach us about how chemicals might increase risk?

How might chemicals increase breast cancer risk?

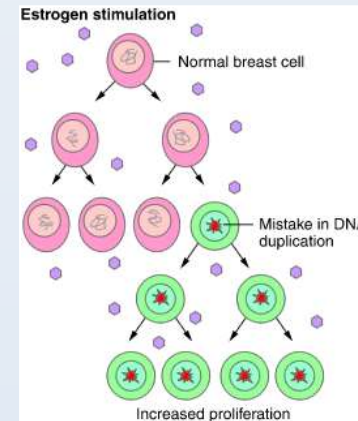
- Damaging DNA

Ionizing radiation



- Promoting tumor growth

HRT



- Disrupting development -> vulnerability

DES



Ionizing radiation and breast cancer

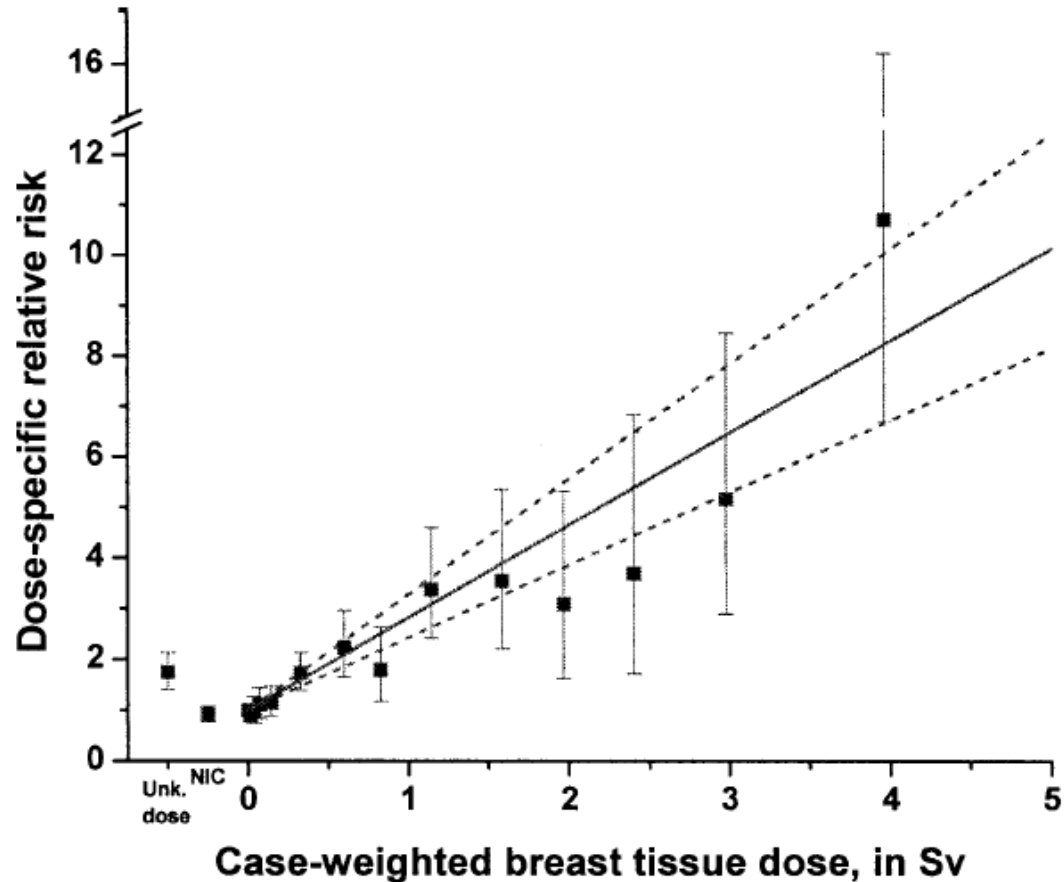
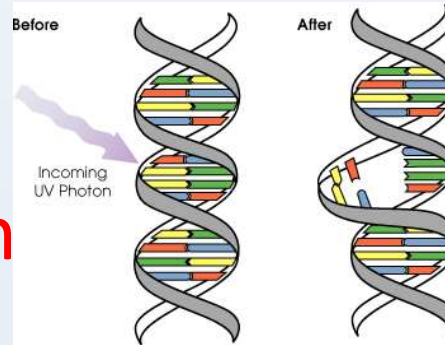


FIG. 1. Estimated relative risk of breast cancer, with 90% confidence limits, by exposure status and radiation dose, with fitted linear dose response for exposed subjects with dose estimates. All ages combined.

How might chemicals increase breast cancer risk?

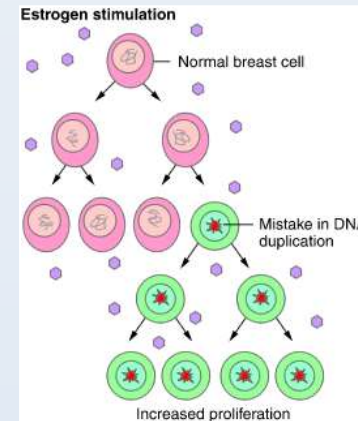
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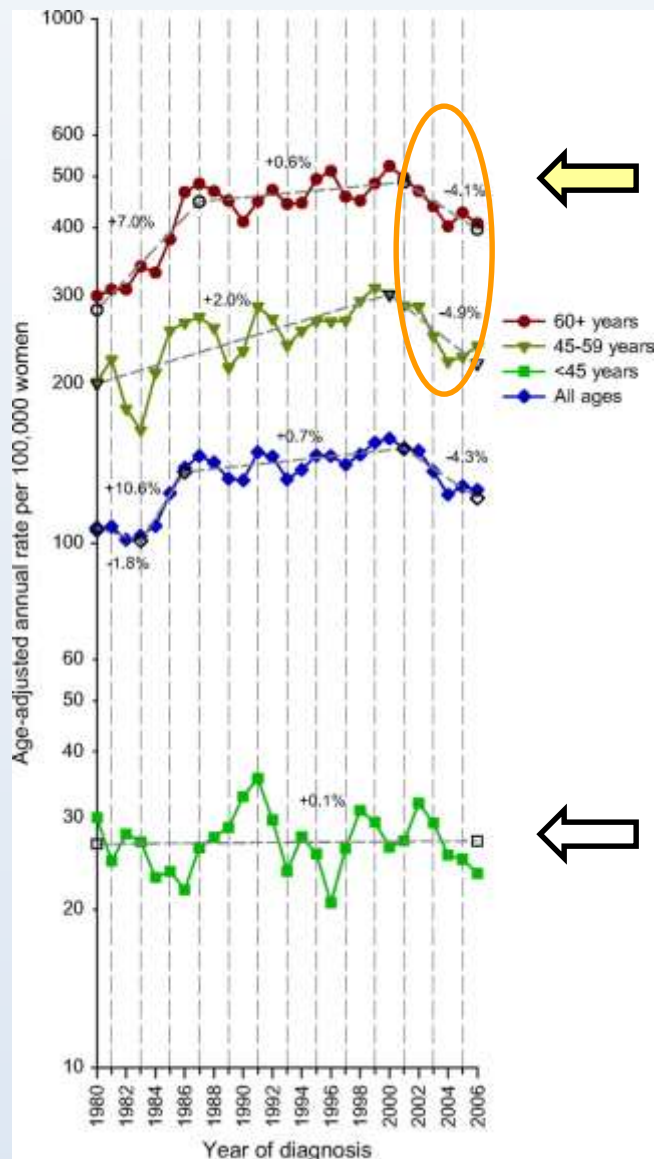
- Disrupting development -> vulnerability

DES



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

Prevention is powerful!



← Women 45+

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

← Women < 45

Subsequent economic analysis by Roth et al. 2014

The WHI scenario resulted in:

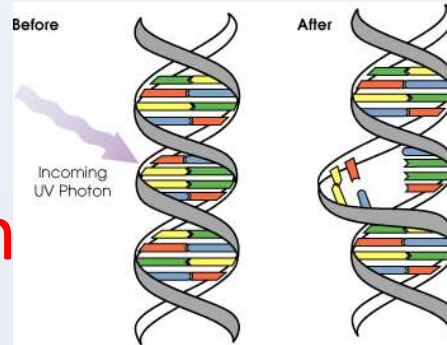
- 4.3 million fewer cHT users
- 126,000 fewer breast cancer cases
- expenditure savings of \$35.2 billion

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

How might chemicals increase breast cancer risk?

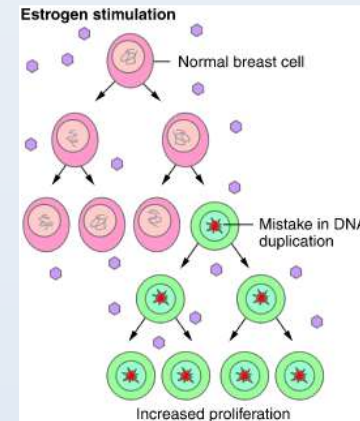
- Damaging DNA

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DES



Diethylstilbestrol (DES)

Prescribed to pregnant women in 1940s-60s



60+ years to develop human evidence of breast cancer link

THE NEW ENGLAND JOURNAL OF MEDICINE

ORIGINAL ARTICLE

Adverse Health Outcomes in Women Exposed In Utero to Diethylstilbestrol

Hoover et al, 2011

Breast cancer risk factors

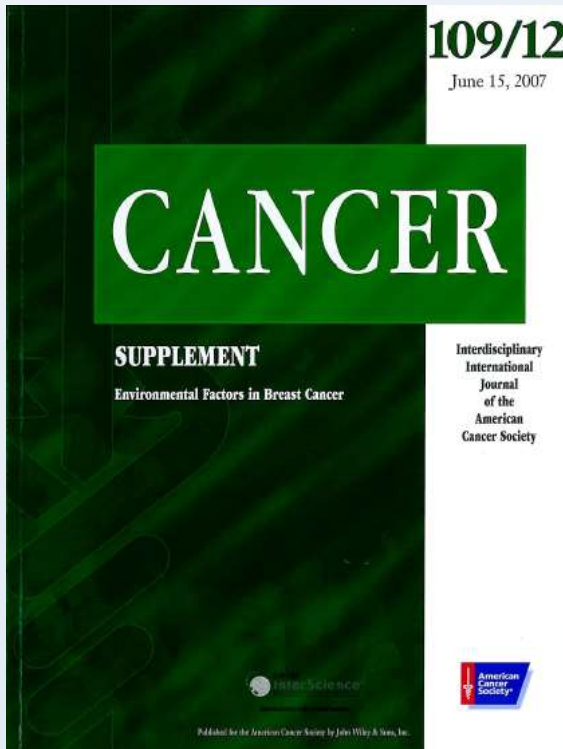
- Family history

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



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
- genotoxics (esp DSB-inducing)
- PAHs
-
- ```
graph LR; A[reproductive factors] <--> B[environmental EDCs]; C[Rx hormones] <--> B; D[alcohol] <--> E[solvents]; F[ionizing radiation] <--> G[genotoxics (esp DSB-inducing)]; H["(tobacco smoke)"] <--> I[PAHs];
```

# Animal and human studies- generally consistent

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

|     |                                      |
|-----|--------------------------------------|
| +   | Stronger evidence of association     |
| (+) | Limited evidence of association      |
| (-) | Limited evidence for no association  |
| -   | Stronger evidence for no association |

Rudel et al. 2014. Environmental Health Perspectives

# What does chemical safety testing have to do with breast cancer?

## *Goals*

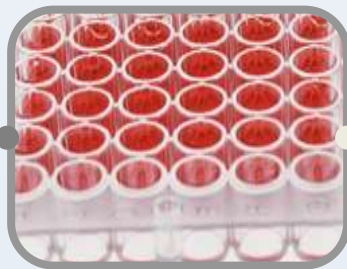
- chemicals evaluated for safety*
- tests relevant to breast cancer*

*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.*

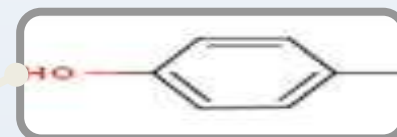
# Revolution in toxicology – high throughput screening



Robots



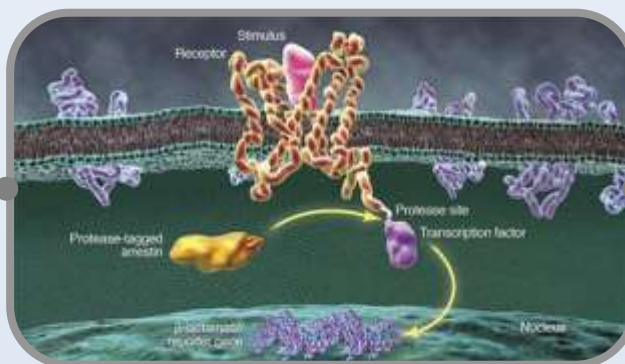
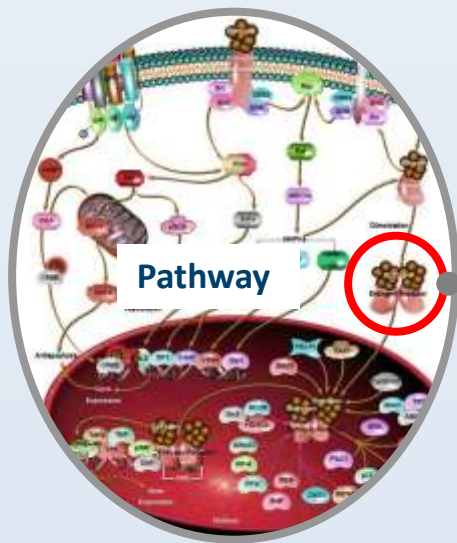
96-, 384-, 1536  
Well Plates



Chemical Exposure



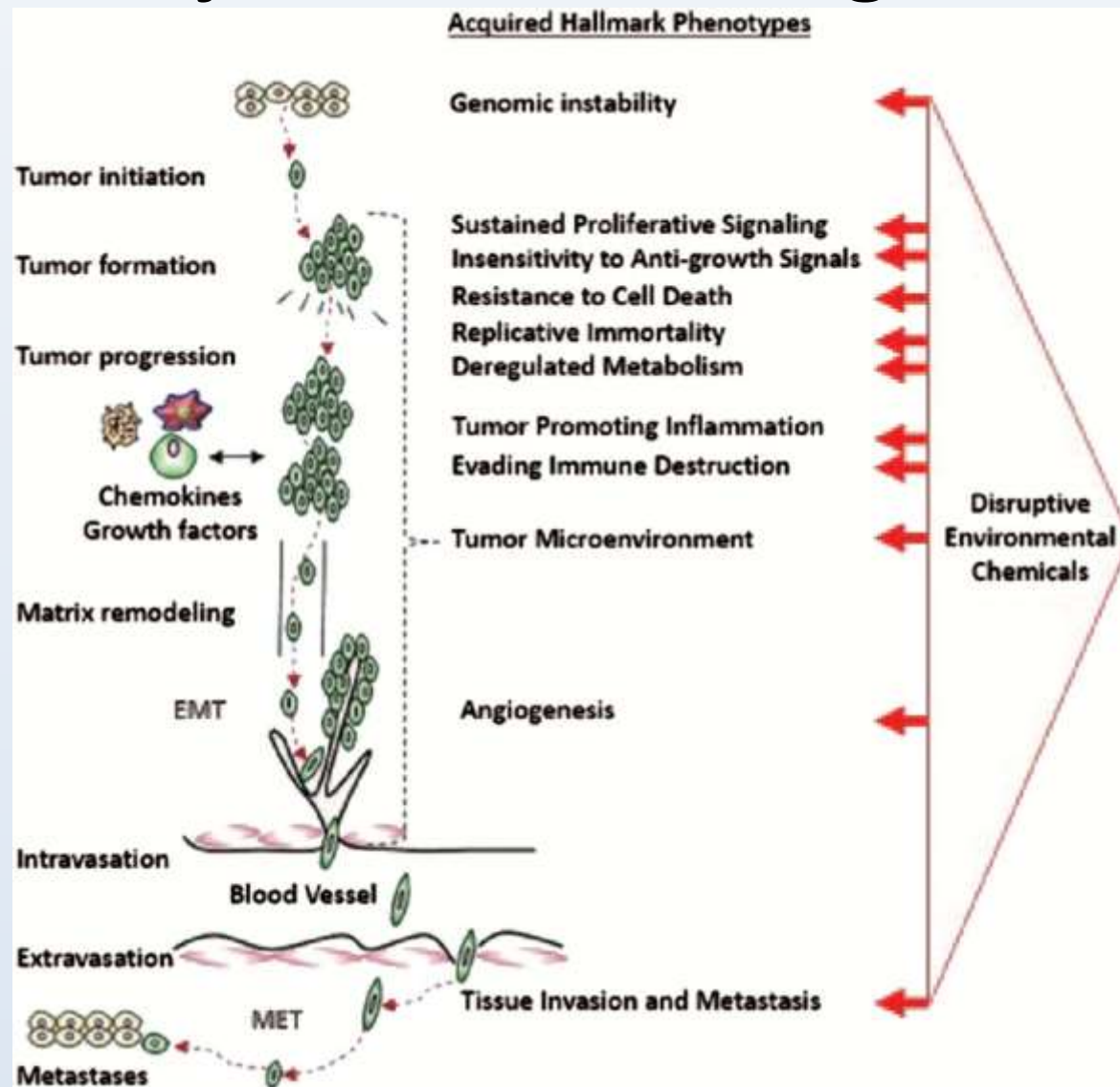
Cell Population



Target Biology  
(e.g., Estrogen Receptor)



# Halifax Project – Redefining Carcinogens



## Events in biological processes potentially associated with breast cancer

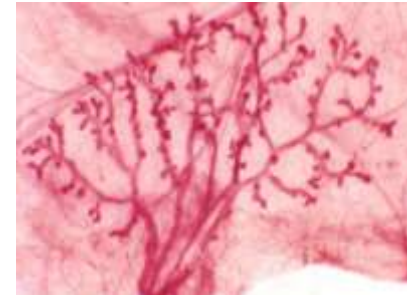
### Cellular & Molecular Events

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



### Tissue Changes

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



### Susceptibility Factors

|                                   |                                               |
|-----------------------------------|-----------------------------------------------|
| Obesity                           | Genetic polymorphisms in metabolizing enzymes |
| Early onset of breast development | Duration of lifetime estrogen exposure        |
| Alterations in cyclicality        |                                               |



Schwarzman et al. 2015. Environmental Health Perspectives

# Breast cancer-related endpoints in EPA's ToxCast

Schwarzman et al. 2015, *EHP*

## Steroid hormones

### Covered

ERa; AR; estrogen metabolism; steroid intermediates

### Some gaps

ERb  
Progesterone receptor  
Aromatase

## Other endocrine (molecular)

### Covered

Thyroid receptor, AhR, PPAR, ROR; glucocorticoid

### Some gaps

ERR

### No assays

Other thyroid endpoints; Her2; prolactin

## Carcinogenesis

### Covered

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

### Some 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

# 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

## Funders

- California Breast Cancer Research Program – Special Research Initiatives
- Avon Foundation for Women

# Thank you!

## Visit [www.silentspring.org](http://www.silentspring.org)

## p.s. We're hiring!

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

FORGING NEW FRONTIERS IN ENVIRONMENTAL RESEARCH TO PREVENT BREAST CANCER

A GALA CELEBRATION  
OCTOBER 20, 2015

With Guest Speaker  
**Siddhartha Mukherjee, M.D., Ph.D.**  
author, *The Emperor of All Maladies: A Biography of Cancer*



### FEATURED RESEARCH



NEW

Lower doses of common product ingredient might increase breast cancer risk.



NEW

Study measures carcinogenic flame retardants in people



NEW

Study identifies priority breast carcinogens and biomonitoring methods

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