

# Data integration, analysis, and interpretation of eight academic CLARITY-BPA studies (Heindel et al.,2020)



## A Combined Morphometric and Statistical Approach to Assess Nonmonotonicity in the Developing Mammary Gland of Rats in the CLARITY-BPA Study.

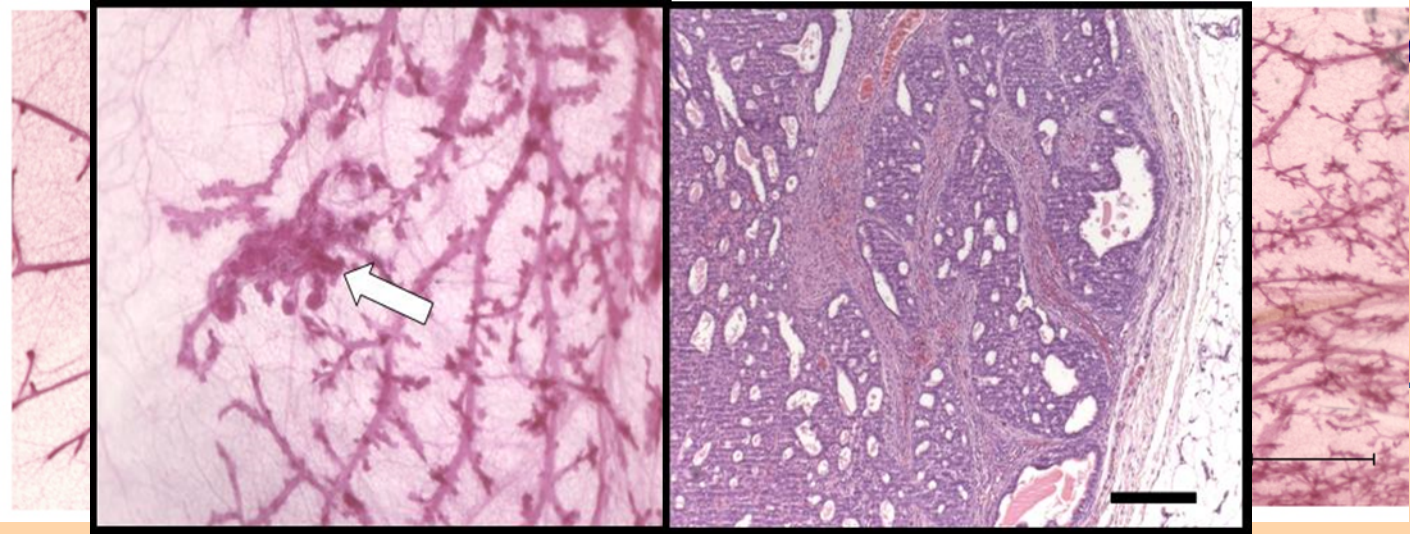
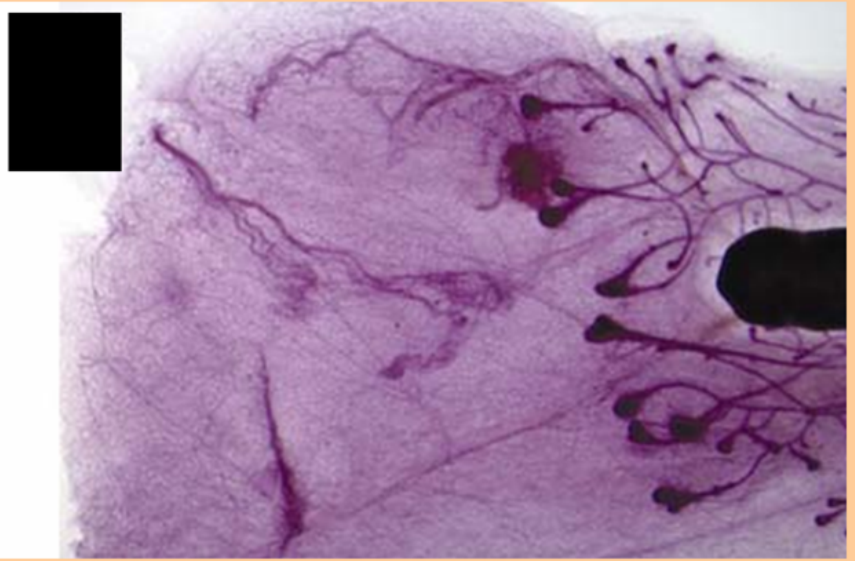
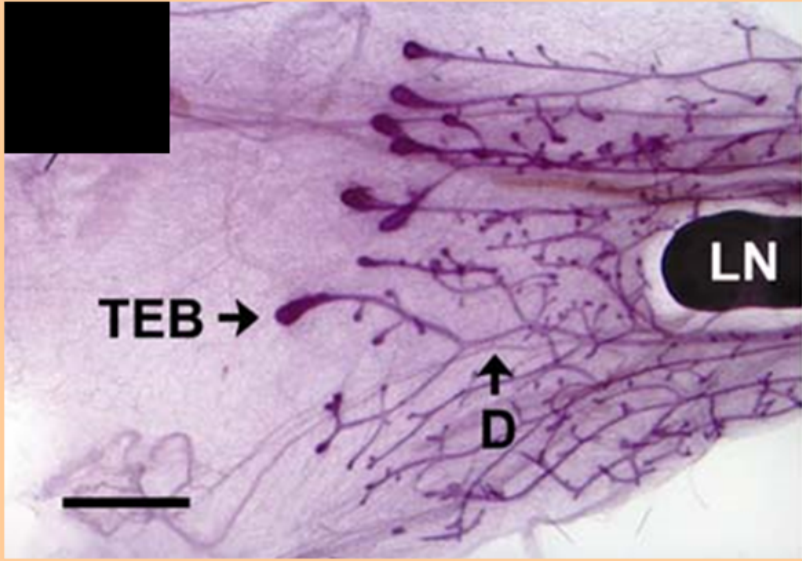
Montévil M, Acevedo N, Schaeberle CM, Bharadwaj M, Fenton SE, Soto AM

*Malka, watercolor © by Luisa Soto*

# Our lab's findings before the CLARITY study

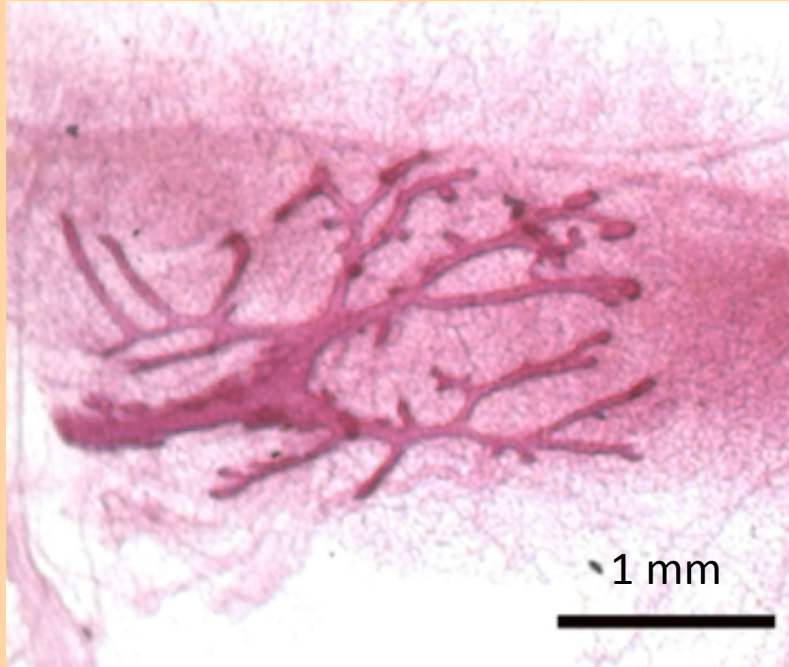
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- The mammary gland is exquisitely sensitive to BPA (altered hormone responses observed after fetal exposure to 25 ng BPA/Kg/day).
- Perinatal exposure to BPA induces abnormal fetal, post-pubertal and adult development of the mammary gland.
- Perinatal BPA exposure increases the propensity to develop mammary cancer.
- BPA does not produce exactly the same effects as the ovarian estrogen of reference, estradiol (sometimes BPA=EE2, sometimes BPA≠EE2).
- Estradiol and BPA induce non-monotonic dose-response curves.

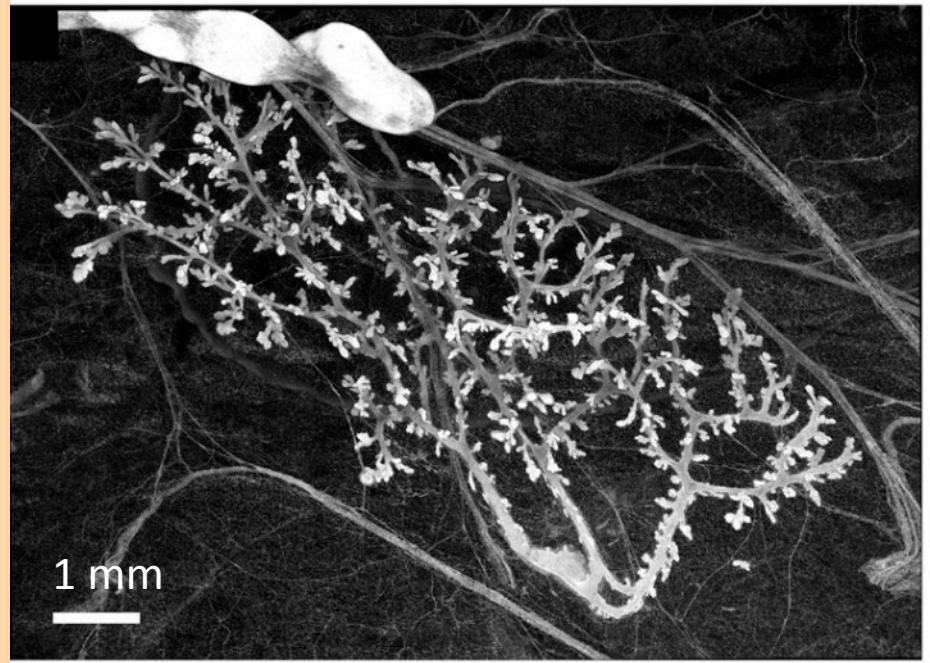


To reproduce these end points we needed  
to develop new methodology

CD1 MOUSE



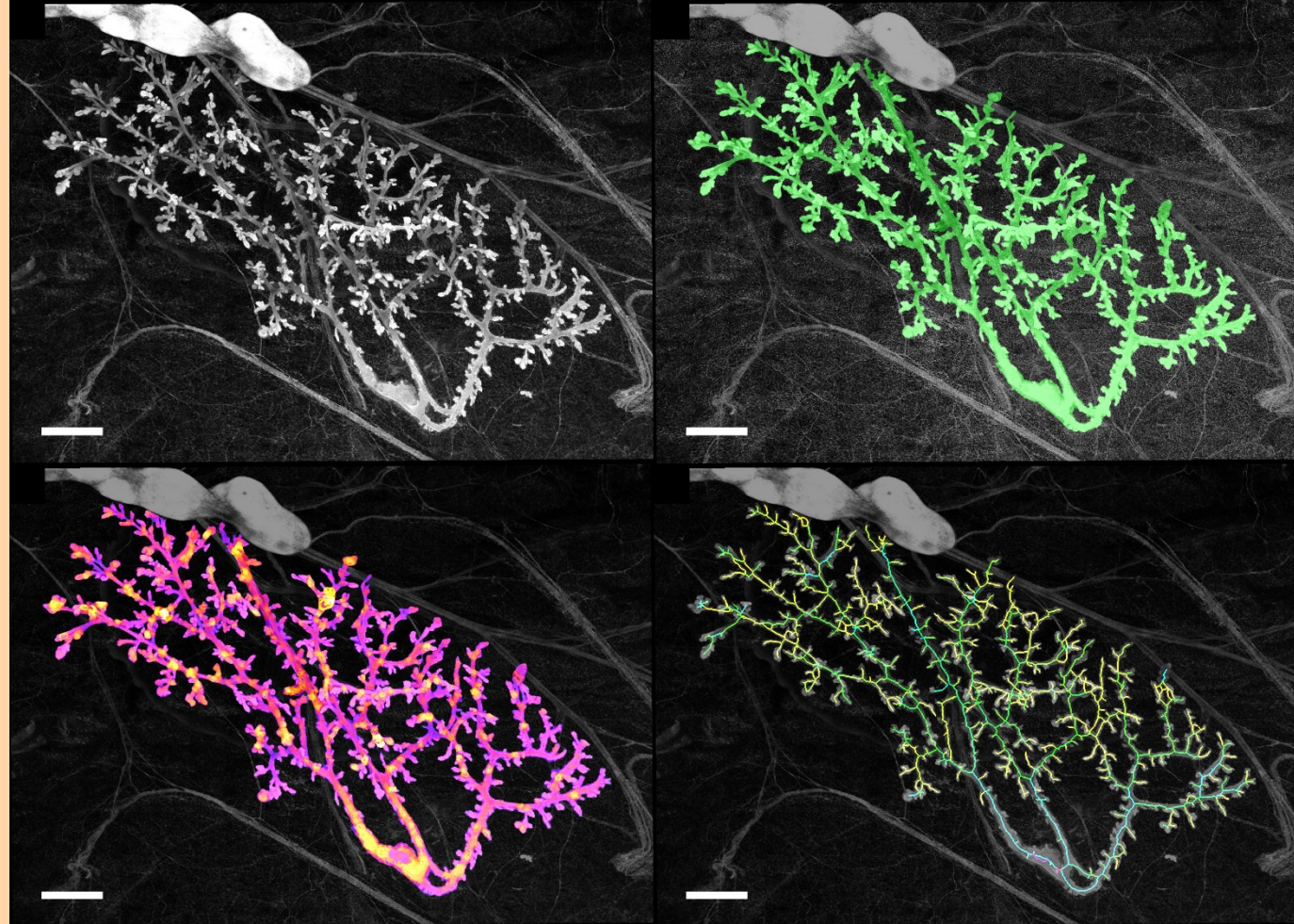
SD RAT



# INNOVATION

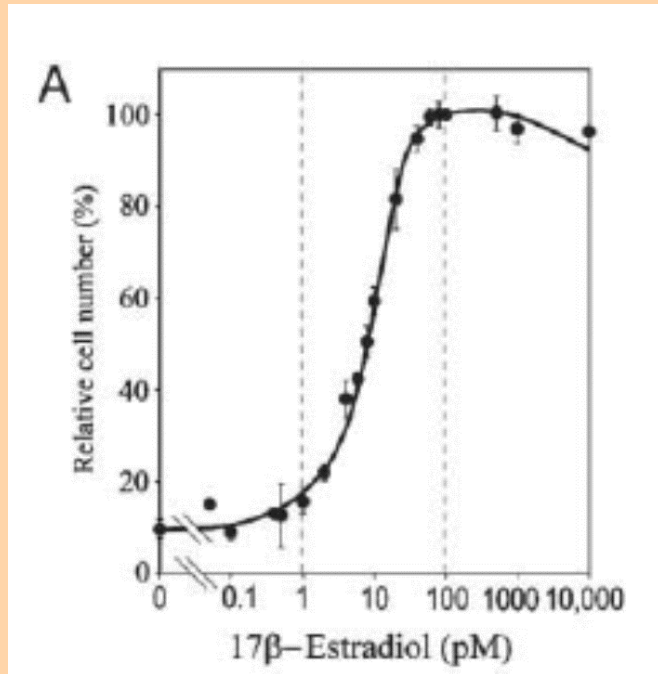
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1. Automated software for image reconstruction
2. Software for Quantitative Analysis of >90 features
3. Use of powerful Statistical tools (first time that permutation analysis is used in EDCs research)

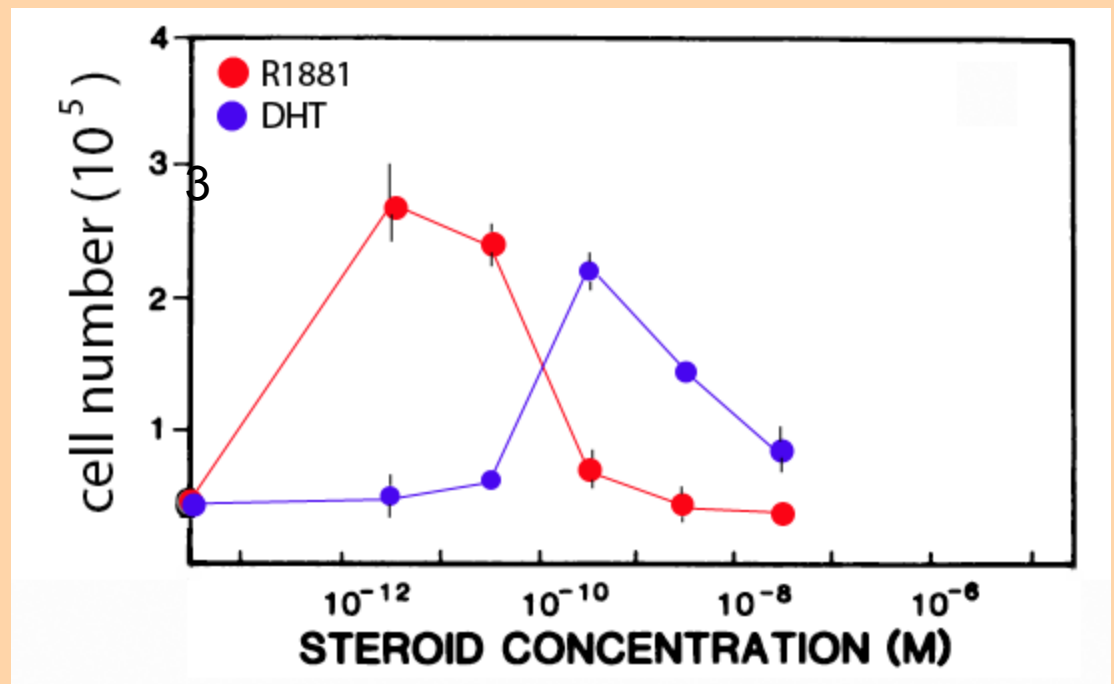


# Dose-response curves

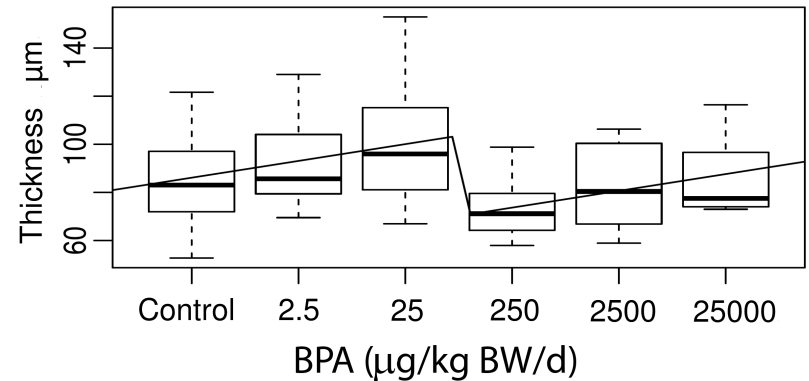
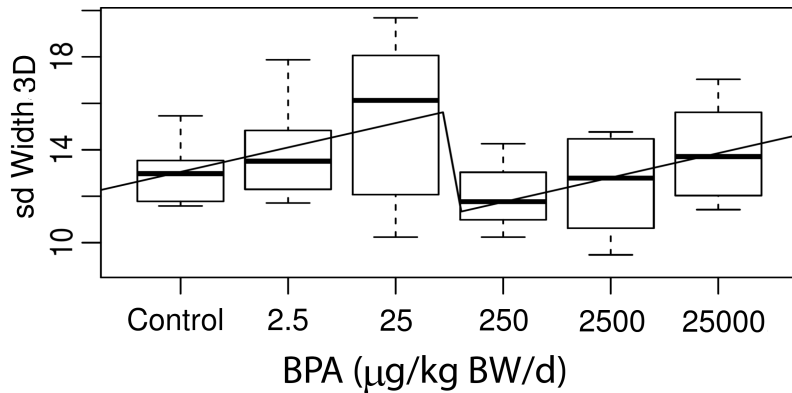
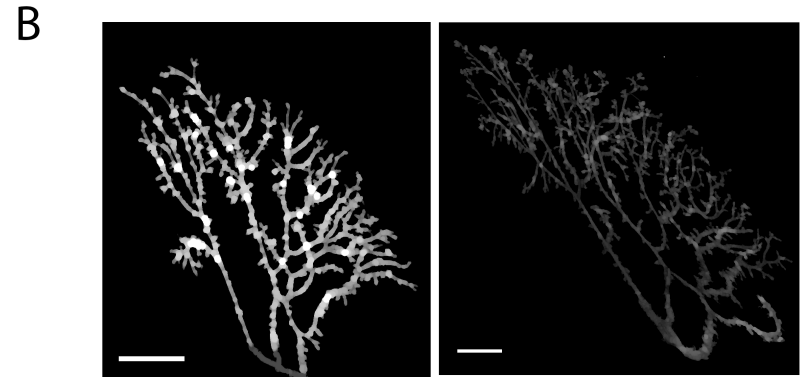
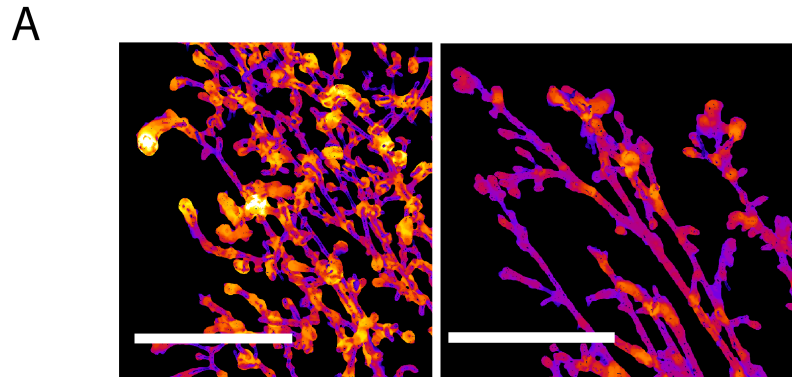
Monotonic



Non-Monotonic



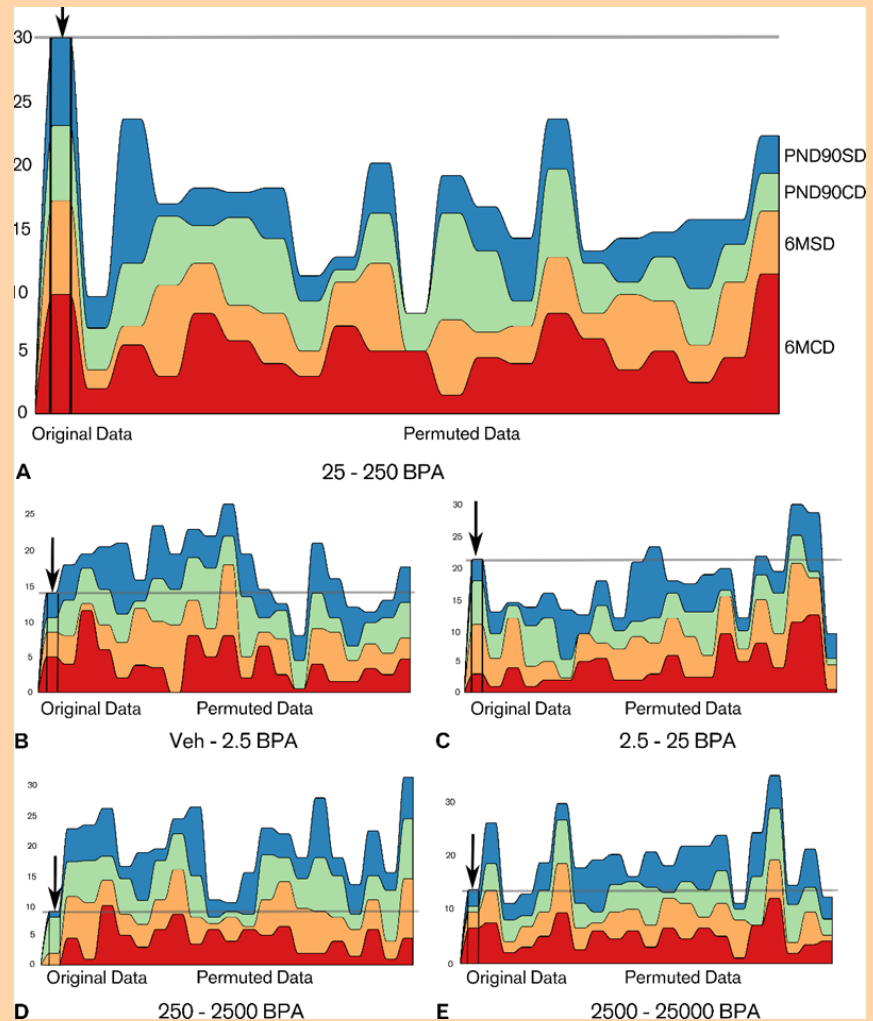
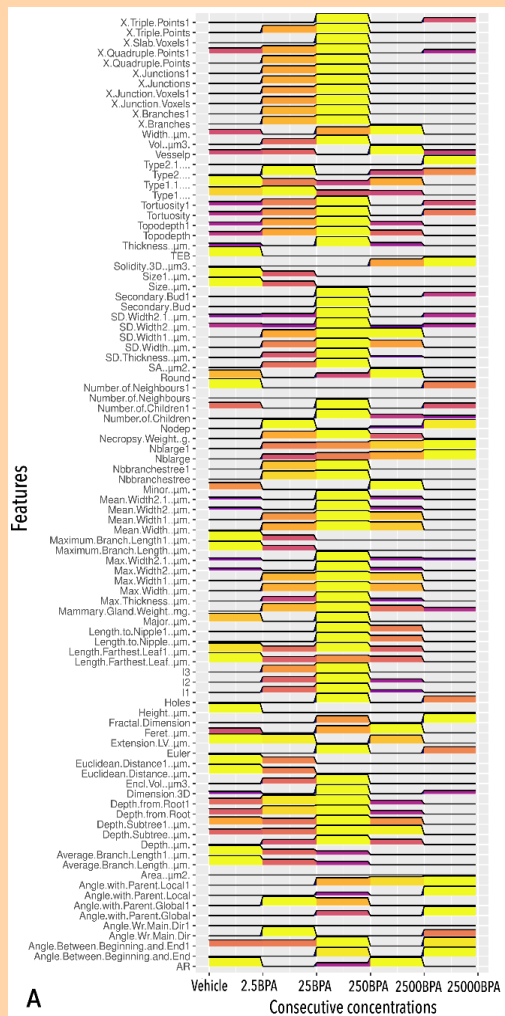
# Clear demonstration of NMDRC



Budding

thickness of the ductal tree

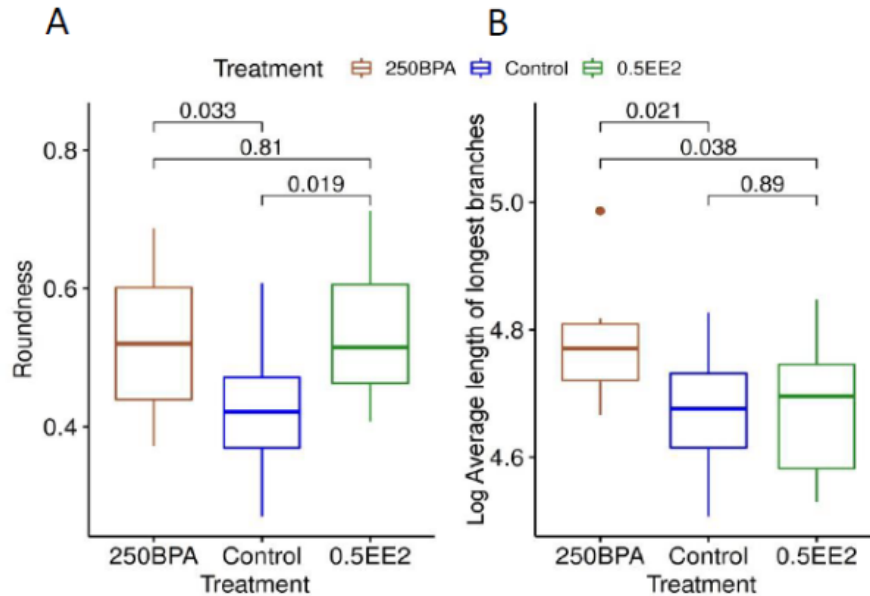
# Observations



8 Our CLARITY results confirm our previous results and demonstrate that the low dose results are not random but represent a real dose-related biological outcome.



# Effects of EE2 versus BPA



**Fig. 7.** Box plots of (A) roundness (ratio between smallest and largest axes of gland) and (B) log of average length of longest branches (length > 75  $\mu\text{m}$ ) of postnatal day (PND) 21 animals treated with control, 250  $\mu\text{g}$  BPA/kg/day, or 0.5  $\mu\text{g}$ /kg/day EE (n=8–10 animals per group). *P*-values correspond to pairwise t-test.

CLARITY results confirm our previous results

# Our CLARITY study results reproduced our pre-CLARITY study results

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- Perinatal exposure to BPA induces abnormal fetal, post-pubertal and adult development of the mammary gland.
- Perinatal BPA exposure increases the propensity to develop mammary cancer.
- BPA does not produce exactly the same effects as the ovarian estrogen of reference, estradiol (sometimes BPA=EE2, sometimes BPA $\neq$ EE2).
- Estradiol and BPA induce non-monotonic dose-response curves.
- The most important effects are observed at the lowest dose.

# Conclusions-1

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- We developed new tools and quantitative end points for assessing alterations of mammary gland development that correlate with mammary gland carcinogenesis.
- When PROPER statistical methods for non-monotonicity are used, clear statistical evidence of **non-monotonic dose response curves** of developmental exposure to BPA for multiple measurements become apparent.
- A break point occurs in the DRC between doses of 25 and 250 ug BPA/kg body weight/day.
- Non-monotonic dose response curves exist **at all ages** of the animals studied, with the same breaking point.

# Conclusions-2

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- This provides a counterpoint to the earlier statements made by the US FDA and National Center for Toxicological Research that the low-dose effects, including **mammary cancer at the lowest dose tested**, were due to random events (“chance fluctuation in incidences of a common rat neoplasm”). **BPA induces cancer.**
- Our study shows clear statistical evidence **that different estrogens can produce either similar or very different effects, depending on the endpoints being measured.** This contradicts the hypothesis that BPA and ethinyl estradiol would always have similar effects.
- Deleterious effects occurred at the lowest dose tested. Hence the **TDI have to be decreased accordingly (to a value 20,000 lower than the current standard).**

Question: When is enough enough regarding BPA?



The CLARITY study strengthens what was already known from academic studies.