Non-monotonic dose-responses: useful to organisms, challenging for regulators

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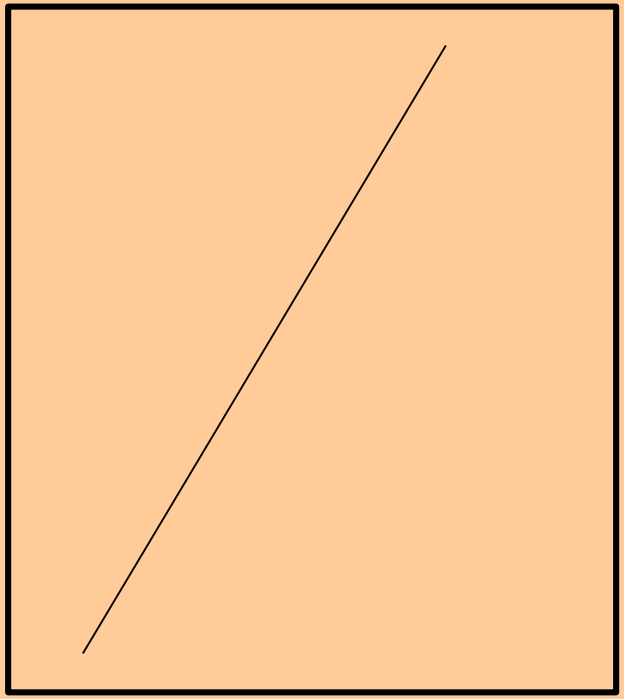
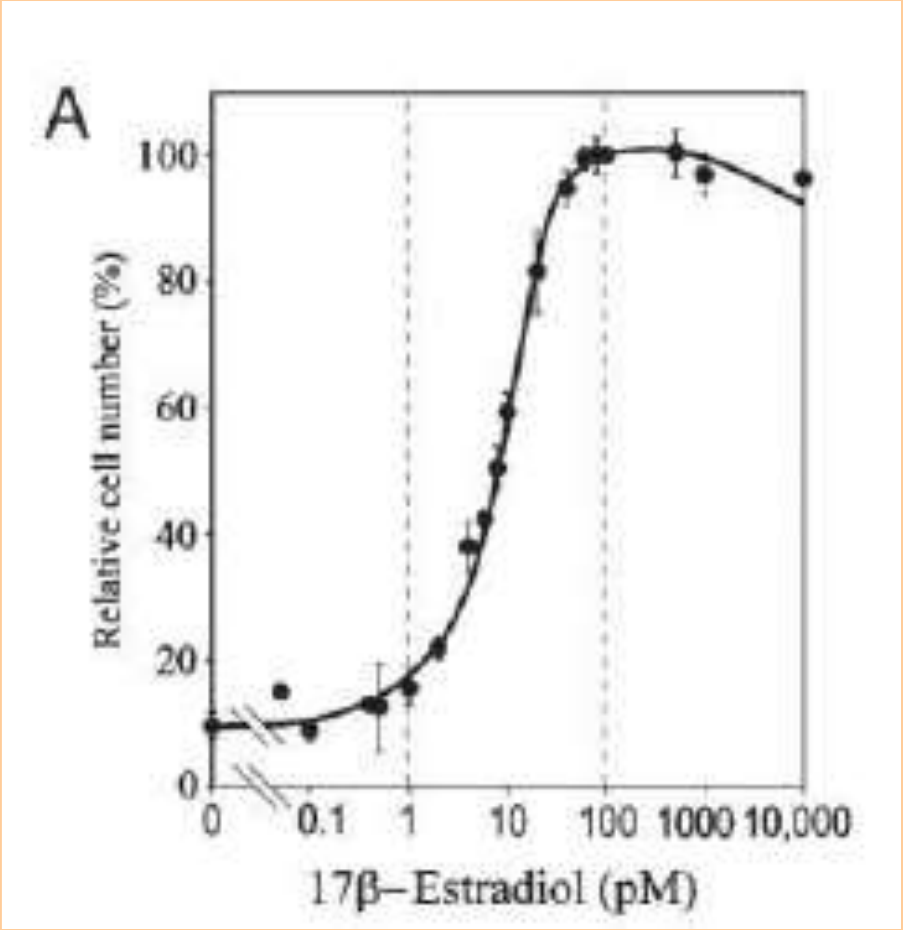
Typical monotonic dose-response curves

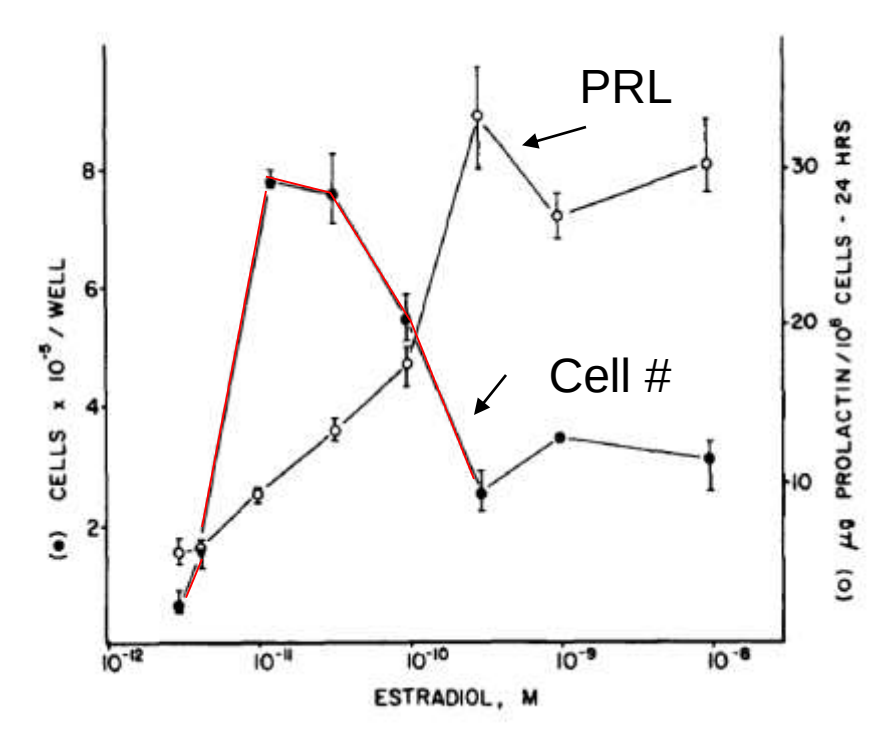
The slope does not change sign





Kosser et al

Non-monotonicity versus monotonicity: same cells, different end points

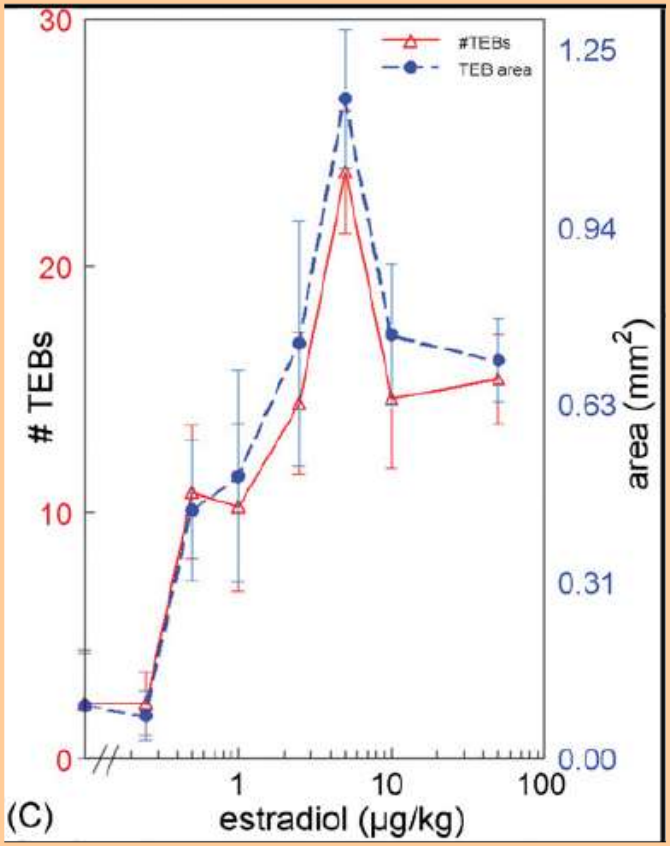


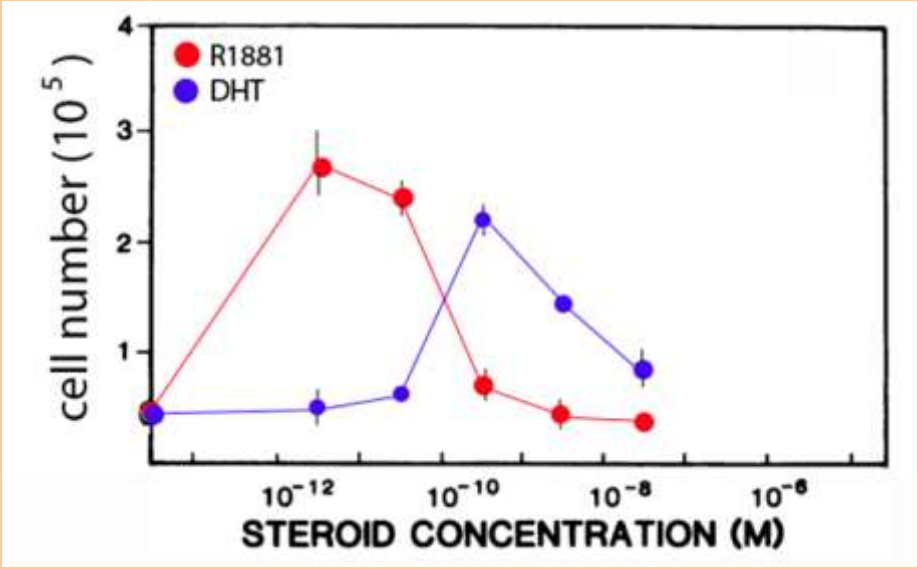
Amara JF, Dannies PS. 17 beta-Estradiol has a biphasic effect on gh cell growth. Endocrinology. 1983 Mar;112(3):1141-3. PubMed PMID: 6822206.

An organismal perspective: the usefulness of non-monotonicity

- The same hormone is able to modulate its own effect.
- The proliferative and inhibitory phases occur through different mechanisms.
- Androgens increase prostate size by inducing cell proliferation. When the adult size is reached, androgens inhibit cell proliferation.
- Animal strains that develop pituitary cancer lack the estrogen induced proliferative shutoff.
- Chronic administration of high estrogen levels inhibit mammary cancer development.

Non-monotonic dose-response curves (NMDRC) are remarkably common in endocrinology

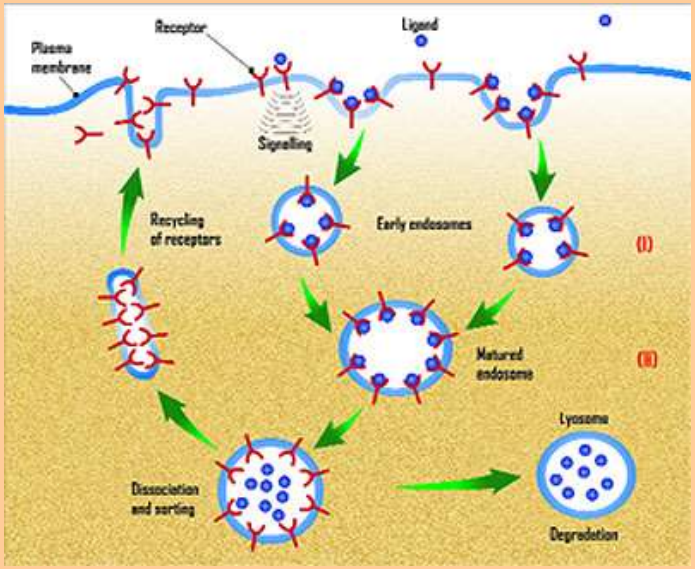


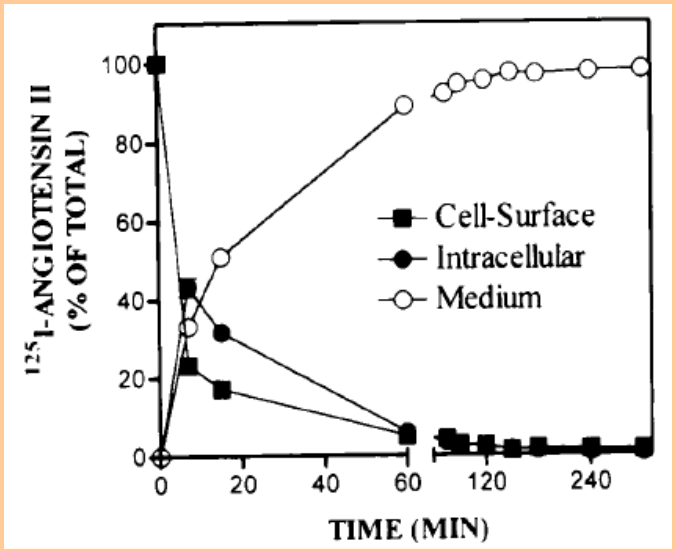


NMDRC: Mechanisms

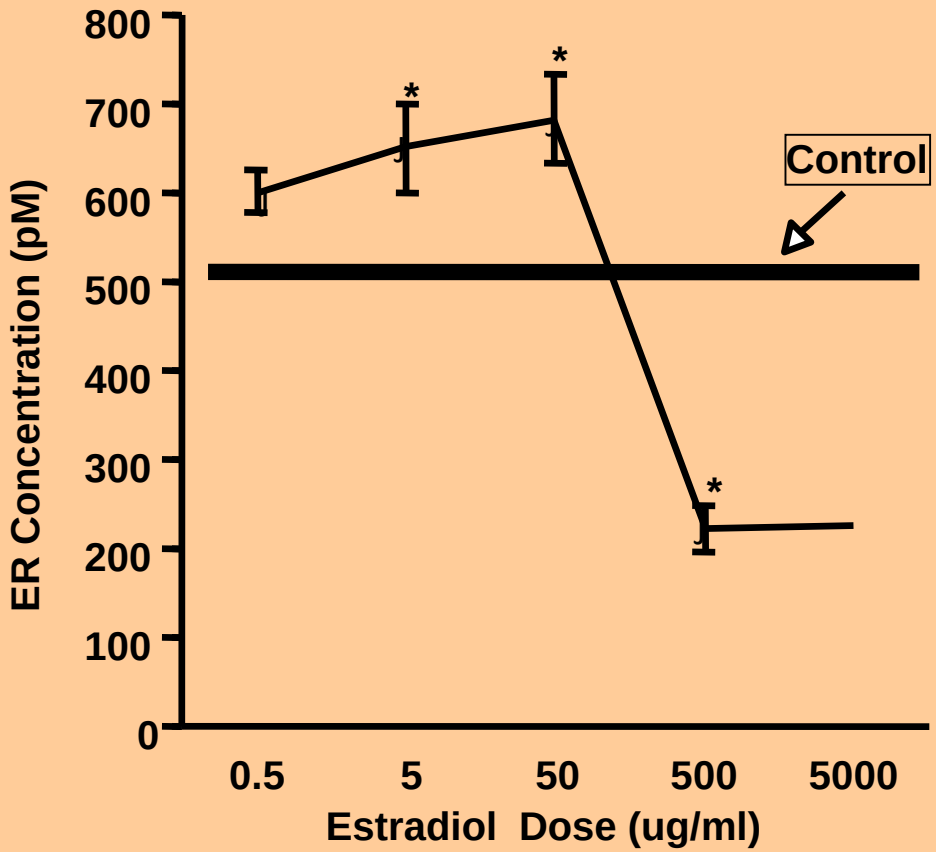
- Receptor down-regulation & desensitization
- Cell- and tissue-specific receptors and cofactors
- Receptor selectivity
- Receptor down-regulation & desensitization
- Endocrine negative feedback loops
- Tissue interactions

Mechanisms: Receptor down-regulation



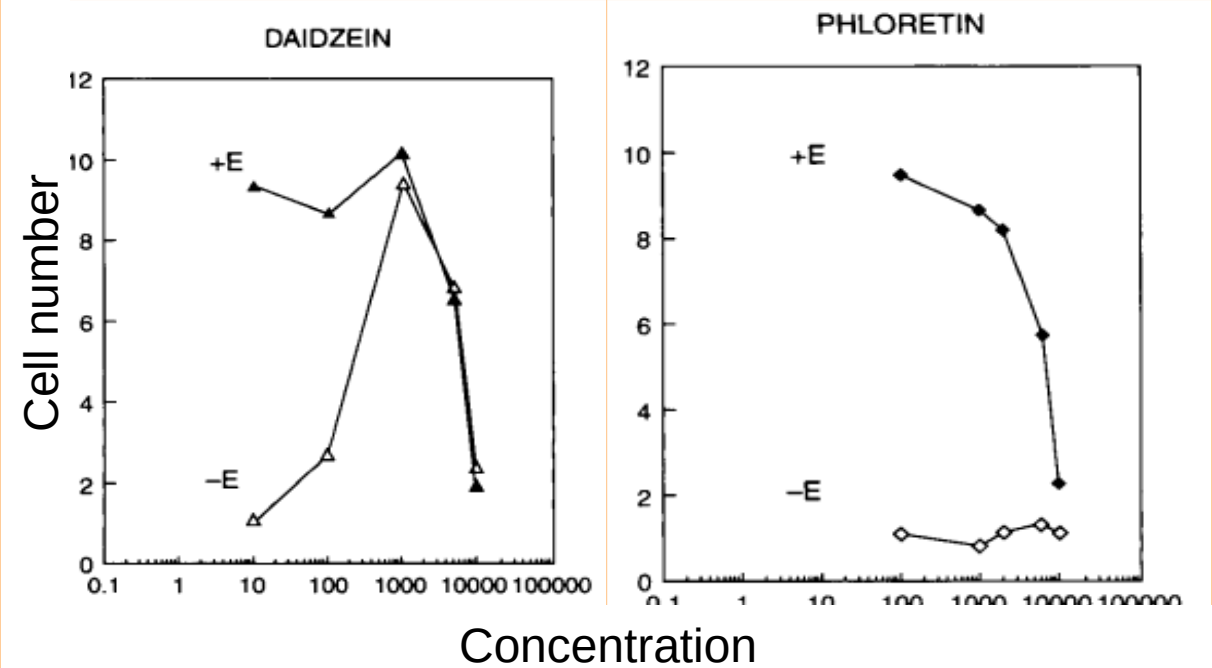


Modrall et al. 2001 [Ref 529]



Medlock et al. 1991 [FDA scientists at NCTR]

Cell- and tissue-specific receptors and cofactors: phytoestrogens and the proliferation of MCF7 cells



+E= 100 pM

low doses=increased cell proliferation (ER-mediated)

High doses=inhibition of cell proliferation (non-ER mediated)

Soto et al, J. Med Food, 1999

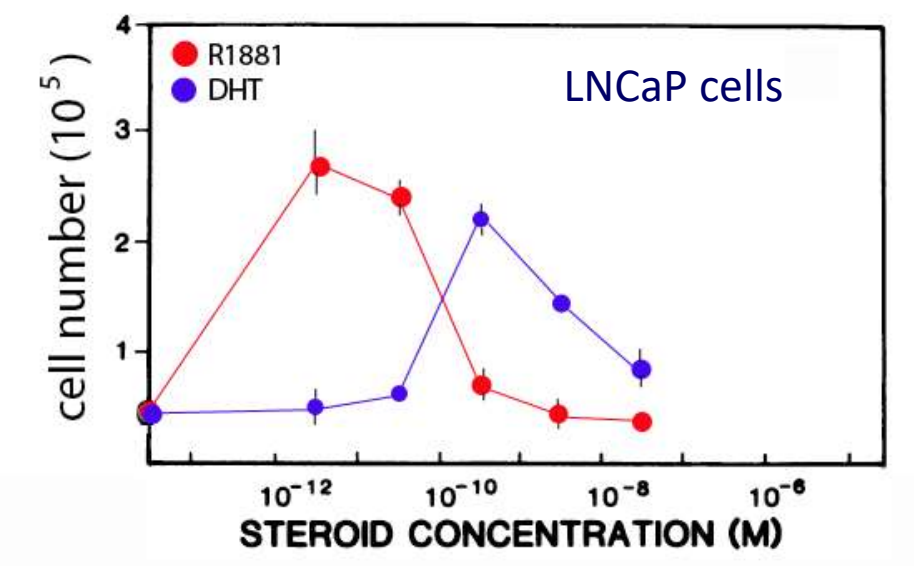
The ER-independent effect is inhibition of cell proliferation

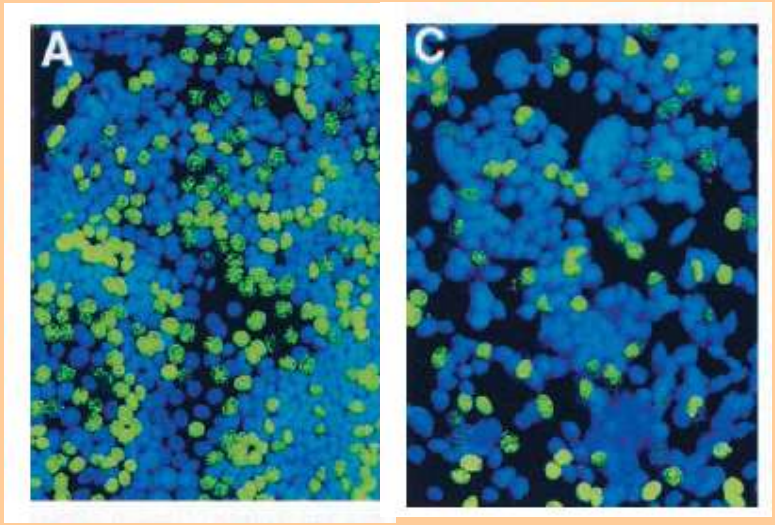
TABLE 1. CELL CYCLE ANALYSIS OF THE EFFECT OF 24-HOUR EXPOSURE TO PHYTOCHEMICALS ON THE PROLIFERATION OF MCF-7 CELLS[1]

Treatment	% G_1	% S	% G_2M
Control (5% CDFBS)	89.9	5.2	4.9
100 pmol/L E_2	58.0	30.1	11.9
10 μmol/L daidzein	88.6	6.5	4.9
10 μmol/L daidzein + 100 pmol/L E_2	89.9	4.8	5.3
10 μmol/L genistein	91.0	5.4	3.5
10 μmol/L genistein + 100 pmol/L E_2	90.4	5.7	3.9
100 nmol/L ICI 182780	89.8	6.6	3.7
100 nmol/L ICI 182780 + 100 pM E_2	89.2	6.3	4.4
5% FBS (t = 0)	68.3	19.7	12.0

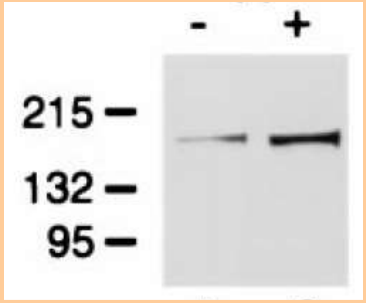
[1]MCF-7 cells were grown in 5% FBS. At time = 0 the medium was changed to 5% CDFBS alone (negative control), and 5% CDFBS plus 100 pmol/L estradiol (E_2) (positive control). Phytochemicals and the full antagonist ICI 182780 were added to 5% CDFBS at the concentrations indicated. Cells were detached by trypsin-EDTA treatment, washed, and resuspended in 0.1% Triton X-100 and 0.1 mg/ml propidium iodide in PBS (Szelei et al., 1997).

Same receptor, different androgen-receptor occupancy (cell culture)



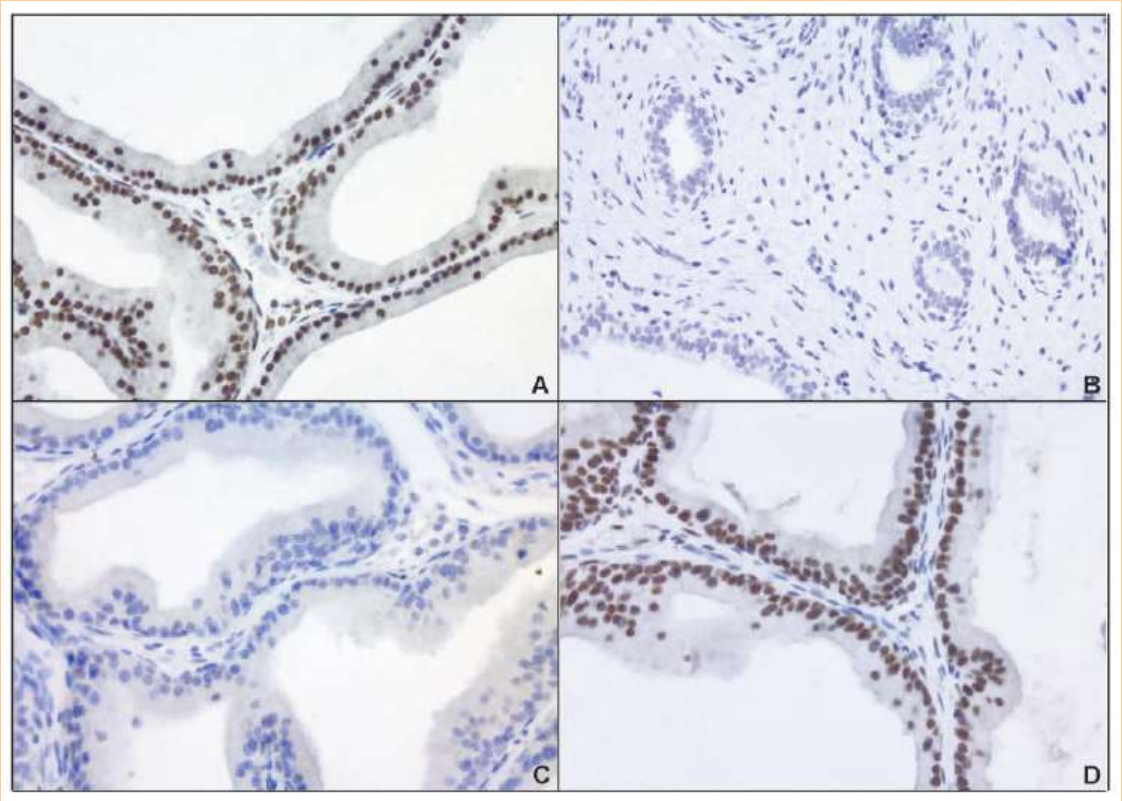


Sonnenschein et al. 1989 [Ref 499]



At high doses androgens induce a transcription factor, **aprin**, that inhibits cell proliferation producing a Go arrest

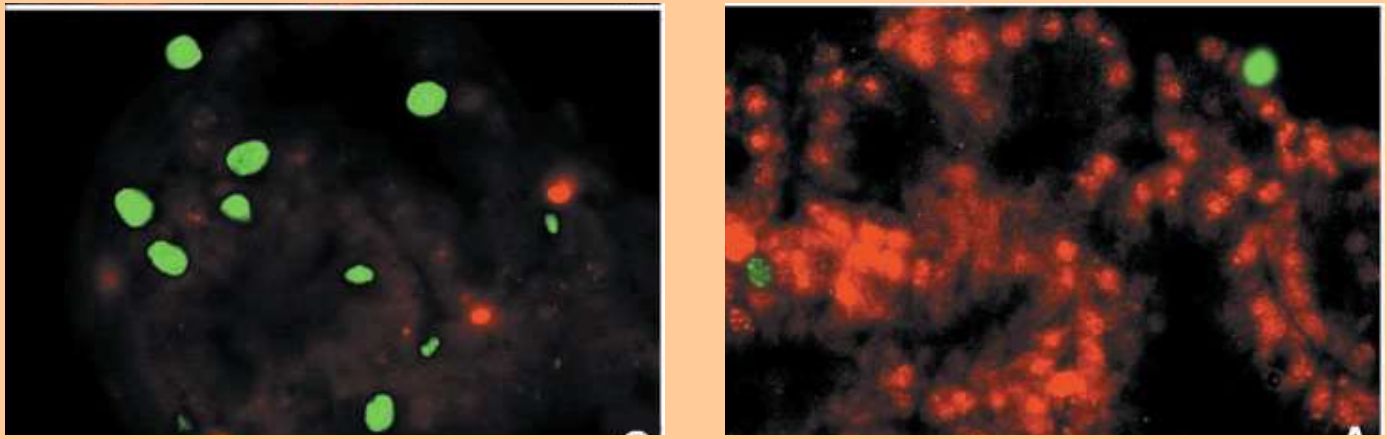
Dissociation of the proliferative and quiescence-promoting effect of androgen in the rat prostate



Aprin expression in the rat prostate. A, Intact adult rat; B, chronically castrated rat; C, maximal cell proliferation induced by 3 d androgen replacement in a castrated rat; D, proliferative shutoff after 7 d of androgen treatment in a castrated rat.

Maffini et al, Endocrinology 2002, 143:2708–2714

Dissociation of the proliferative and quiescence-promoting effect of androgen in the rat prostate



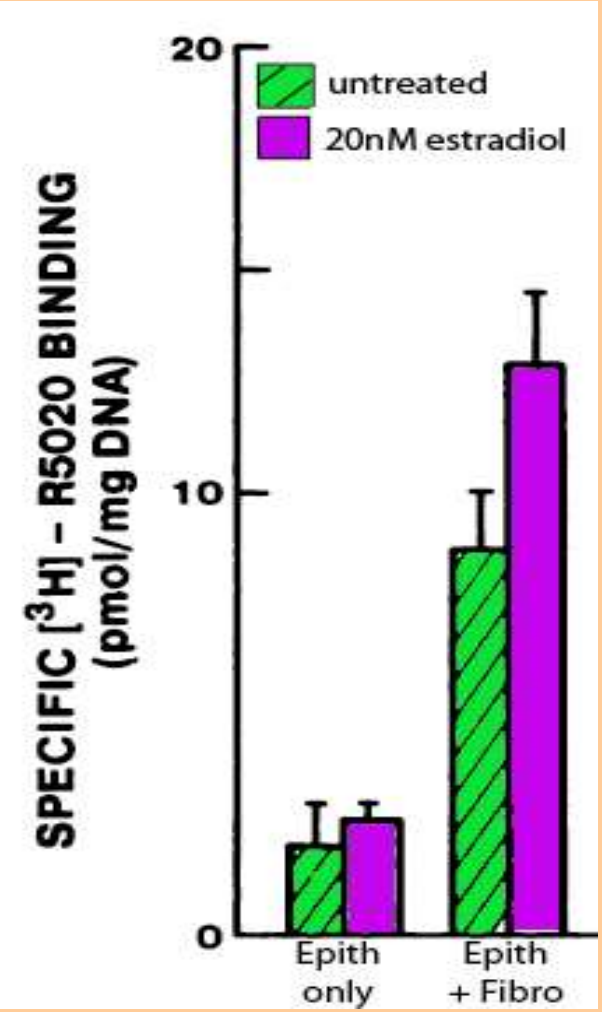
Left: maximal cell proliferation induced by 3 d androgen replacement in a castrated rat; Right, proliferative shutoff after 7 d of androgen treatment in a castrated rat. Green: BrdU, red: Aprin

Maffini et al, Endocrinology 2002, 143:2708–2714

Receptor selectivity: BPA

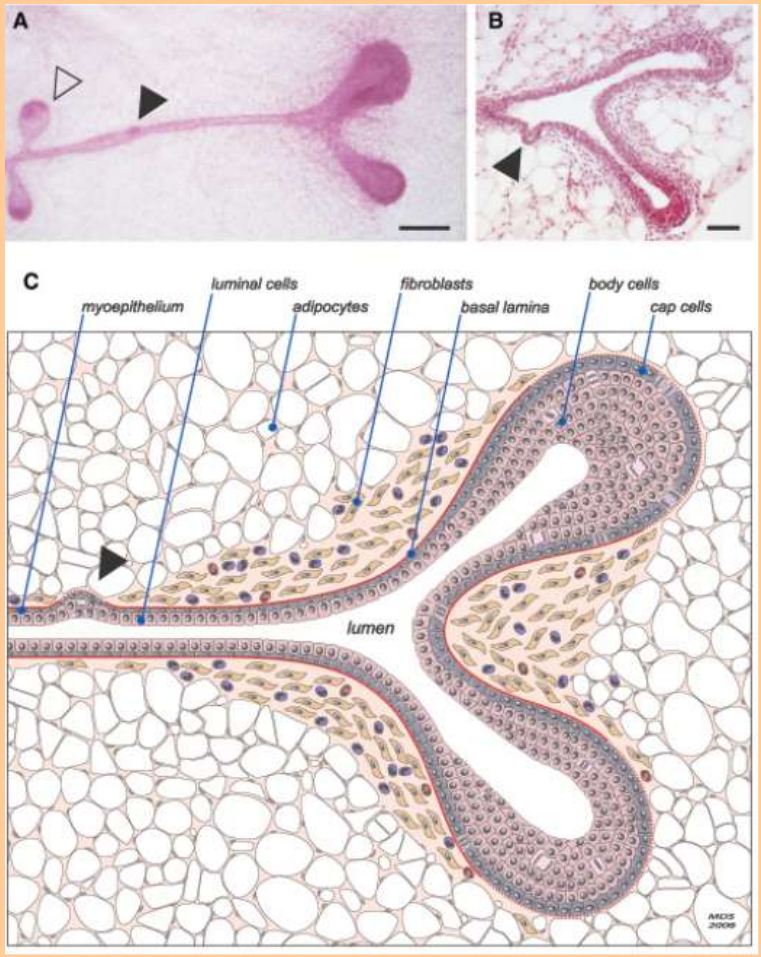
- Very low dose: membrane ERs (Nadal, Watson)
- Intermediate dose: nuclear ERs (Shioda)
- High dose TR
- High dose: AR

Cell-cell interaction

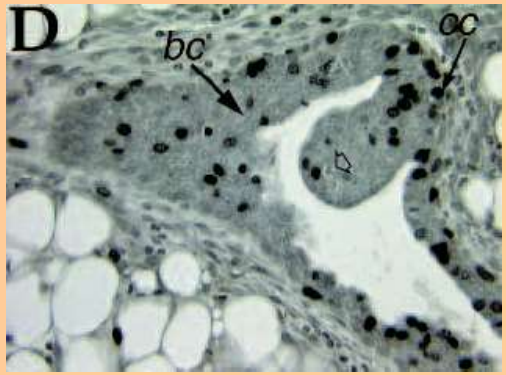


Haslam 1986

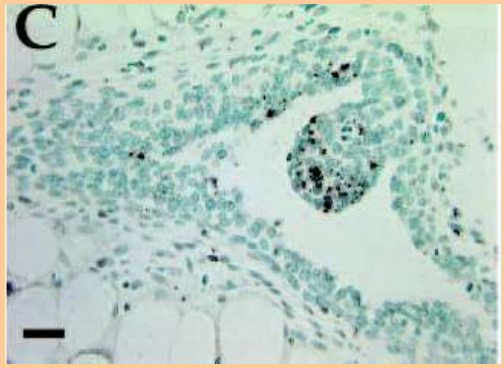
Branching morphogenesis in the mammary gland



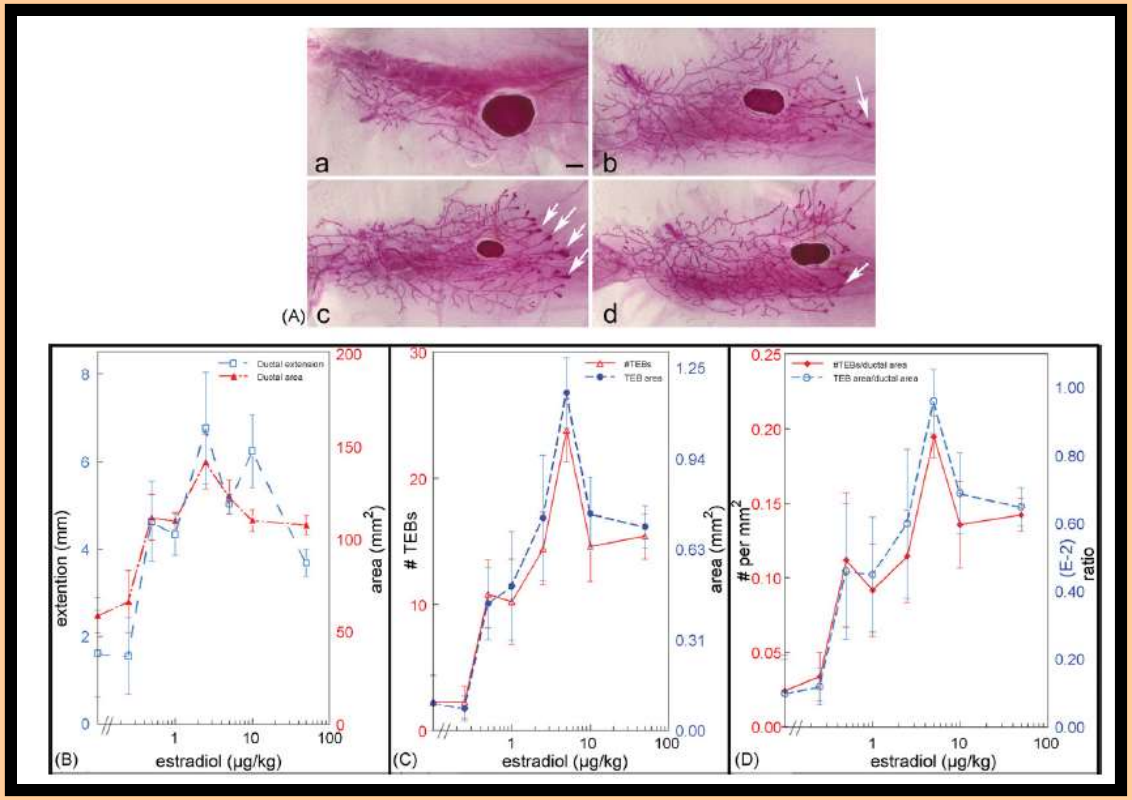
Proliferation

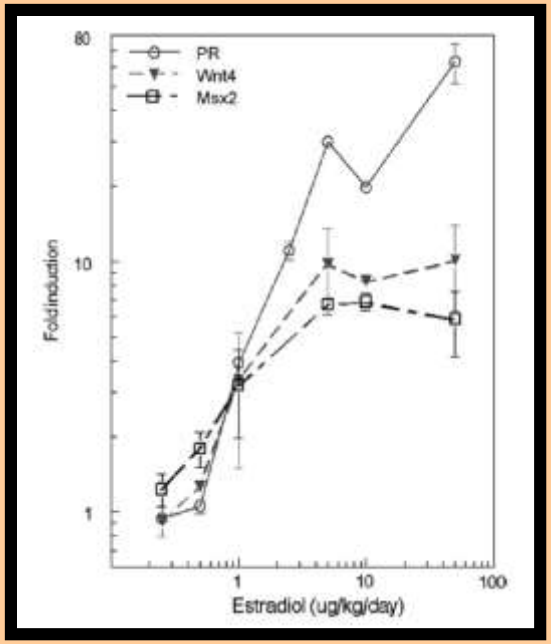


Cell Death



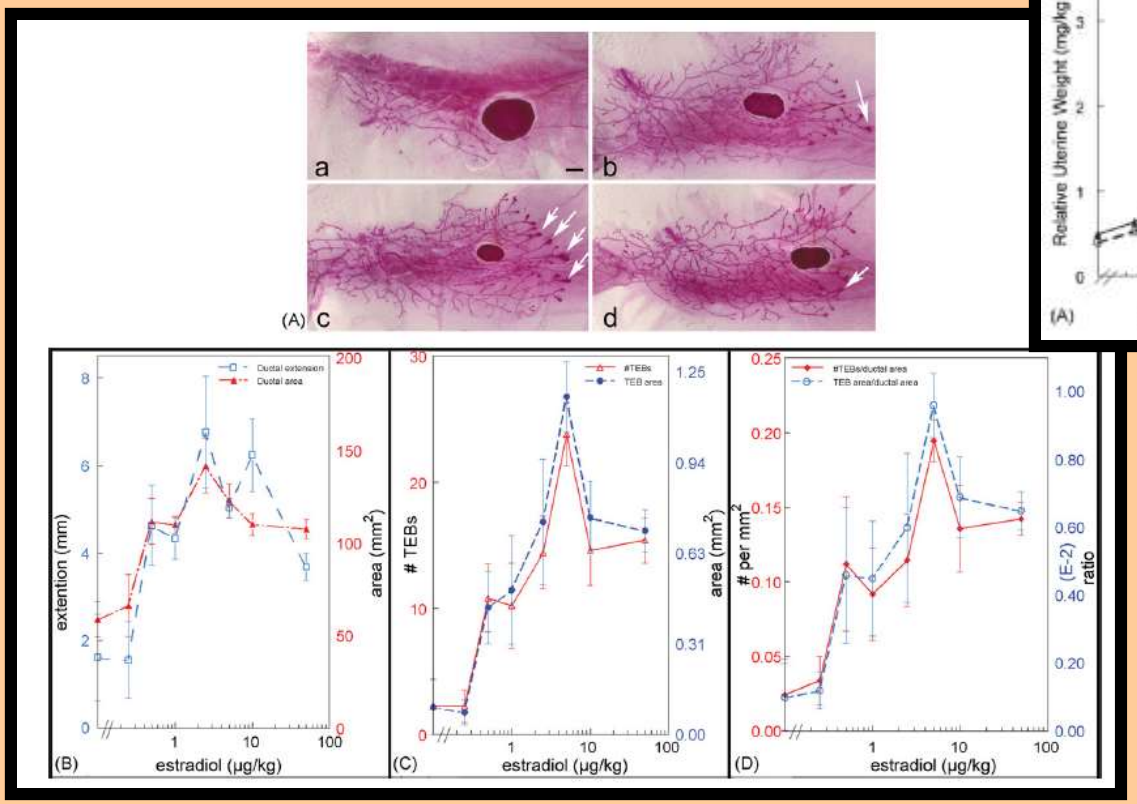
NMDRCs due to tissue interactions

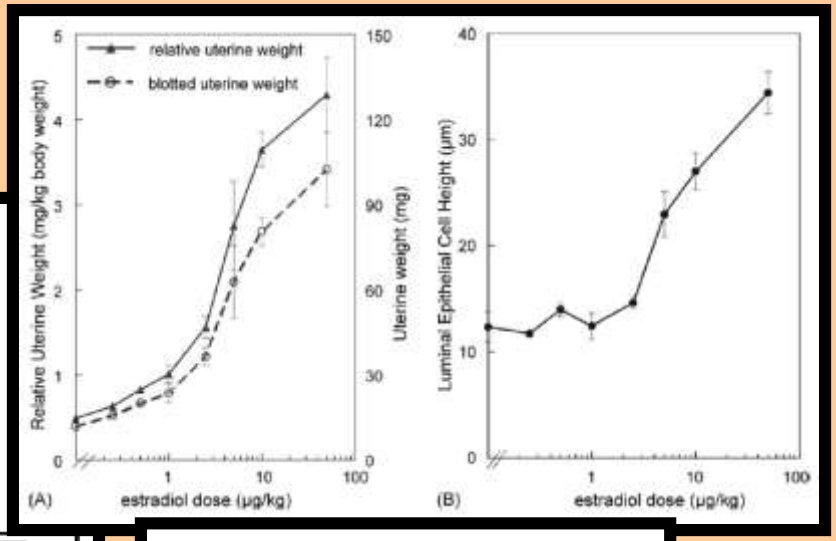


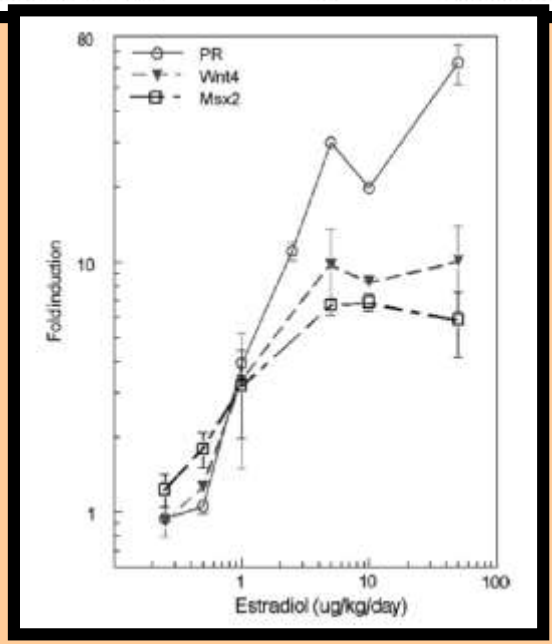


Vandenberg et al. 2006

NMDRCs are specific to doses examined, tissues & endpoints

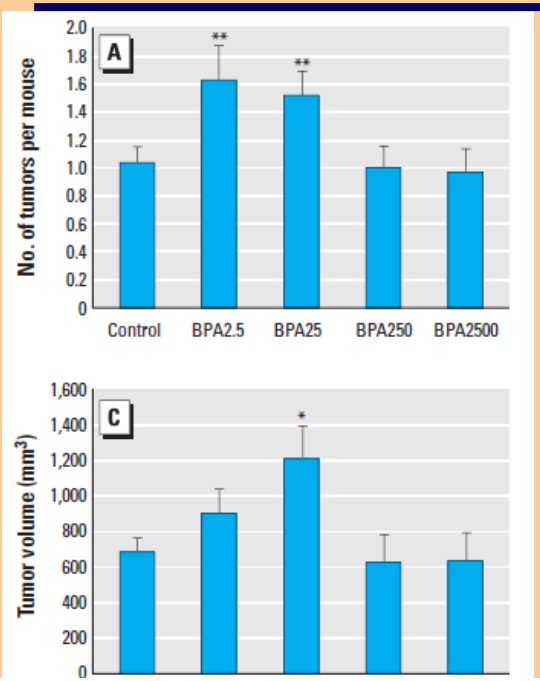


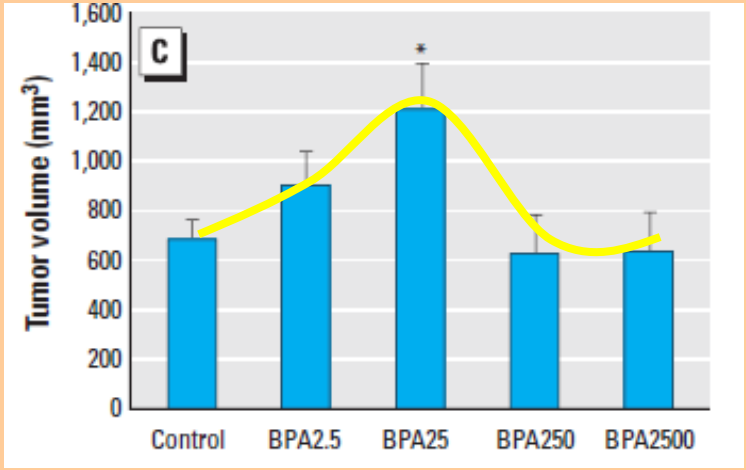


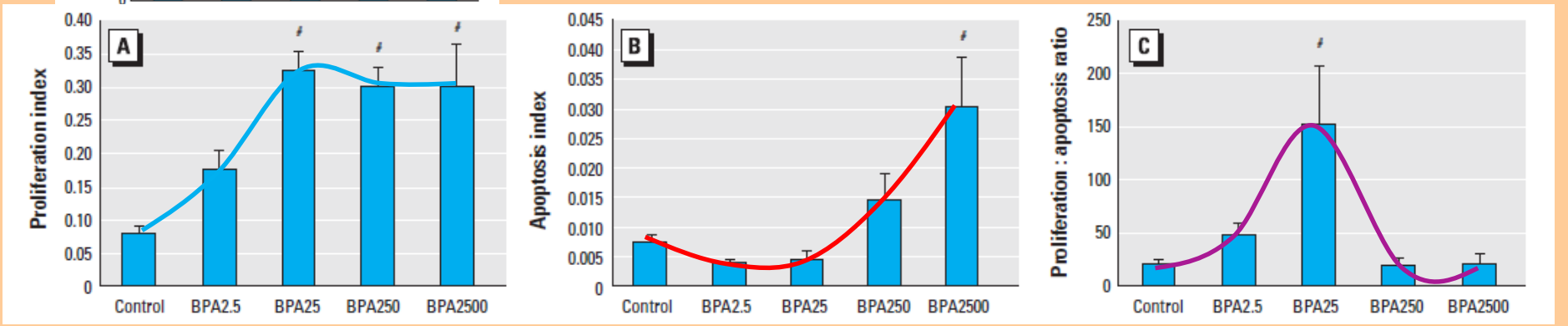


Vandenberg et al. 2006

The same mechanisms manifest in normalcy and in cancer







Jenkins et al. 2011 [Ref 293]

Clarity Study

- CLARITY-BPA is a collaboration of guideline-compliant studies and academic hypothesis-based studies to assess the effects of BPA.

- Objectives: To determine BPA's effects on the developing rat mammary gland and to develop a software tool for semi-automatic evaluation of quantifiable aspects of the mammary ductal tree

- Methods: Sprague Dawley rats were exposed to BPA, vehicle, or positive control (ethinyl estradiol, EE2) by oral gavage beginning on gestational day 6 and continuing with direct dosing of the pups after birth. Glands were harvested at multiple time points; whole mounts and histological specimens were analyzed blinded to treatment.

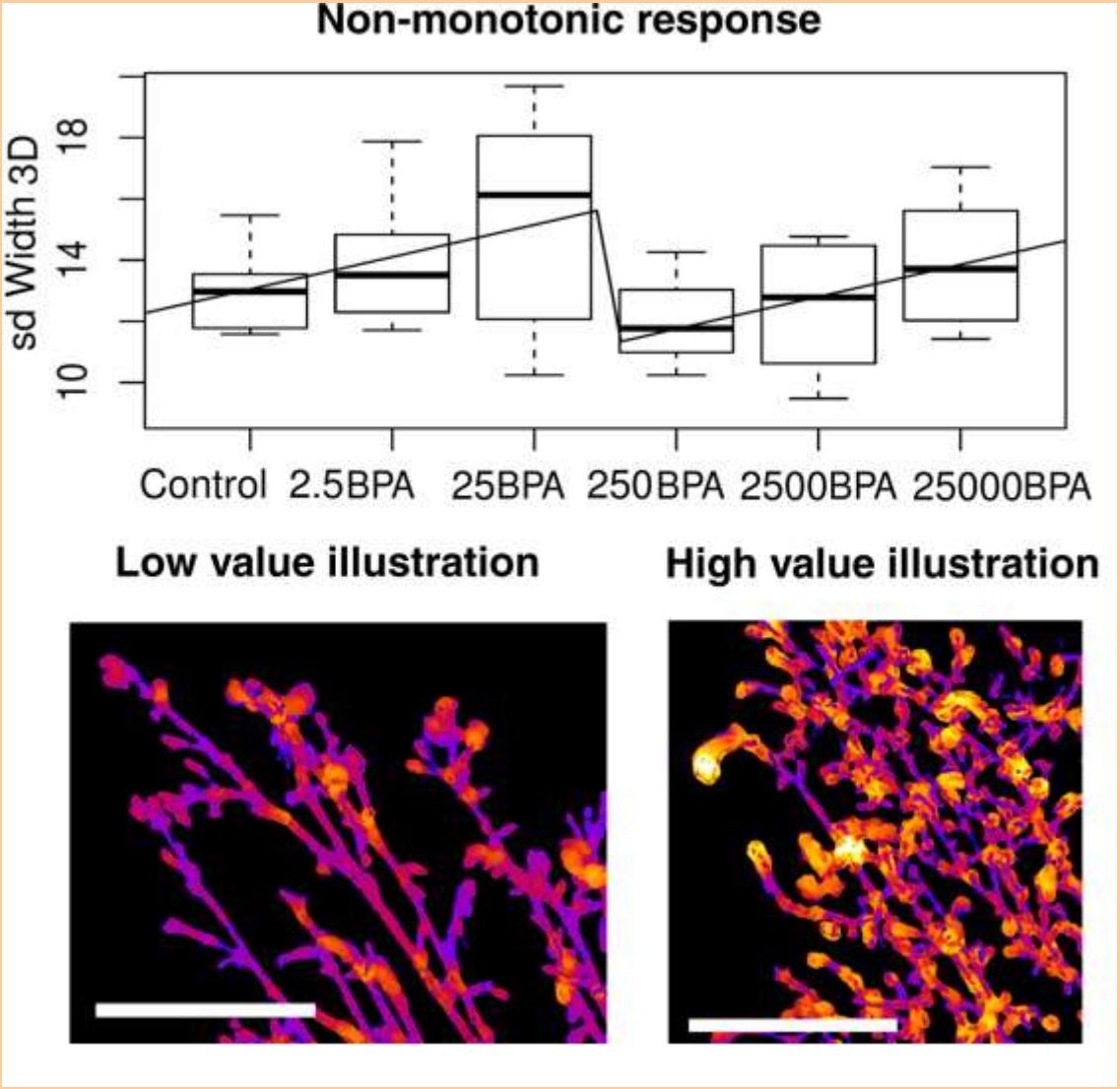
Montévil et al -http://biorxiv.org/cgi/content/short/783019v1

Clarity Study

- Results: Quantitative, automatized analysis of the PND21 MG displayed **non-monotonic BPA** effects with a breaking point between the 25 and 250 ug/kg/day doses. This breaking point was also observed at PND90 and 6 months. The BPA response was different from the EE2 effect for many features.

- Conclusions: The quantitative unsupervised analysis used a set of 91 measurements and produced the most striking non-monotonic dose-response curves. At all-time points, lower doses resulted in larger effects, consistent with the core study which revealed a significant increase of mammary adenocarcinoma incidence at the lowest BPA dose tested.

Clarity Study



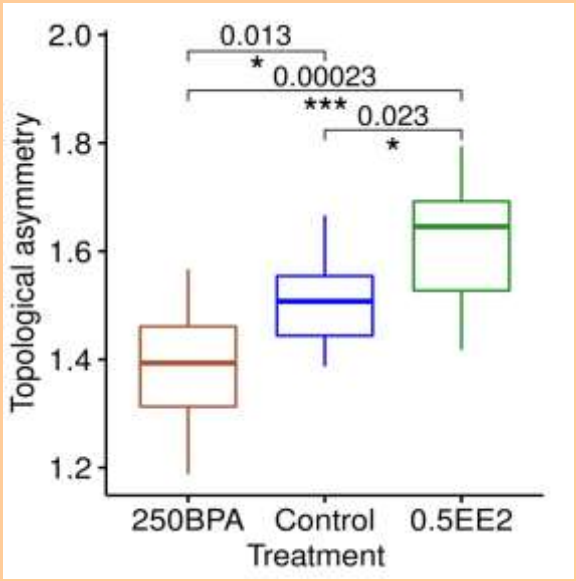
Clarity Study

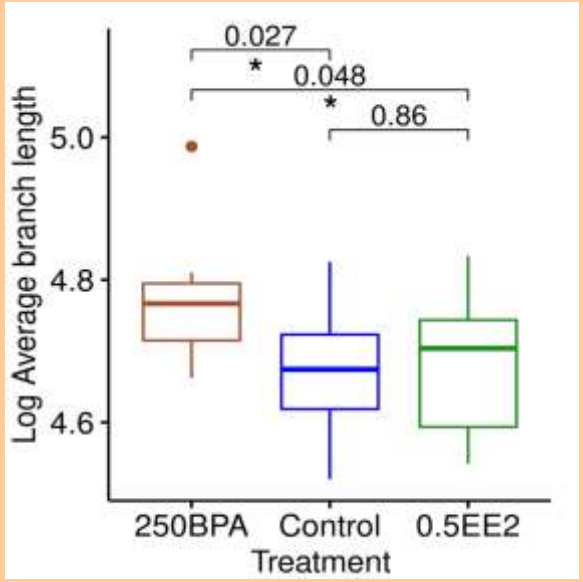
Table 1 *Number of observed quantities where the largest difference is between each consecutive condition in the PND21C data set.*

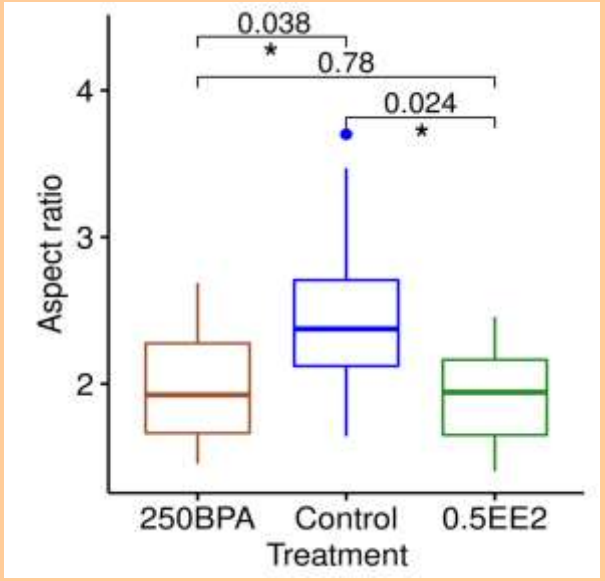
p_{thr}	Control – 2.5BPA	2.5 – 25BPA	25 - 250BPA	250 - 2500BPA	2500-25000BPA
0.05	3	0	17	0	0
0.5	14	5	57	6	9
1 (no threshold)	15	6	52	7	9

Note: Differences are counted when the significance of this difference has a p value lower than p_{thr} for a t-test (criterion $B(p_{thr})$). For all values of p_{thr} the interval 25-250BPA is the one that shows the largest value.

Clarity Study







Mechanisms *In Vivo*

- Many of the same mechanisms that operate *in vitro* are observed *in vivo*.
- Tissues are more complex: receptor expression changes at different developmental periods; multiple cell types are present and in contact with varying levels of receptor expression; tissue compartments interact and influence each other.

Do we need to know the underlying mechanisms in order to accept the existece of NMDRC?

Example: Since time immemorial, humans castrated animals to make them useful to them. Our ancestors **did know** that castrated animals would not reproduce. They **did not know** why. Mechanistic explanations were generated thousands of years after this practice became common.

Conclusion: No, we do not need to know mechanisms in order to accept the existence of a phenomenon!

Conclusions

- NMDRC occur in at all levels of biological organization
- Both natural hormones and endocrine disruptors produce NMDRC
- The mechanisms underlying NMDRC are well understood
- The existence of a phenomenon is not defined by the mechanism producing it