

Chemicals that increase synthesis of  
estrogen and progesterone as risk factors  
for breast cancer: A case study for 21st  
century approaches to identifying likely  
carcinogens

**Ruthann Rudel**

Director of Research

Silent Spring Institute

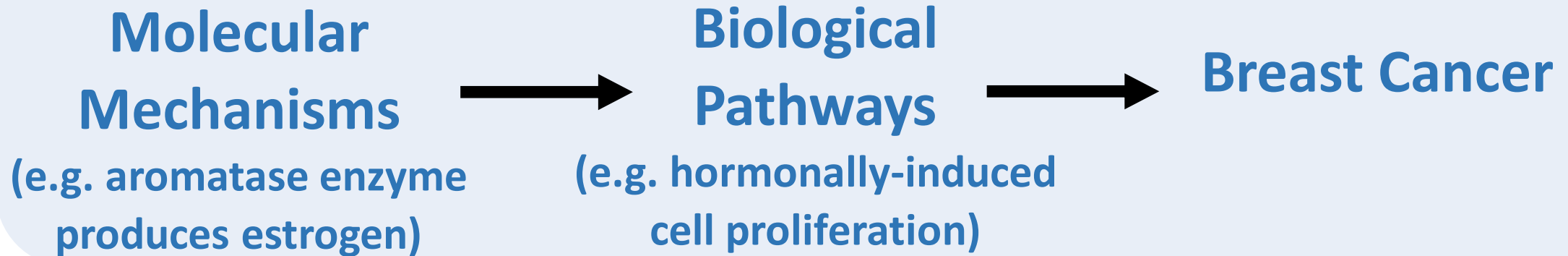


SILENT SPRING INSTITUTE  
Researching the Environment and Women's Health



# What chemical exposures increase breast cancer risk?

## 1) How does breast cancer develop?

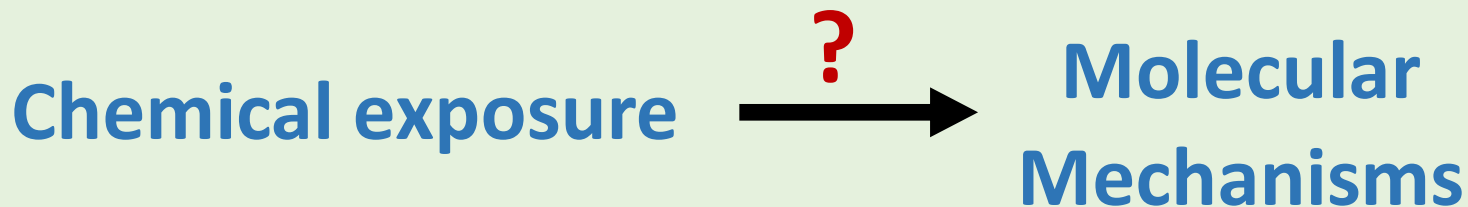


# What chemical exposures increase breast cancer risk?

## 1) How does breast cancer develop?

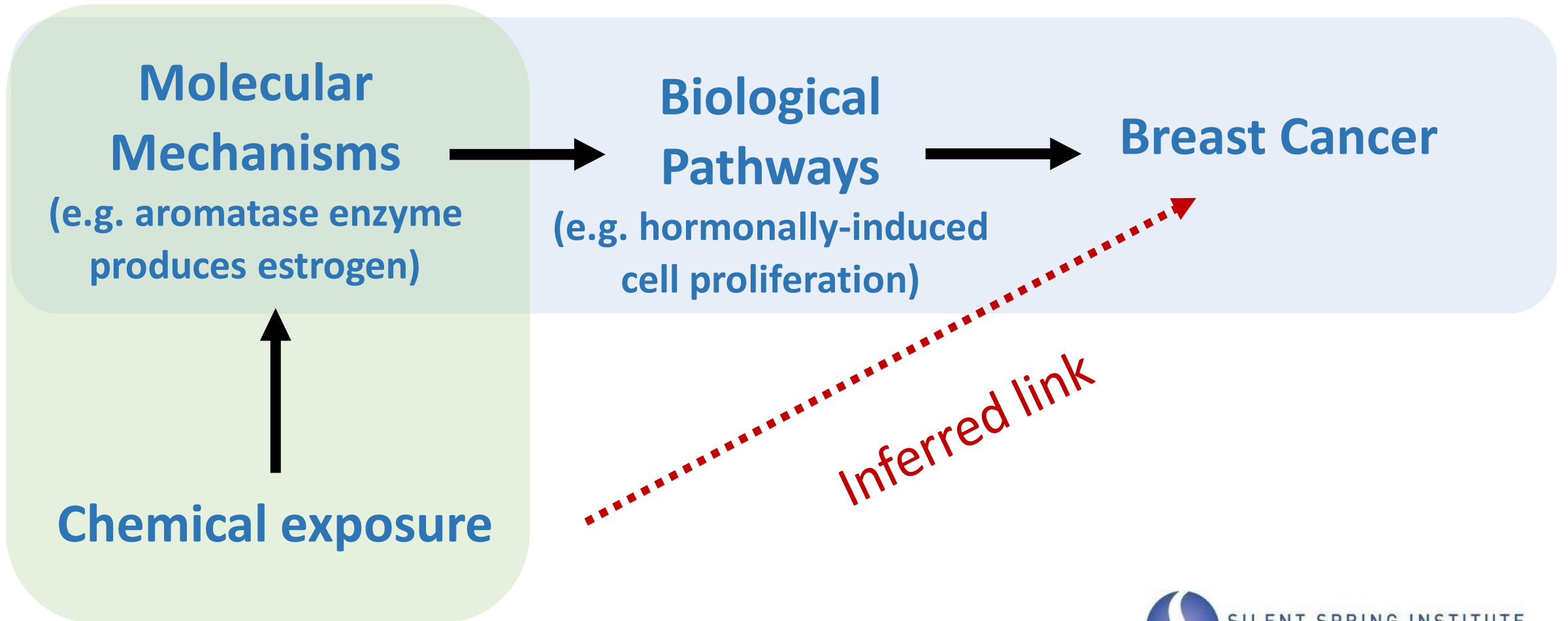


## 2) How do chemicals affect biology?





# What chemical exposures increase breast cancer risk?

## 3) Which chemicals cause effects related to breast cancer?



Vol. 129, No. 7 | Research

## Application of an *in Vitro* Assay to Identify Chemicals That Increase Estradiol and Progesterone Synthesis and Are Potential Breast Cancer Risk Factors

 is accompanied by ▼
Bethsaida Cardona and Ruthann A. Rudel Published: 21 July 2021 | CID: 077003 | <https://doi.org/10.1289/EHP8608> | Cited by: 1
 Sections  PDF

 Supplement

### Abstract


**Background:** Established breast cancer risk factors, such as hormone replacement therapy and reproductive factors, increase estrogen and progesterone (P4) activity.

**Objective:** We aimed to use *in vitro* screening data to identify chemicals that increase the synthesis of estradiol and progesterone, which are potential risks.

**Method:** Using data from a high-throughput (HT) *in vitro* steroidogenesis assay developed for the U.S. EPA's ToxCast program, we identified chemicals that increased estradiol (E2-up) or progesterone (P4-up) in human cells. We prioritized chemicals by their activity. We compiled *in vivo* studies and assessments about carcinogenicity and reproductive/developmental (repro/dev) toxicity. We identified exposure sources and predicted intakes.



**Results:** We found 296 chemicals increased E2 (182) or P4 (185), with 71 chemicals increasing both. *In vitro* data is consistent with this mechanism. Of the E2- and P4-up chemicals, about 30% were likely repro/dev toxicants. About 13% were classified as unlikely. However, most of the chemicals had insufficient *in vivo* data to evaluate their risk. We identified 29 chemicals associated with mammary gland effects, and also tested in the H294R assay, 29 increased E2 or P4, including the known carcinogen 7,12-dimethylbenz(a)anthracene. E2- and P4-up chemicals include pesticides, consumer products, and drinking water contaminants.

**Discussion:** The U.S. EPA's *in vitro* screening data identified several hundred chemicals that should be considered potential breast cancer risk factors because they increased E2 or P4 synthesis. *In vitro* data is a helpful addition to current toxicology data sensitive to mammary gland effects. Relevant effects on the mammary gland are often not noticed or are missed in *in vivo* studies. Fifty-three active E2-up and 59 active P4-up chemicals that are in consumer products, including dichlorophenol and cyfluthrin. Fifty-three active E2-up and 59 active P4-up chemicals that are in consumer products, including dichlorophenol and cyfluthrin.


 Open Access


Vol. 129, No. 7 | Invited Perspective

## Invited Perspective: Prioritizing Chemical Testing and Evaluation Using Validated *in Vitro* Assays Relevant to Key Characteristics

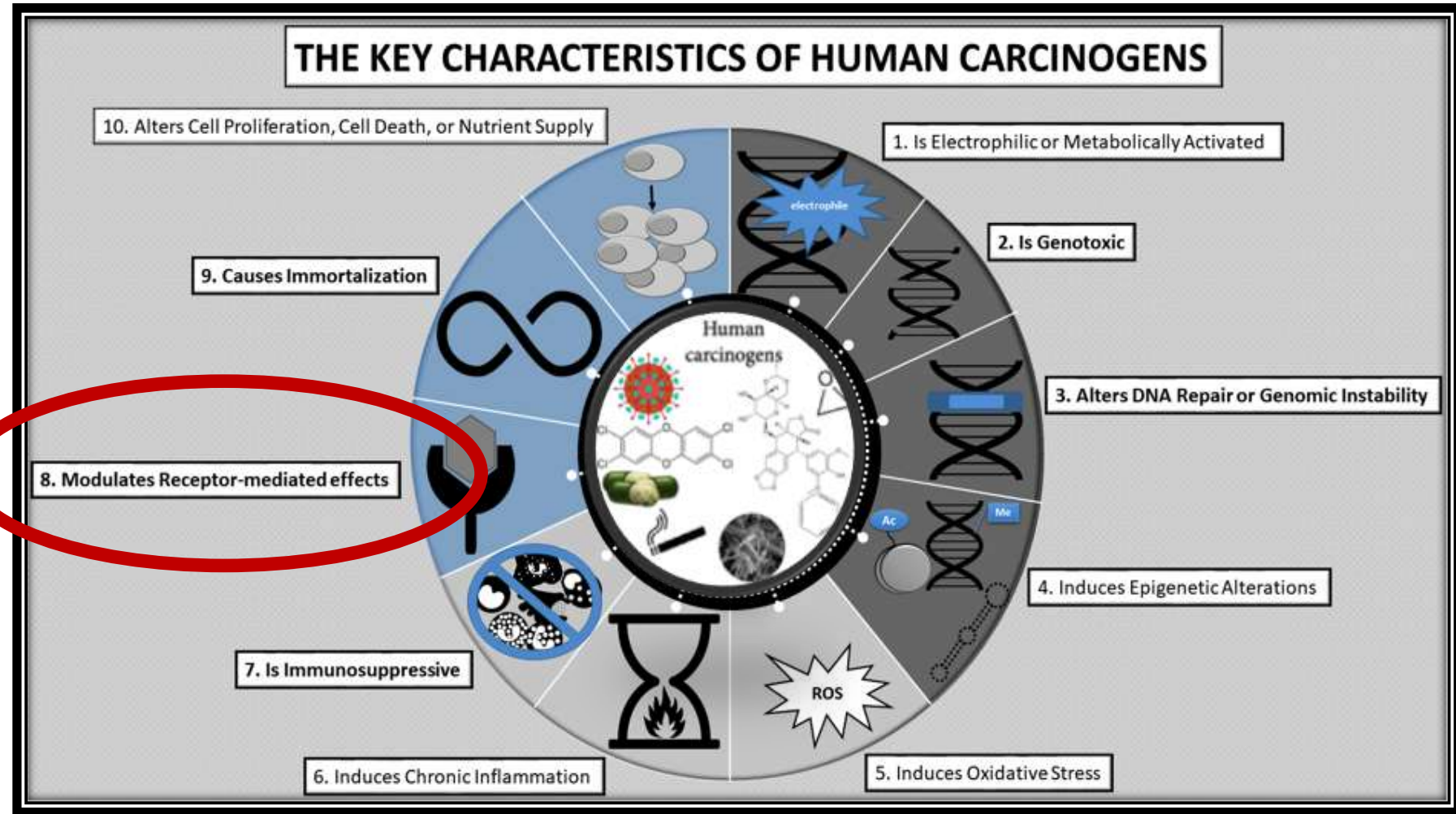
 is companion of ▼
Kathryn Z. Guyton  and Mary K. Schubauer-BeriganPublished: 21 July 2021 | CID: 071303 | <https://doi.org/10.1289/EHP9507>
 PDF

 Tools  Share

 Open Access

By interfering with hormone action, endocrine-disrupting chemicals (EDCs) can increase the risk of various adverse health outcomes, including cancer and reproductive impairment (La Merrill et al. 2020). In their article, Cardona and Rudel (2021) have identified nearly 300 chemicals that increased estradiol, progesterone, or both in an *in vitro* steroidogenesis assay that is internationally validated for use in regulatory contexts. They screened publicly available testing data for more than 2,000 chemicals tested in the ToxCast™ high-throughput *in vitro* steroidogenesis assay in cultured human H295R adrenocarcinoma cells. This U.S. Environmental Protection Agency Tier 1 assay has been used to study chemical impacts on 13 hormones involved in the steroidogenic pathway, including estrogens and progestogens (Haggard et al. 2018; Karmaus et al. 2016). Cardona and Rudel focused specifically on estradiol and progesterone. For the active agents, they systematically compiled available *in vivo* evidence from databases, authoritative evaluations, and published studies. An important consideration was whether

# New Insights for Identifying Cancer Causes



IARC  
@IARCWHO

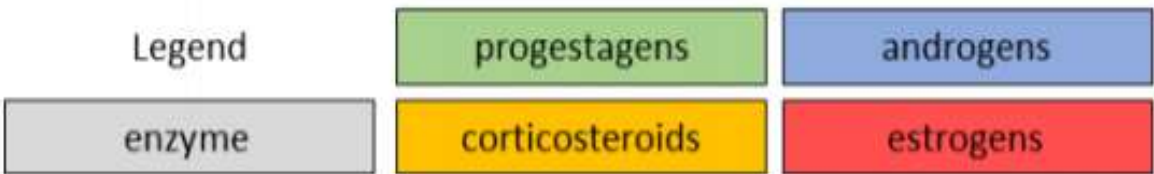
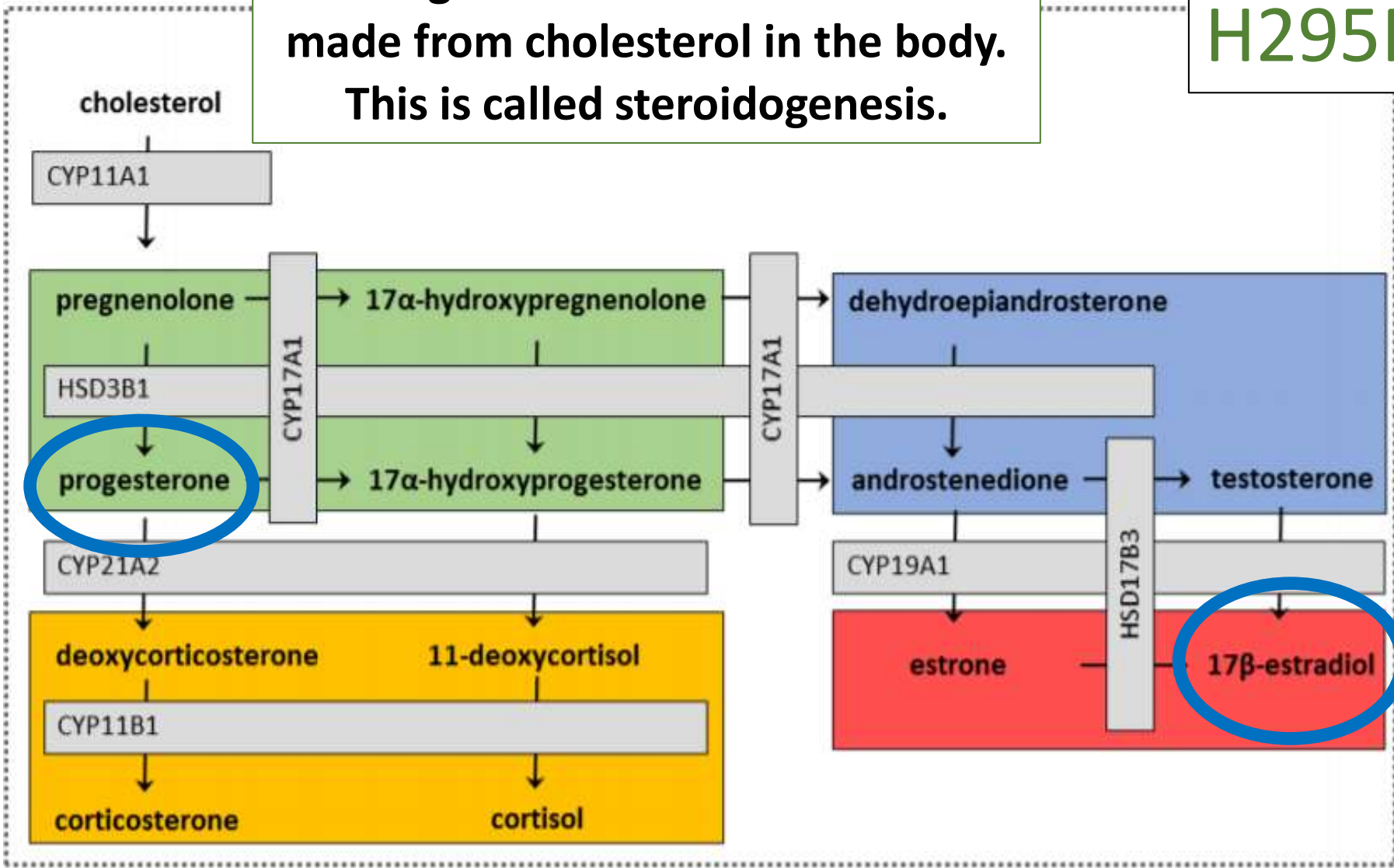
Follow

What causes #cancer? An IARC collaboration offers a fresh approach to this tough question. The key characteristics of carcinogens help ID new cancer causes & make sense of suspected carcinogens. Read the article in @ChemResTox about progress & next steps

- Guyton KZ, Rieswijk L, Wang A, Chiu WA, Smith MT (2018). *Chemical Research in Toxicology*, 31(12): 1290-1292.
- Smith MT, Guyton KZ, Kleinstreuer N, Borrel A, Cardenas A, Chiu WA, Felsher DW, Gibbons CF, Goodson WH, Houck KA, Kane A, La Merrill MA, Lebec H, Lowe L, McHale CM, Minocherhomji S, Rieswijk L, Sandy MS, Sone H, Wang A, Zhang L, Zeise L, Fielden M (2020). *Cancer Epidemiol Biomarkers Prev.* 29(10):1887-1903.
- For more on the key characteristics of hazardous exposures, see: <https://keycharacteristics.org/>

Estrogen and other steroids are made from cholesterol in the body. This is called steroidogenesis.

H295R cell



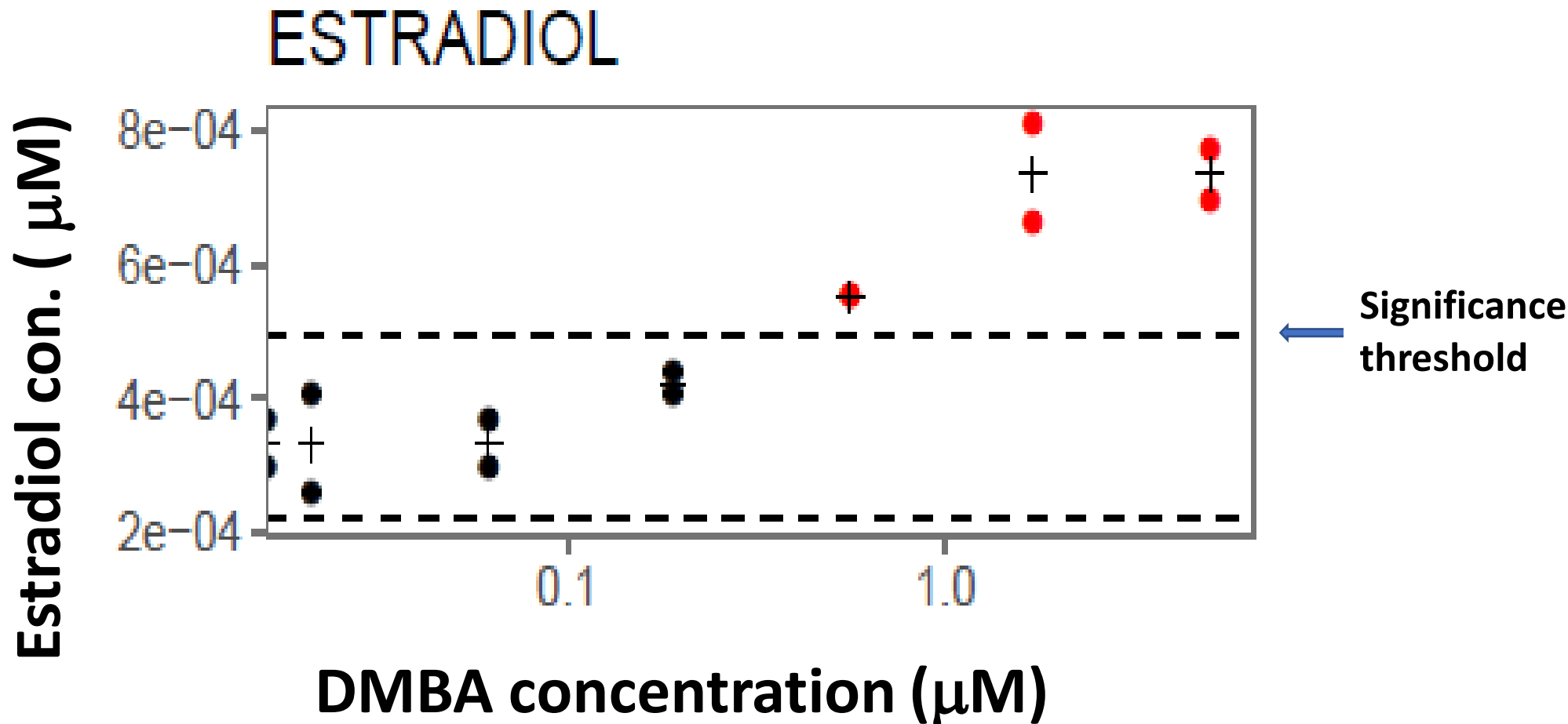


# Why estradiol and progesterone?

- Women at high inherited risk sometimes remove ovaries to reduce risk
- E+P HRT increases breast cancer risk
- Women with a gene for elevated aromatase (increases estrogen) have poor survival for ER+/PR+ breast cancer
- Treatments for ER+/PR+ breast cancer block estrogen action with...
  - Tamoxifen
  - Aromatase inhibitors

# How does the test work?

How big is the hormone effect?



H295R adrenocortical carcinoma cells

Dose: How much chemical to get an effect?

# Risk map

**Higher  
hormone  
effect**

**Worst chemicals:  
High effect at  
low dose**

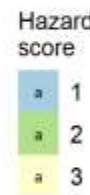
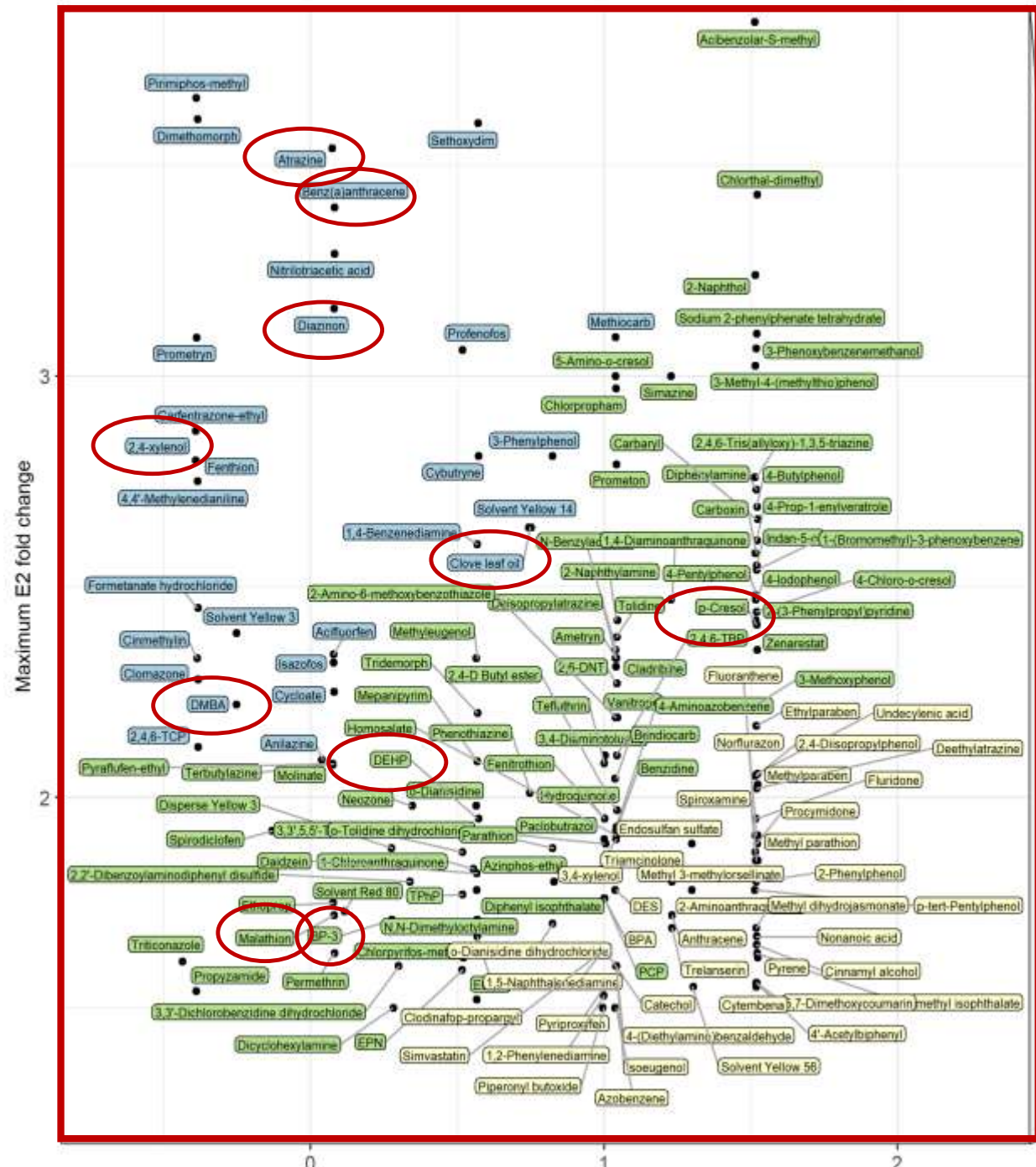
**Not so good: Especially  
problematic for common  
chemicals**

**Higher chemical dose**



# Risk map for some E2-up chemicals

(N = 182)



# *In vivo* effects of top 10 E2-up chemicals, including 8 pesticides

## Effects in rodents

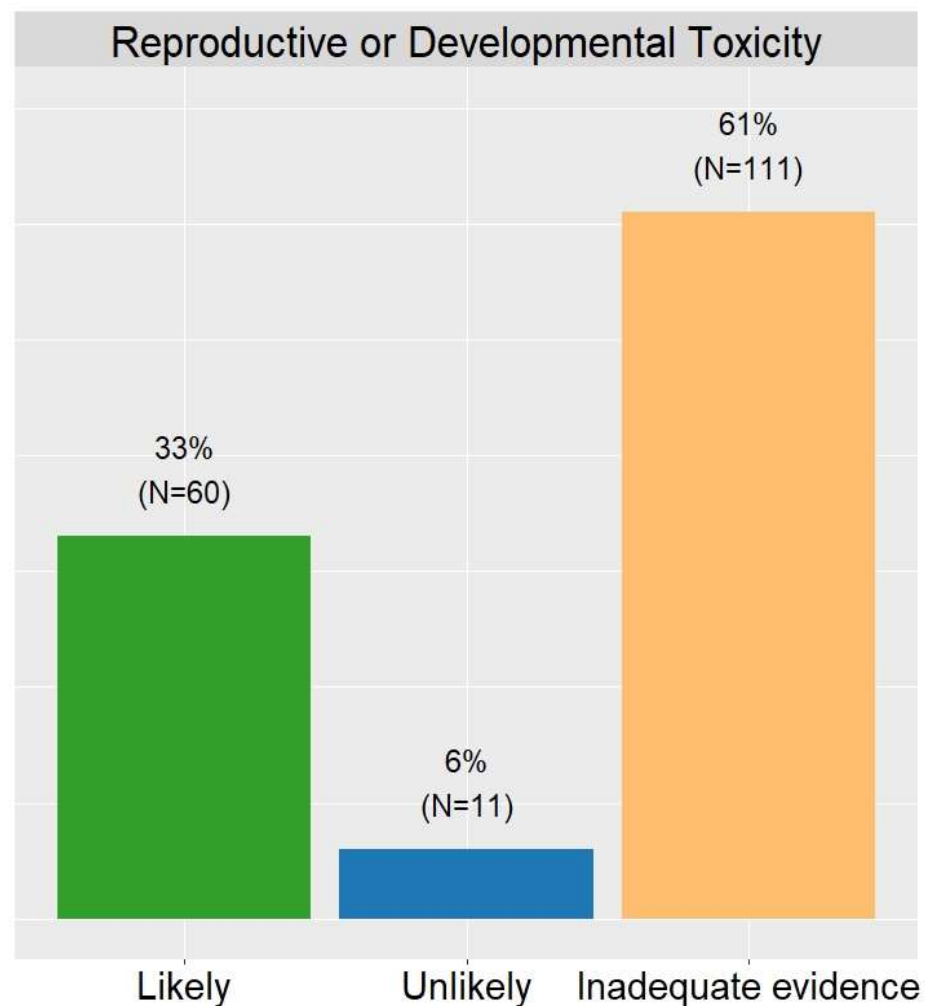
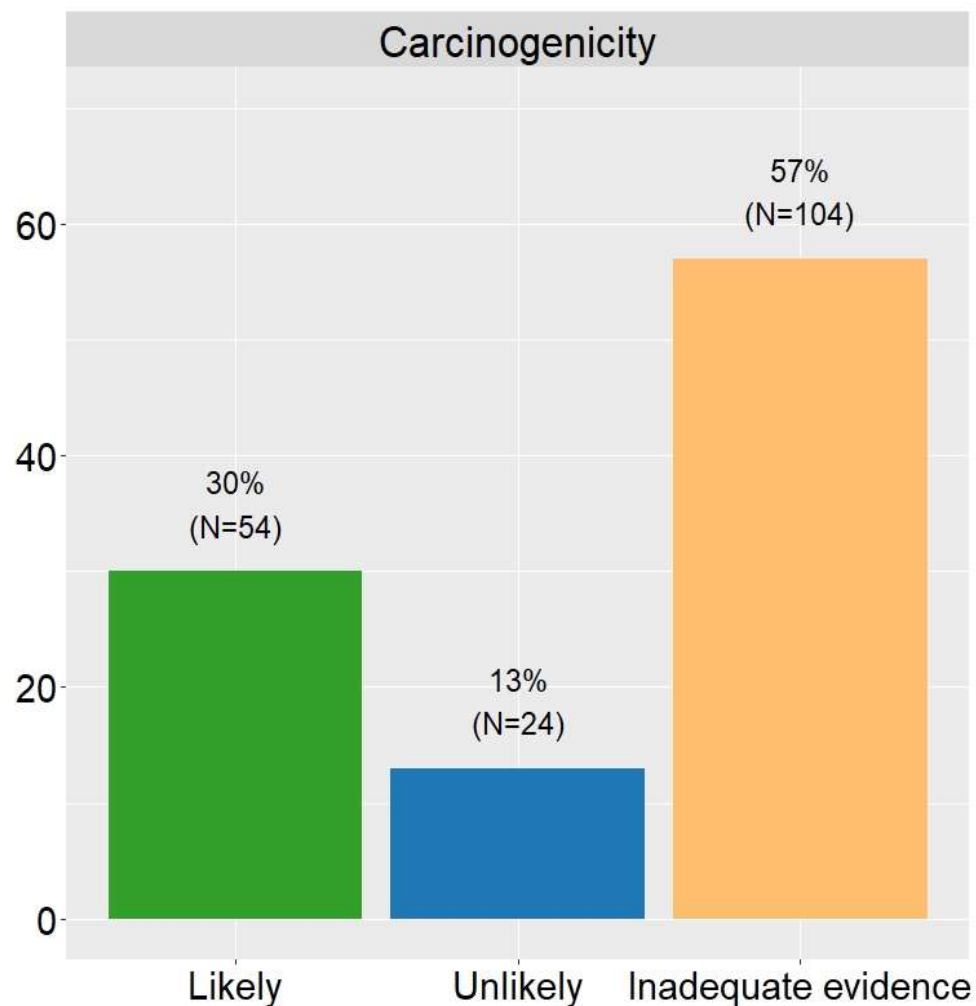
1. Mammary tumors
2. Mammary swelling, whitening and stiffening
3. Increased uterine weight of weanling or sexually immature mice
4. Lower pup body weight or body weight gain (e.g., possible lactation effect)
5. Lower lactation index and lower viability during lactation
6. Delayed vaginal opening
7. Less implantation sites or higher post-implantation loss
8. Increased reabsorptions
9. Lower number of live births
10. Decreased litter size

**7** of the top **10** chemicals had at least one effect

**3** had **4** effects

**Several have incomplete data**

# Substantial concordance with earlier assessments



*Cardona and Rudel 2021*

Classifications from EPA ToxVal database and other sources

# Who can use the new “breast cancer list”?

- Regulatory agencies can use in risk assessments (e.g., EPA, FDA, State of California, European Chemicals Agency)
- International Agency for Research on Cancer (IARC)
- Manufacturers and product certifiers
- Consumers and advocates
- Epidemiologists
- Computational chemists




## Other points to consider

- Mixtures: We are exposed to many of these chemicals simultaneously
- Not all chemicals that are positive in this test cause breast cancer







We found ~ 300  
chemicals that increase  
estradiol or progesterone

Testing for breast-  
related effects is lax, and  
misses effects

Happy Women's History Month!

# Cancer Prevention Science

**Biological  
mechanism**



**Human exposure**

**Basis for  
action**

*Exposure reduction  
= Opportunities for  
prevention*

*Strength of  
evidence, not  
“proof”*

**Educate  
Regulate  
Reformulate**

# Acknowledgements

## **Silent Spring Institute**

- Toxicology - Bethsaida Cardona
- Leadership - Julia G. Brody
- Communications – Alexandra Goho
  
- **US EPA**
  - Katie Paul-Friedman
  - Derik Haggard

**I have no financial interests related to the material presented**

## **FUNDERS – THANK YOU!**

- NIEHS Breast Cancer and Environment Research Program
- California Breast Cancer Research Program
- Cedar Tree Foundation
- Charitable donations to Silent Spring Institute's Safer Chemicals Program