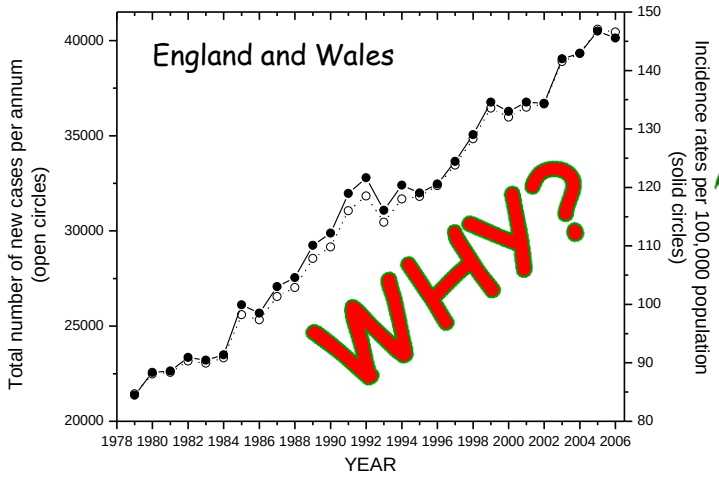
ROLE OF ENVIRONMENTAL CHEMICALS IN THE DEVELOPMENT OF BREAST CANCER

Philippa Darbre
School of Biological Sciences
University of Reading

- Breast cancer is not a new disease
- BUT incidence continues to rise
- Risk factors identified but causes uncertain



**Until causes are known,
prevention cannot become a reality**

RISK FACTORS FOR BREAST CANCER

- GENETICS
  - Female
  - Loss of function of the BRCA1 / BRCA2 genes
- DIET /ALCOHOL / RADIATION
- OBESITY
- HORMONAL EFFECTS (lifetime exposure to oestrogen)
  - Physiological (age of puberty/menopause)
  - Childbirth (age of first child, breast feeding)
  - Personal decisions (use of OC or HRT)
- CHEMICALS FROM THE ENVIRONMENT WHICH CAN ENTER THE HUMAN BREAST?
- IF EXPOSURE TO OESTROGEN IS A MAIN RISK FACTOR, HOW ABOUT EXPOSURE TO OESTROGENIC CHEMICALS?

Darbre 2001 Eur J Cancer Prevent 10, 389-393; Darbre 2003 J Appl Toxicol 23, 89-95; Harvey & Darbre 2004 J Appl Toxicol 24, 167-176;
Darbre 2009 Breast Cancer Research 11 (S3); Darbre 2010 Anticancer Research 30, 815-828 Darbre PD 2010 CML-Breast Cancer 22, 113-122
Darbre 2012 Hormone Molecular Biology and Clinical Investigation 9, 65-85.

ENVIRONMENTAL OESTROGENIC CHEMICALS WHICH CAN ENTER THE HUMAN BREAST

- OUTDOOR ENVIRONMENT
  - Use of pesticides / herbicides
- DIET
  - Agrochemicals (Animal fat)
  - Polychlorinated biphenyls, dioxins (Animal fat)
  - Phytoestrogens (Plant material)
- HOUSEHOLD PRODUCTS
  - Plastics (Bisphenol A, phthalates)
  - Flame retardants (Polybrominated diphenyl ethers)
  - Non-stick coatings –polytetrafluoroethylene (PTFE)(teflon)
  - Stain resistant coatings -perfluorooctanoic acid (PFOA)
  - Detergents (alkyl phenols)
- DERMAL ABSORPTION OF PERSONAL CARE PRODUCTS
  - Parabens, triclosan (preservatives, deodorant)
  - Aluminium (antiperspirant)
  - UV filters (absorb UV light)
  - Musks, Lilial, benzyl salicylate (fragrance)
  - Cyclosiloxanes (conditioning/spreading)
  - Antiageing

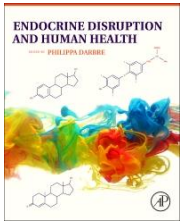
CAN EXPOSURE TO ENVIRONMENTAL OESTROGENS INFLUENCE HUMAN HEALTH?

- WOMEN PRESCRIBED DIETHYLSTILBOESTROL DURING PREGNANCY
  - Women have increased breast cancer incidence
    - Greenberg et al 1984. New Eng J Med 311: 1393-1398
  - Daughters have increased breast cancer incidence
    - Troisi et al 2007. Int J Cancer 121: 356-360

- THE MORTICIANS MYSTERY
  - Absorption of oestrogenic chemicals from embalming creams caused loss of libido, decreased testicular size, breast development
    - Finkelstein et al 1988. New Eng J Med 318: 961-965.

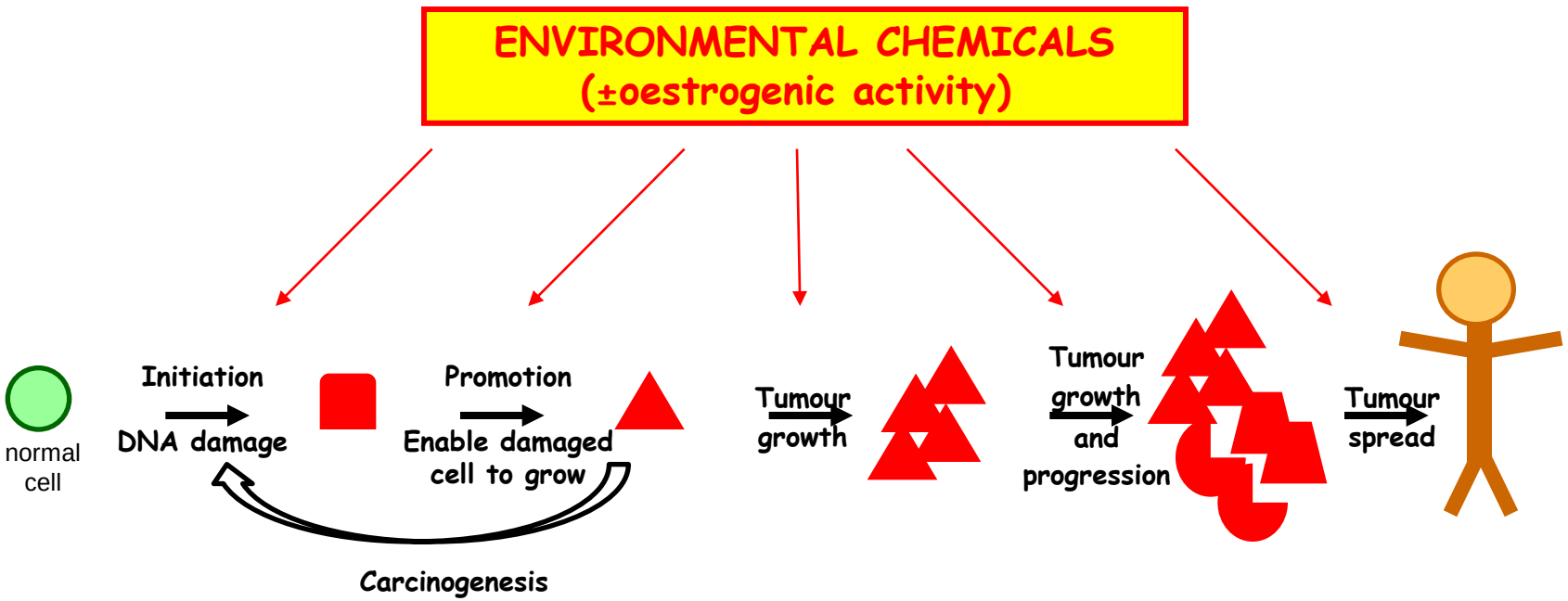
- THELARCHE IN A 36-MONTH GIRL FROM EXPOSURE TO MOTHER'S SHAMPOO
    - Guanari et al 2008. Clin Toxicol 46: 762-764.

- ABNORMAL BLEEDING IN A 93-YEAR OLD AFTER USING AN ETHYNYLOESTRADIOL-CONTAINING BODY CREAM
    - Komori et al 2008. Menopause 15: 1191-1192.



Darbre 2015. Endocrine Disruption and Human Health. Elsevier-Academic Press.

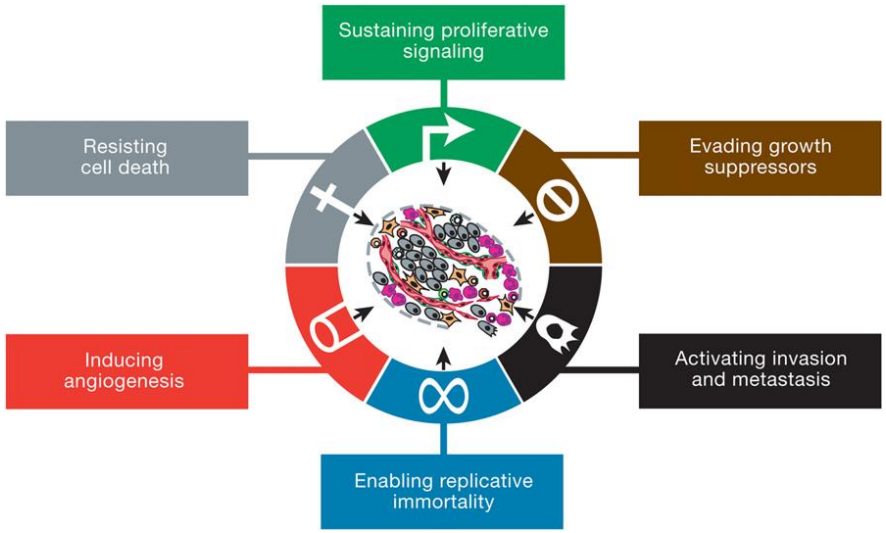
COULD ENVIRONMENTAL CHEMICALS INFLUENCE THE DEVELOPMENT OF BREAST CANCER



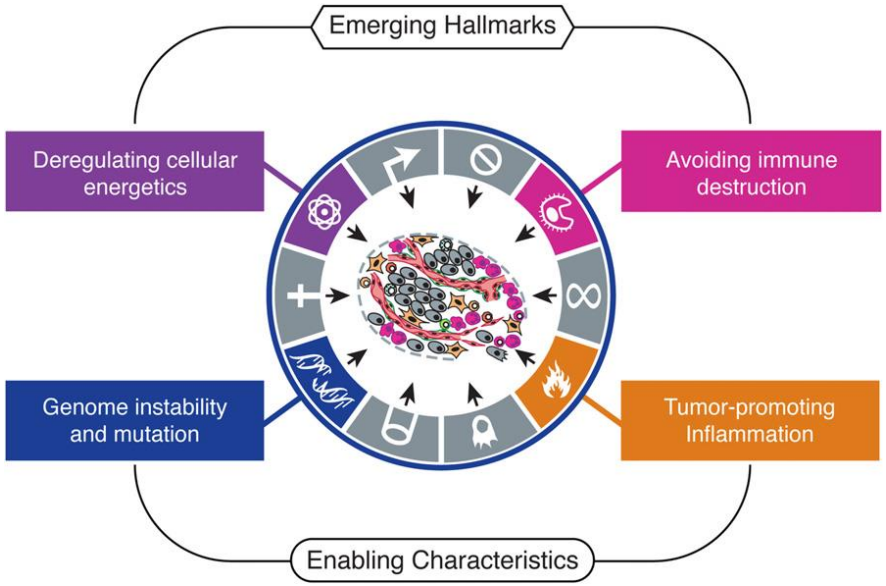
COULD ENVIRONMENTAL CHEMICALS INFLUENCE DEVELOPMENT OF HUMAN BREAST CANCER?

Hanahan and Weinberg, 2011
Cell 144, 646-674.

The six hallmarks of cancer



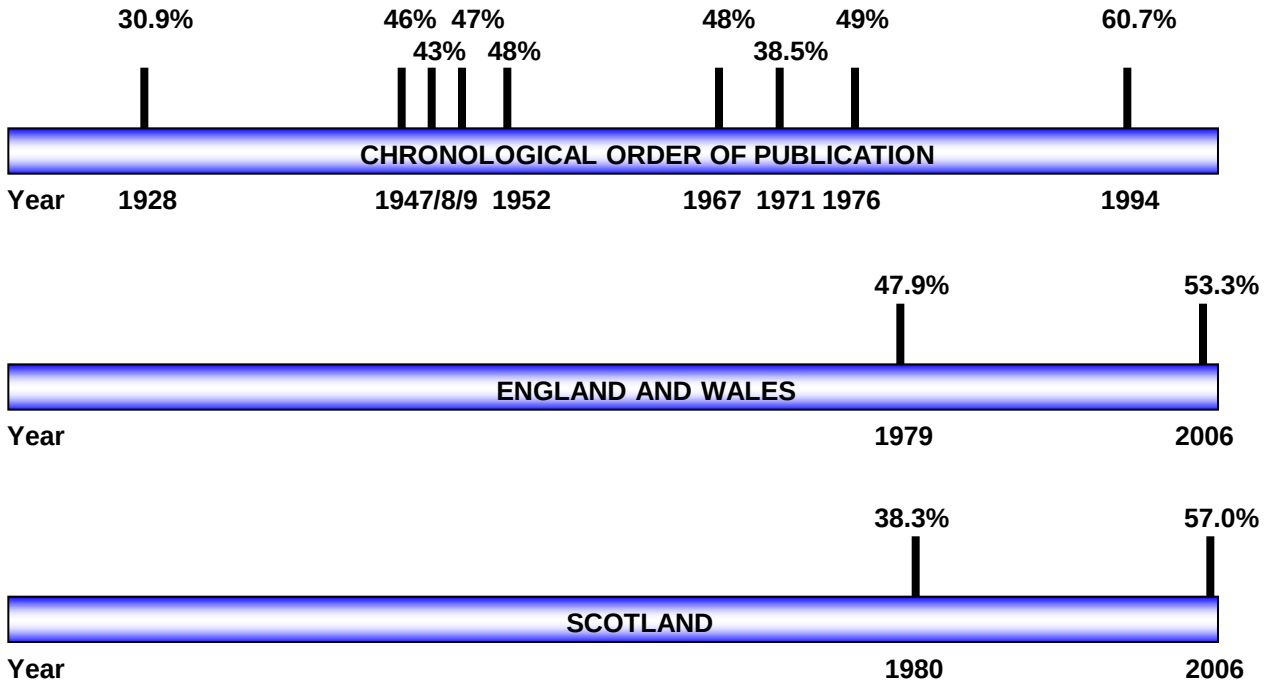
Emerging hallmarks and Enabling characteristics



NEW HYPOTHESIS IN 2001: COULD UNDERARM COSMETICS CONTRIBUTE TO CAUSALITY OF BREAST CANCER?

Darbre 2001 European Journal of Cancer Prevention 10: 389-393

The disproportionate incidence of breast cancer in the upper outer quadrant of the breast



This rising incidence is inconsistent with the disproportionality relating solely to more epithelial tissue in that region.

Darbre & Charles. 2010. Anticancer Research 30, 815-828

THE CASE FOR AN INVOLVEMENT OF COSMETIC CHEMICALS IN BREAST CANCER

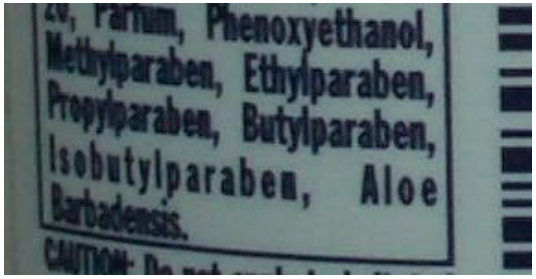
- **Exposure**
  - Applied to underarm and breast area
  - Left on skin allowing for continuous exposure
  - Antiperspirants, deodorants, body lotions, body creams, body sprays, moisturising creams, breast firming/enhancing creams, tanning creams, sun-care creams.
- **Chemical overload / individual susceptibility**
  - Used with increasing frequency & quantity
  - Used by ever younger children & babies
- **Molecular basis**
  - DNA damage – genotoxic chemicals?
  - Cell growth – oestrogenic chemicals?
- **Chemical toxicity**
  - Long-term low dose exposure to mixtures of chemicals
  - Absorption, metabolism, clearance
  - Consideration of timing of exposure



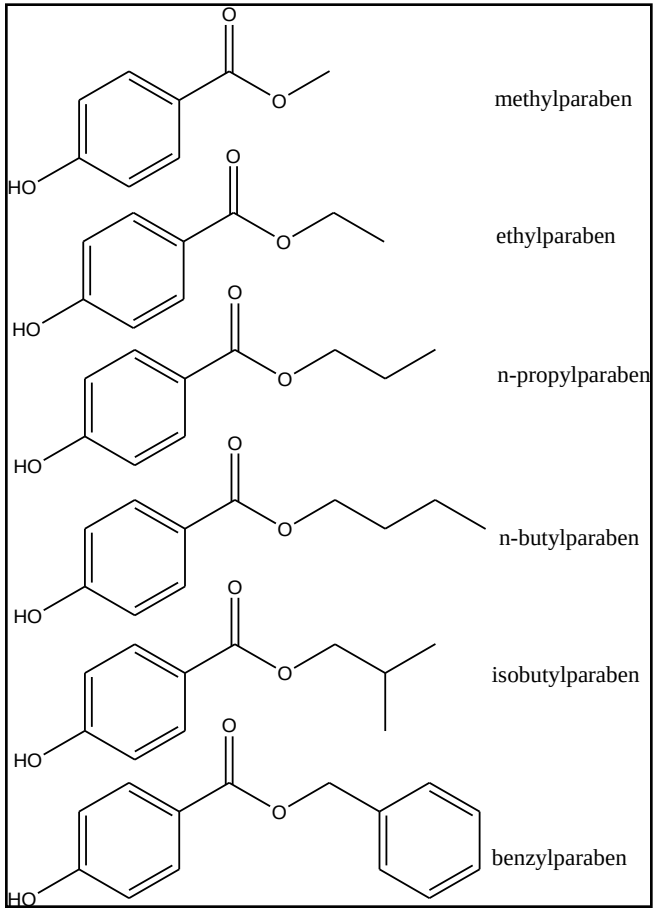
Darbre 2001 Eur J Cancer Prev 10, 389-393; Darbre 2003 J Appl Toxicol 23, 89-95
Darbre 2006 Best Pract & Res Clin Endocrinol Metab 20, 121-143; Darbre 2009 Breast Cancer Research 11 (S3) 1-5.
Darbre 2010 Anticancer Research 30, 815-828; Darbre PD 2010 CML-Breast Cancer 22, 113-122

PARABENS
The alkyl esters of *p*-hydroxybenzoic acid

- **Used as preservatives:**
  - Personal care products
  - Foods
  - Pharmaceuticals
  - Paper products







Reviews:

Harvey and Darbre 2004 J Appl. Toxicol. 24: 297-306
Darbre, and Harvey 2008 J Appl. Toxicol 28, 561-578
Darbre and Harvey 2014 J. Appl. Toxicol. 34: 925-938

PARABENS
WHAT RESEARCH HAVE WE DONE?

- Parabens have oestrogenic activity in *in vitro* and *in vivo* assays
  - lifetime exposure to oestrogen is the main risk factor for breast cancer
- Parabens have been measured in human breast tissue
- Parabens can enable the development of multiple hallmarks of cancer

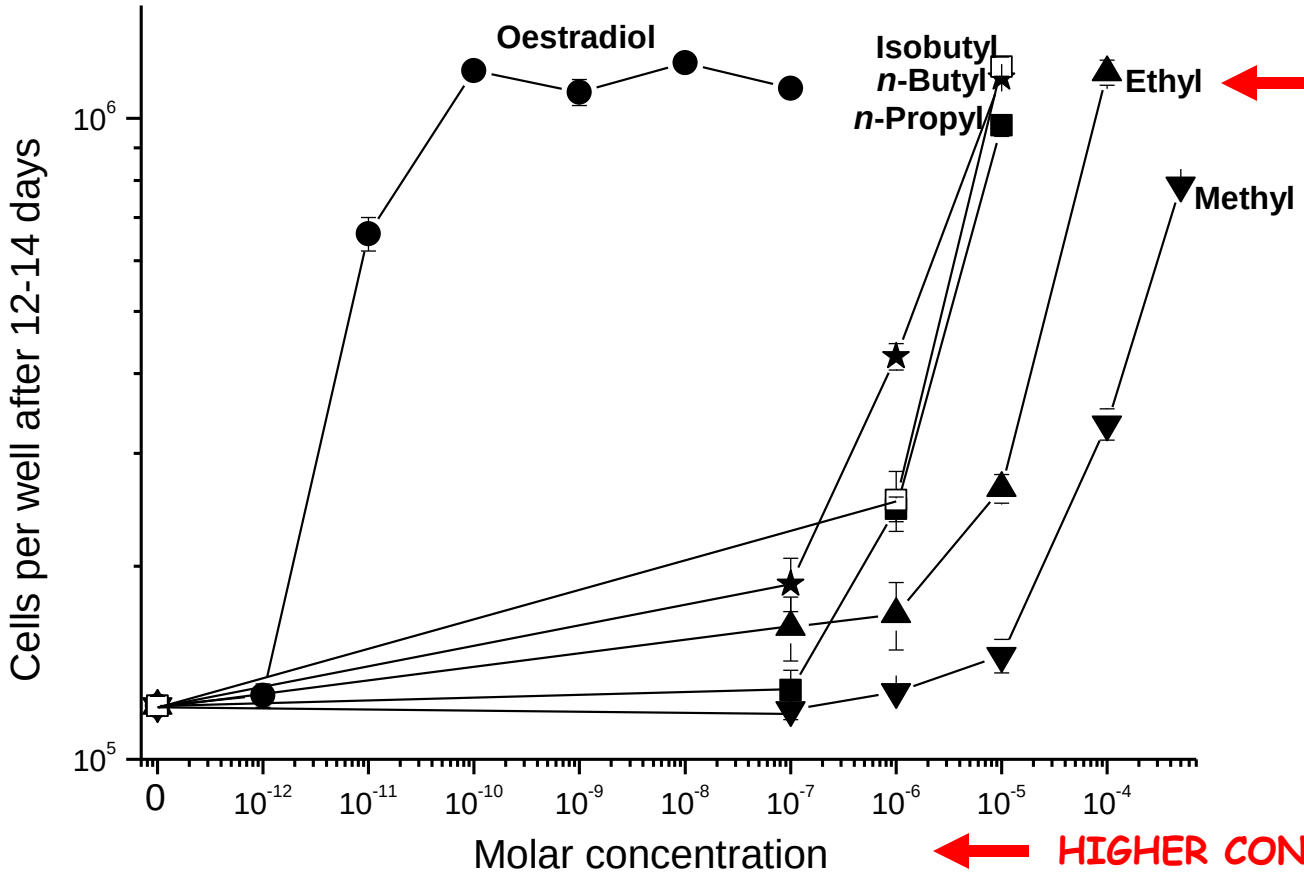
EVIDENCE FOR OESTROGENIC ACTIVITY OF PARABENS

- BIND TO OESTROGEN RECEPTORS
- REGULATE OESTROGEN-RESPONSIVE GENE EXPRESSION
- INCREASE GROWTH OF OESTROGEN-DEPENDENT HUMAN BREAST CANCER CELLS
- INCREASE UTERINE WEIGHT IN IMMATURE RODENT

Reviewed in: Darbre, Harvey 2008 J Appl. Toxicol 28, 561-578
Darbre, Harvey 2014 J Appl Toxicol 34, 925-938

PARABENS INCREASE PROLIFERATION OF MCF7 HUMAN BREAST CANCER CELLS

-DO NOT HAVE LOW EFFICACY
-full agonists if sufficient concentration is given



EFFICACY SIMILAR TO OESTRADIOL

HIGHER CONCENTRATIONS DUE TO REDUCED BINDING AFFINITY

Byford *et al* 2002 JSBMB 80,49; Darbre *et al* 2002 J Appl Toxicol 22, 219;
Darbre *et al* 2003 J Appl Toxicol 23, 43; Pugazhendhi *et al* 2005 J Appl Toxicol 25, 301.

PARABENS MEASURED IN HUMAN BREAST TISSUE

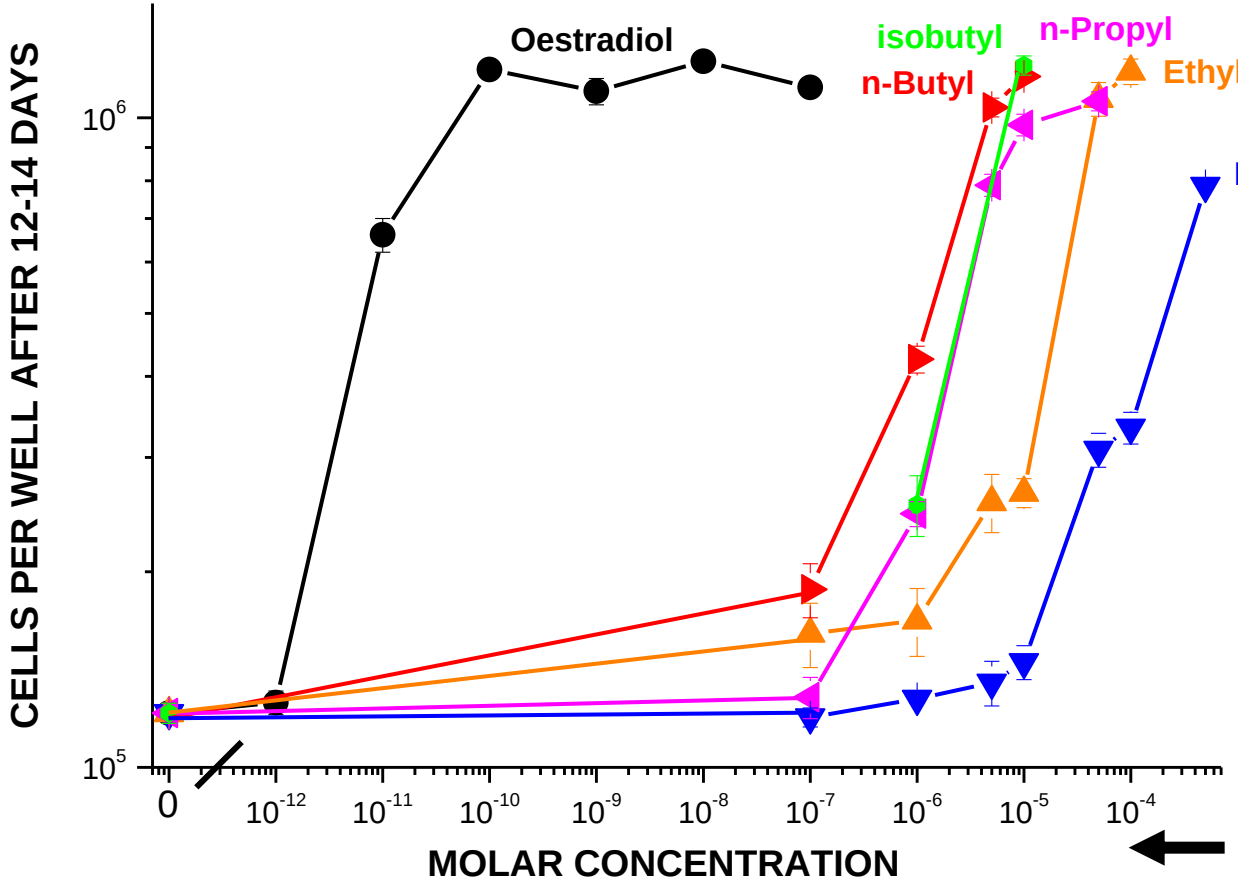
- 2004 study - 20 human breast tumour samples *(J Appl Toxicol 24: 5-13, 2004)*
  - average 20.6 ng/g tissue

- 2012 study – 160 human breast tissue samples *(J Appl Toxicol 32: 219-232, 2012)*
- - 40 patients with primary breast cancer
- - non-affected tissue
- - 4 serial locations across the breast
- - measured one or more paraben in 158/160 of the samples
- - 96/160 (60%) contained all 5 parabens
  - median 85.5 ng/g tissue

  - Median levels highest for propylparaben (16.8ng/g) and methylparaben (16.6ng/g)
  - Higher levels of propylparaben in axilla vs inner regions
  - Varied levels of each paraben
    - Between the same breast region of different patients
    - Between different regions within the same breast
  - High levels of one paraben did not associate with high levels of another paraben

- Question – Are these parabens at functionally significant levels?

GROWTH OF MCF7 CELLS



EFFICACY SIMILAR TO OESTRADIOL

HIGHER CONCENTRATIONS NEEDED DUE TO LOWER ER BINDING AFFINITY

Methylparaben 0-5102.9ng/g

Ethylparaben 0-499.7ng/g

n-Propylparaben 0-2052.7ng/g

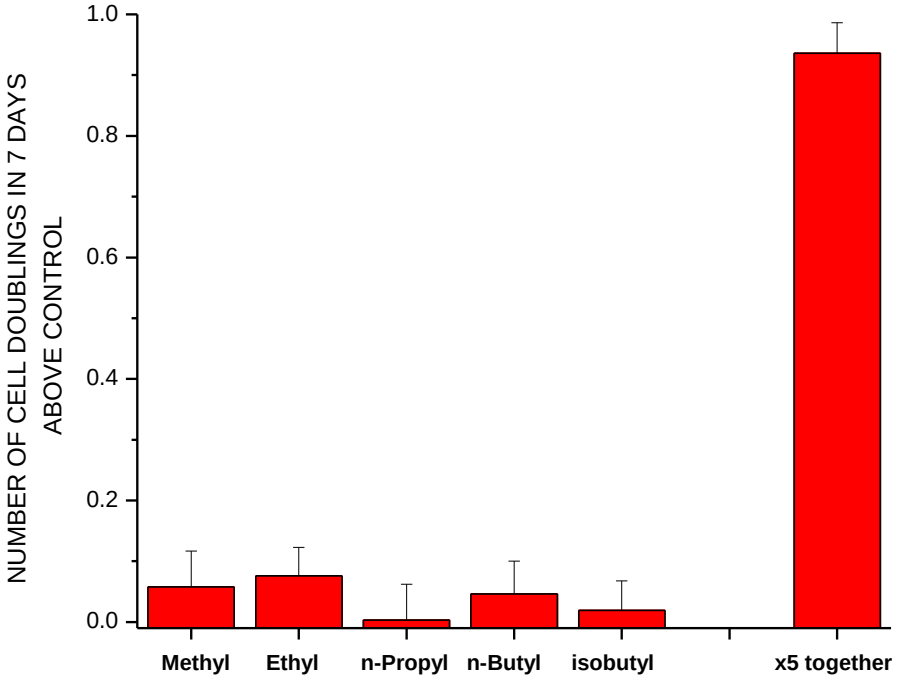
n-Butylparaben 0-95.4ng/g

Isobutylparaben 0-802.9ng/g

RANGE OF CONCENTRATIONS MEASURED IN HUMAN BREAST TISSUE

PARABENS HAVE ADDITIVE EFFECTS ON THE GROWTH OF MCF7 HUMAN BREAST CANCER CELLS

	Concentration at below NOEC (no observed effect concentration)
Methylparaben	**$2x10^{-5}M$**
Ethylparaben	**$8x10^{-7}M$**
***n*-Propylparaben**	**$2x10^{-7}M$**
***n*-Butylparaben**	**$2x10^{-7}M$**
Isobutylparaben	**$10^{-7}M$**

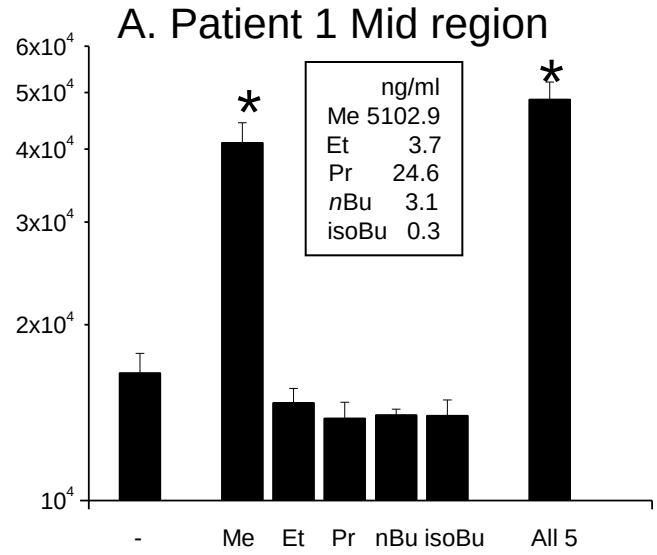


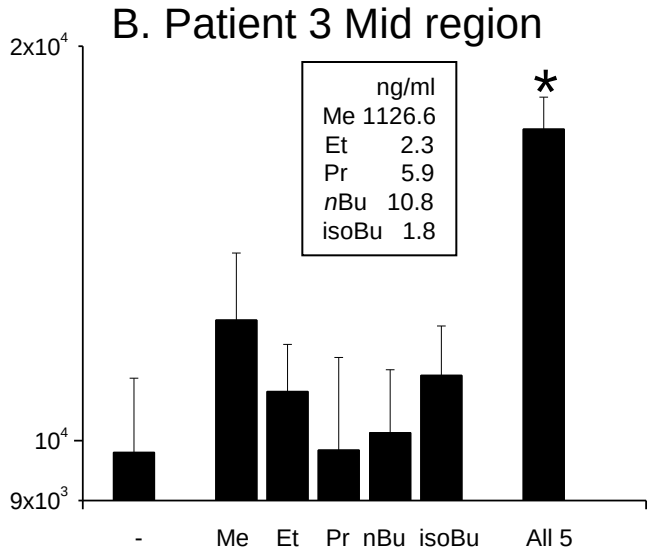
5 PARABENS WERE MEASURED IN HUMAN BREAST

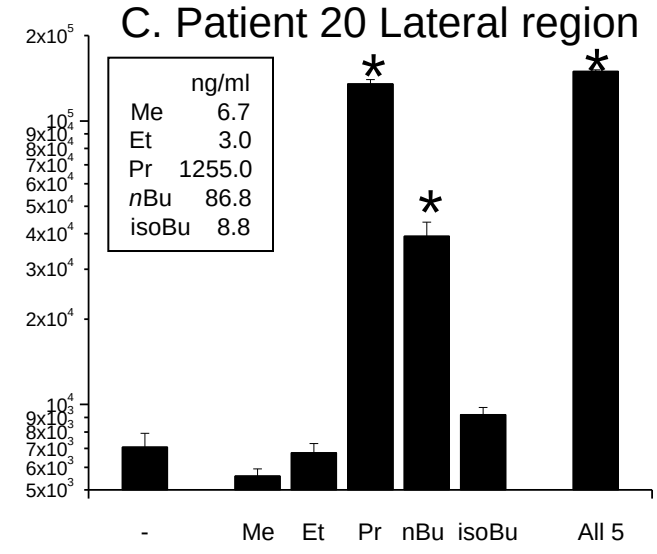
Darbre 2009 Breast Cancer Res 11, S5 1-5
Darbre and Charles 2010 Anticancer Res 30, 815-828

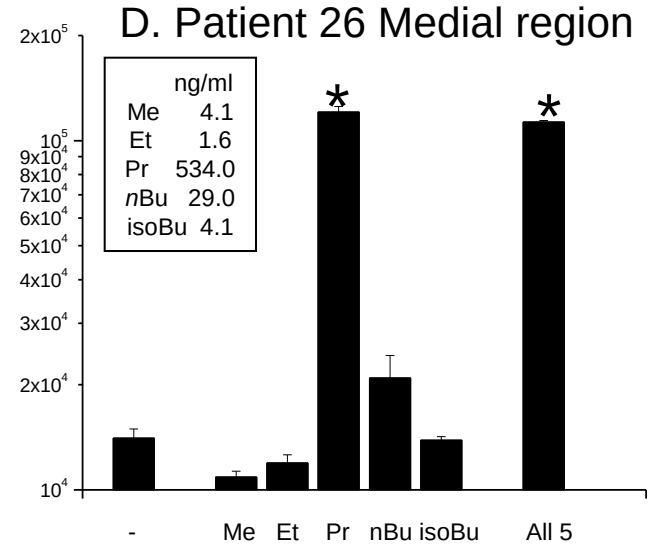
SOME CONCENTRATIONS OF PARABENS MEASURED IN HUMAN BREAST TISSUES ARE SUFFICIENT TO ENABLE GROWTH OF MCF-7 HUMAN BREAST CANCER CELLS

CELLS PER WELL ON DAY 14





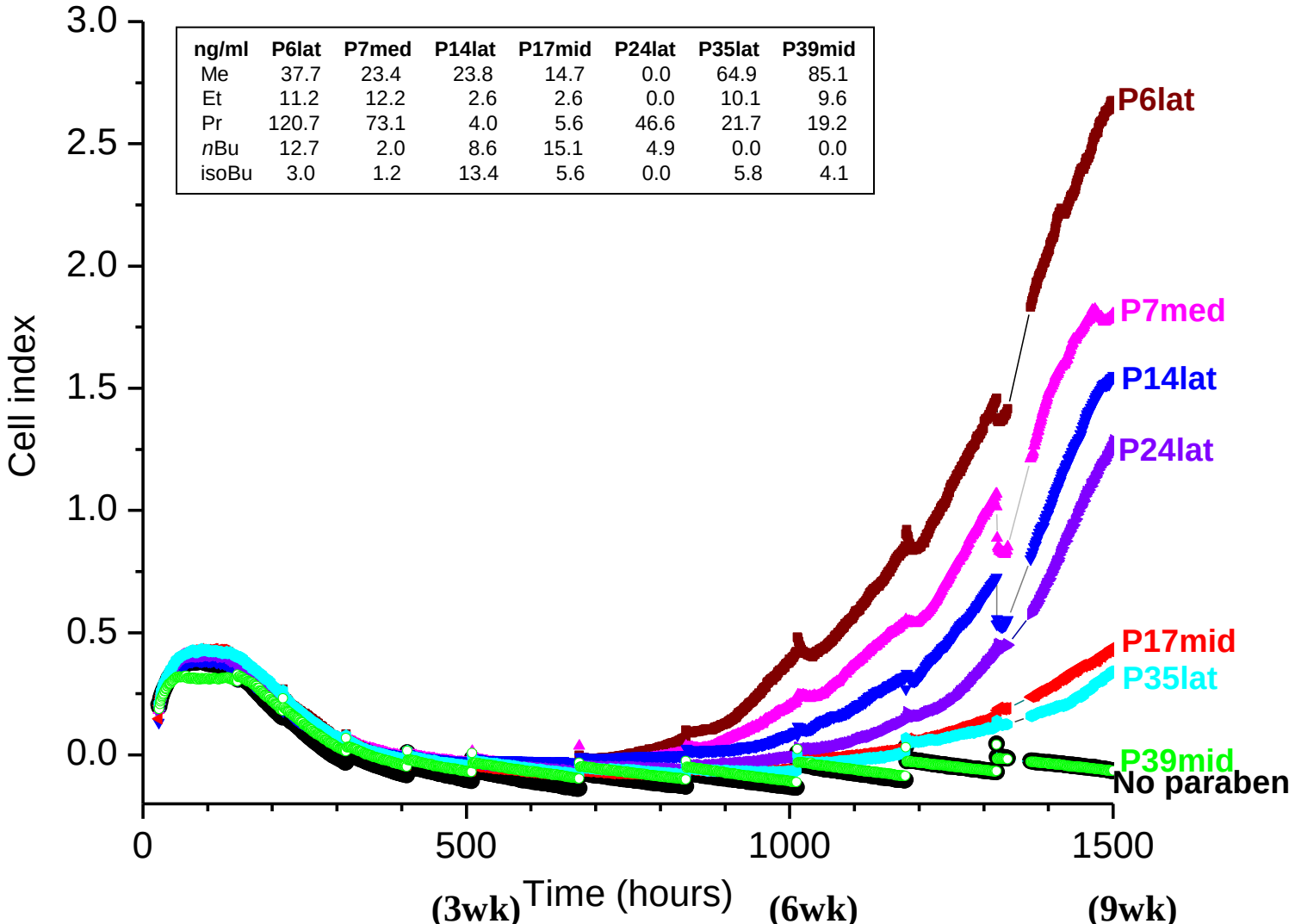




SOME CONCENTRATIONS OF PARABENS MEASURED IN HUMAN BREAST TISSUES ARE SUFFICIENT TO ENABLE GROWTH OF MCF-7 HUMAN BREAST CANCER CELLS

WHEN TIME OF THE ASSAY IS LENGTHENED

ng/ml	P6lat	P7med	P14lat	P17mid	P24lat	P35lat	P39mid
Me	37.7	23.4	23.8	14.7	0.0	64.9	85.1
Et	11.2	12.2	2.6	2.6	0.0	10.1	9.6
Pr	120.7	73.1	4.0	5.6	46.6	21.7	19.2
*n*Bu	12.7	2.0	8.6	15.1	4.9	0.0	0.0
isoBu	3.0	1.2	13.4	5.6	0.0	5.8	4.1



CONCLUSIONS - PARABENS

- Parabens have oestrogenic activity in *in vitro* and *in vivo* assays
  - lifetime exposure to oestrogen is the main risk factor for breast cancer
- Parabens have been measured in human breast tissue
- Parabens can increase proliferation of human breast cancer cells at tissue concentrations
- Parabens enable other cancer hallmarks
  - enable suspension growth of MCF10A non-transformed human breast epithelial cells (marker of transformation)
  - Long-term exposure to parabens can lead to alterations in cell motility and migration

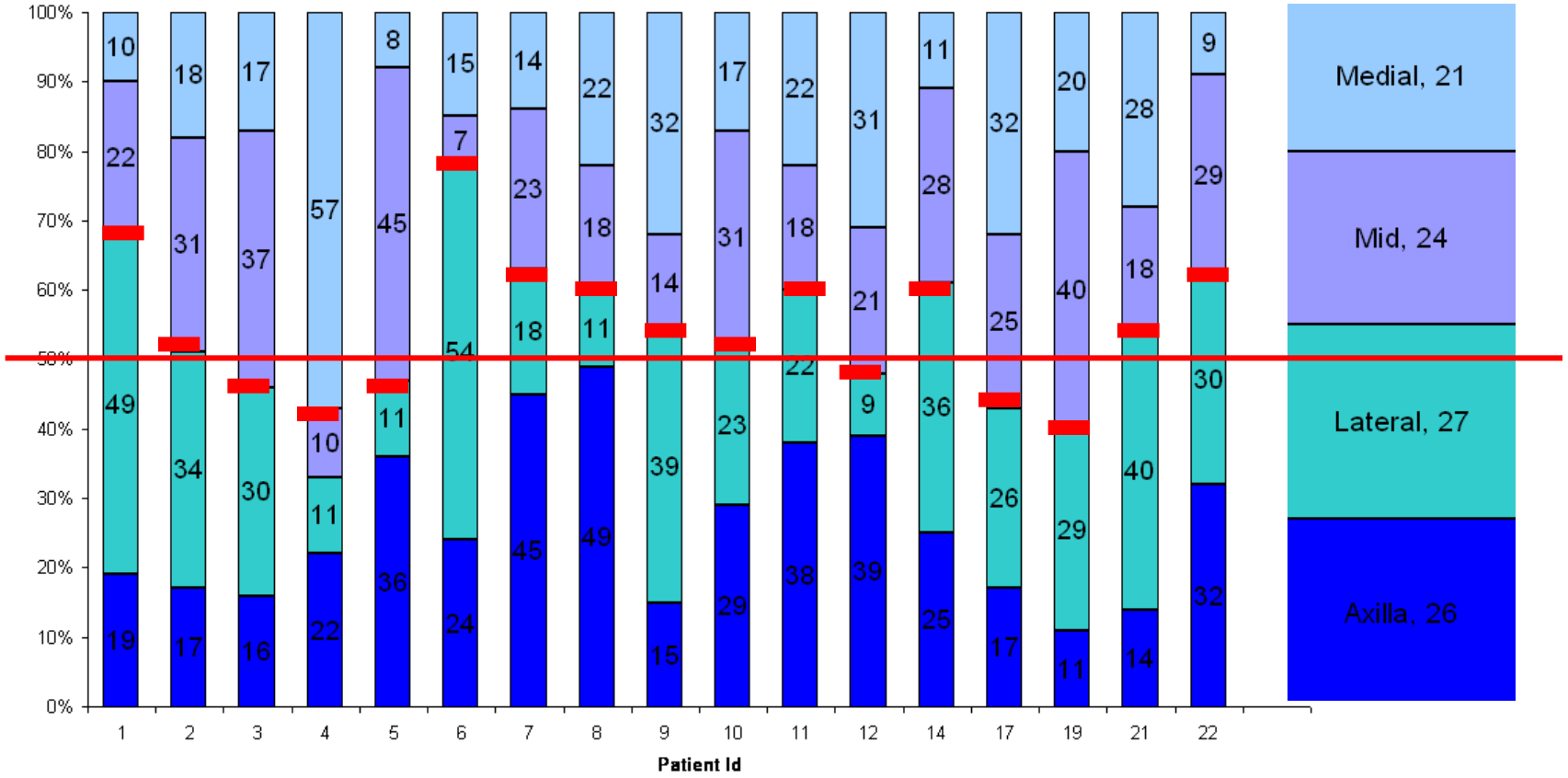
THE CASE FOR AN INVOLVEMENT OF ANTIPERSPIRANT ALUMINIUM SALTS

- Human body is exposed to aluminium from diet, personal care products, vaccine adjuvants
- As underarm antiperspirant, aluminium salts are applied to the breast area at high levels
- Aluminium chlorohydrate can be absorbed through human skin
- Aluminium has been measured in several human breast structures
  - Human breast tissue
  - Milk
  - Nipple aspirate fluid
  - Breast cyst fluid
- Aluminium is genotoxic and a metalloestrogen
- Aluminium can enable several hallmarks of cancer

ALUMINIUM CAN BE MEASURED IN HUMAN BREAST TISSUE

- VARIED FROM 4-437 nmol/gm DRY WT

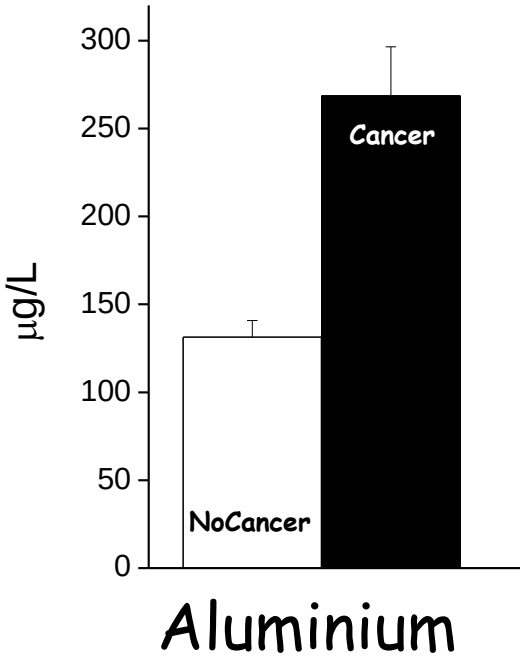
Aluminium content of the outer regions (axilla+lateral) was higher than inner regions (mid+medial) (*P*=0.033)



Exley et al., 2007 J Inorg Biochem 101, 1344-1346

MEASUREMENT OF ALUMINIUM IN HUMAN NIPPLE ASPIRATE FLUID

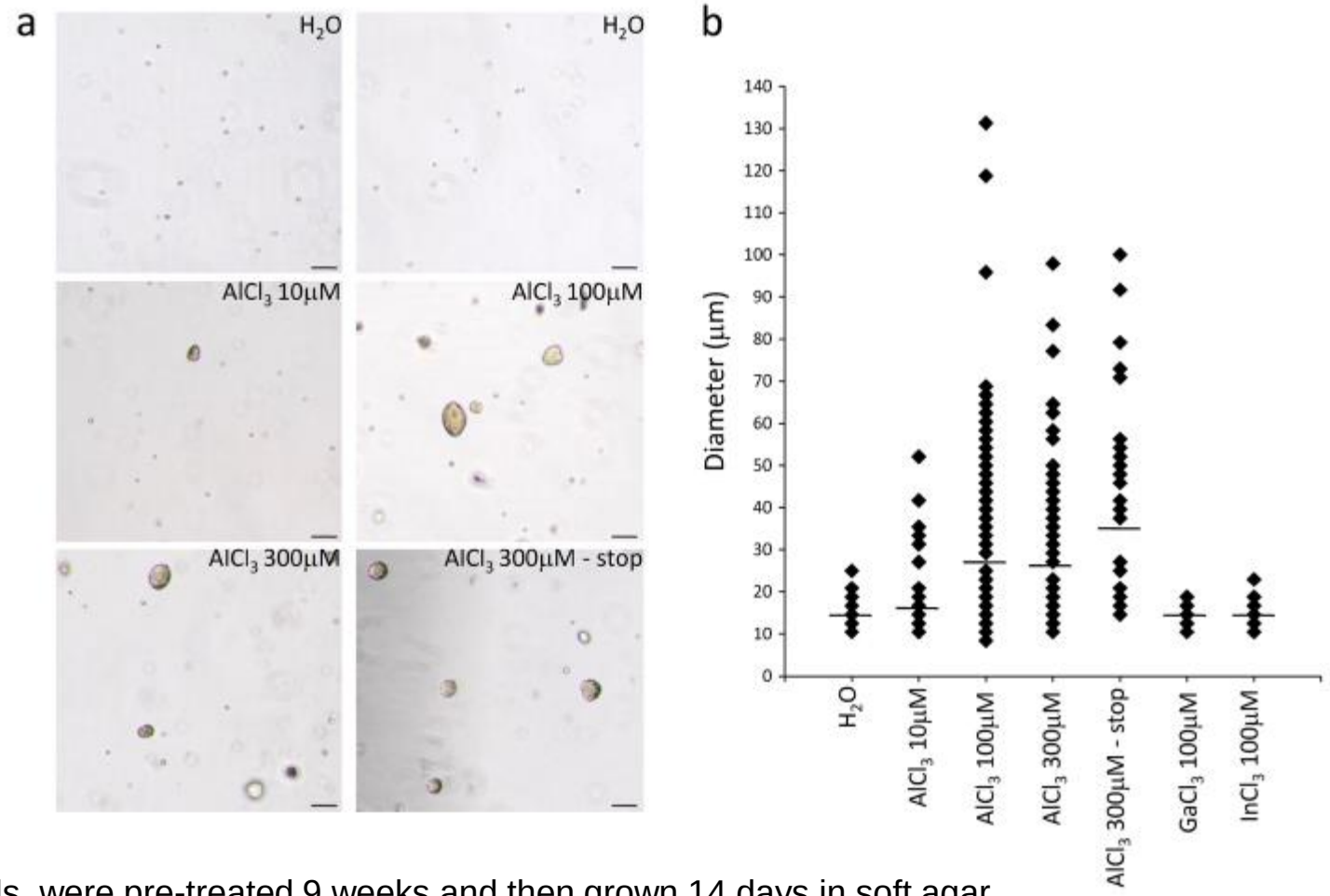
- from healthy women (*NoCancer*)
- from women affected by breast cancer (*Cancer*)



NoCancer n=16
Cancer n=19

Mannello, Tonti, Medda, Simone, Darbre, 2011 J Appl Toxicol 31: 262-269

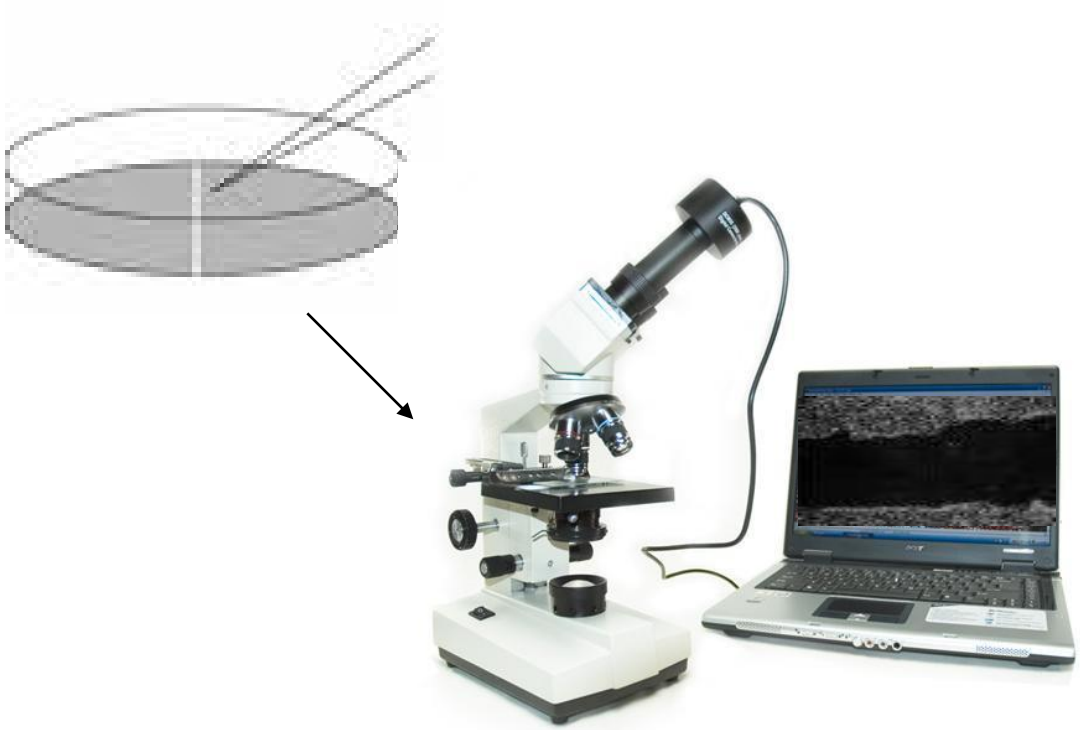
Aluminium chloride induces anchorage-independent growth of MCF10A human non-transformed, immortalised breast epithelial cells



Cells were pre-treated 9 weeks and then grown 14 days in soft agar.

Sappino et al. 2012. J Appl. Toxicol. 32, 233.

MOTILITY OF CELLS CAN BE STUDIED USING A WOUND-HEALING OR SCRATCH ASSAY





0hr

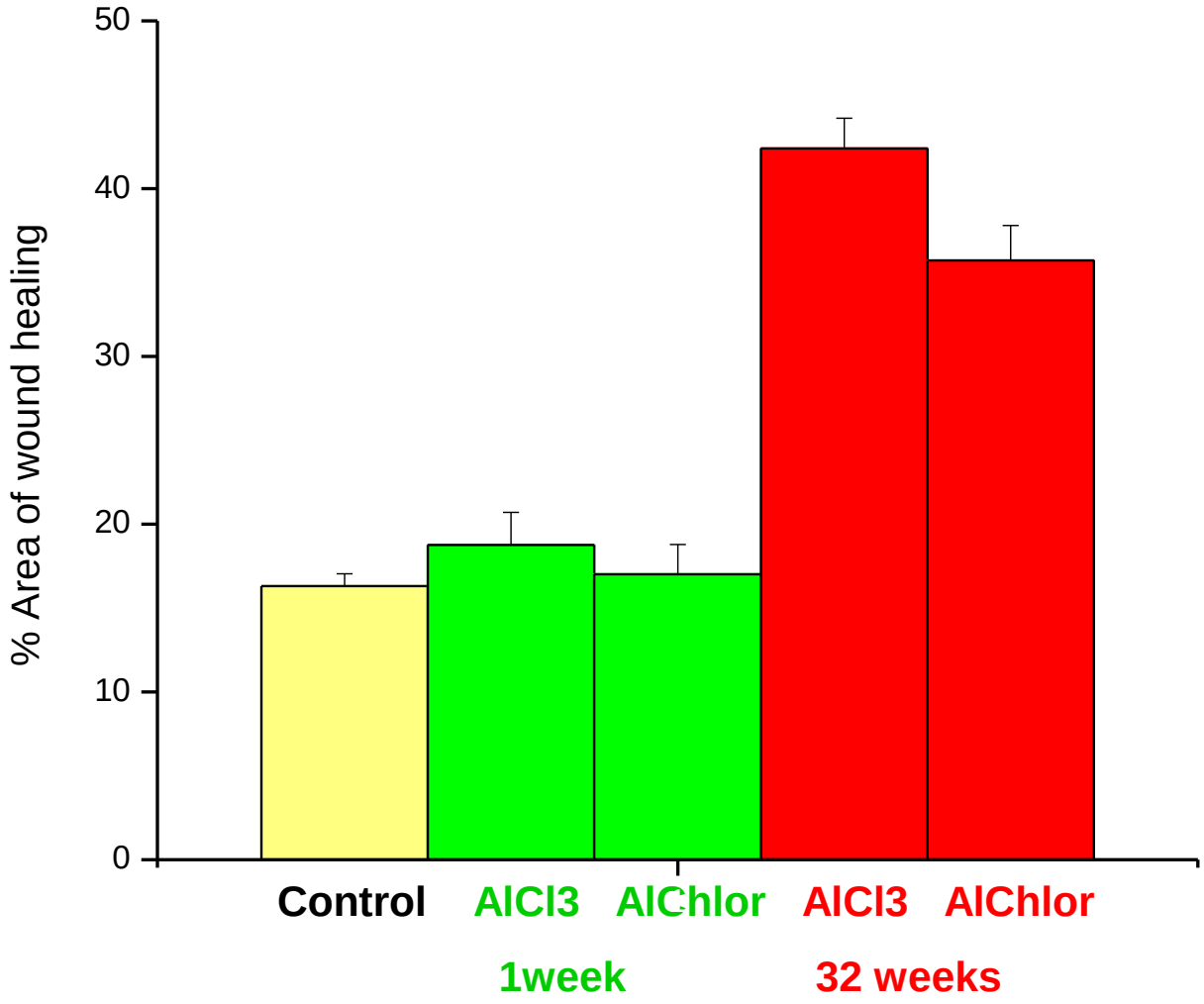


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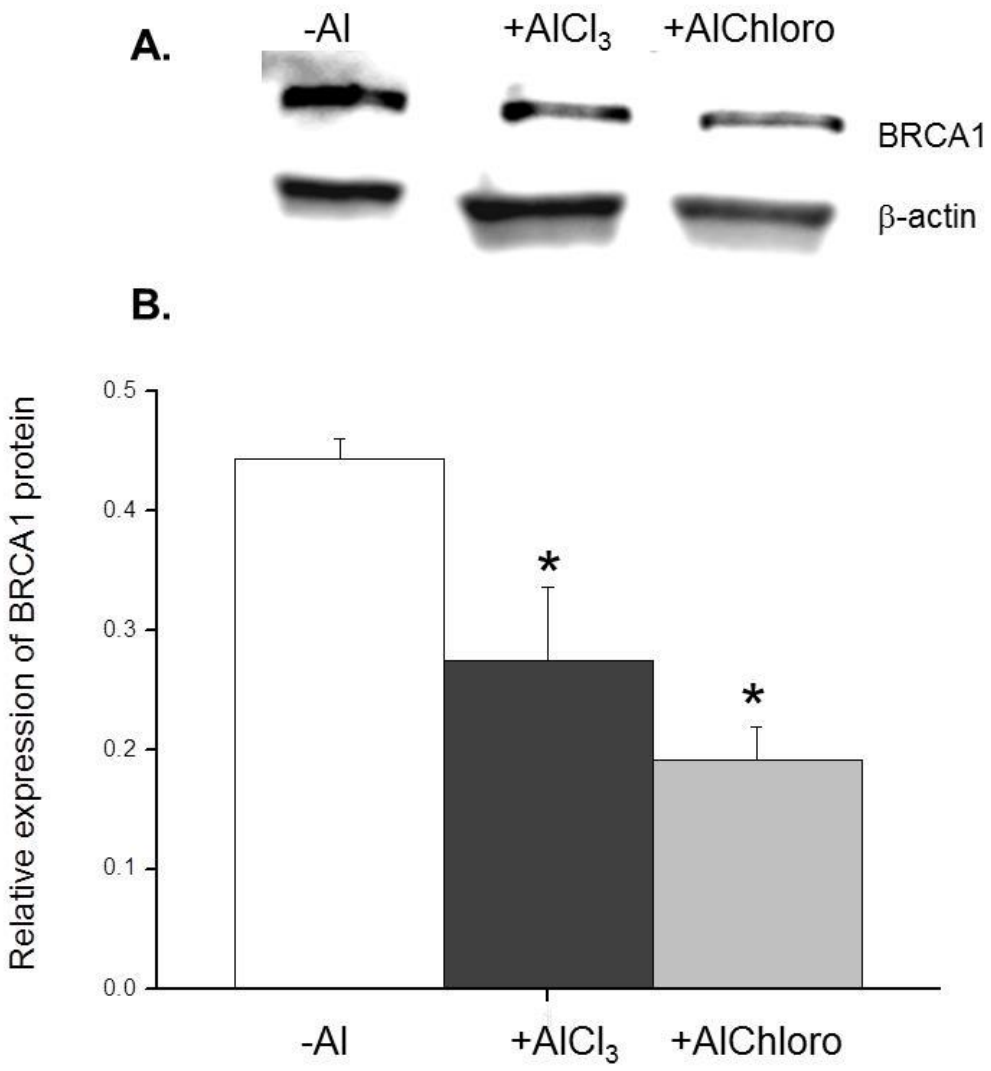
48hr

LONG-TERM EXPOSURE TO ALUMINIUM INCREASES MIGRATION OF MCF-7 HUMAN BREAST CANCER CELLS



Darbre, Bakir, Iskakova 2013 J Inorg Biochem 128: 245-249.

LOSS OF BRCA1 PROTEIN IN MCF10A NON-TRANSFORMED HUMAN BREAST EPITHELIAL CELLS AFTER 30 WEEKS EXPOSURE TO ALUMINIUM

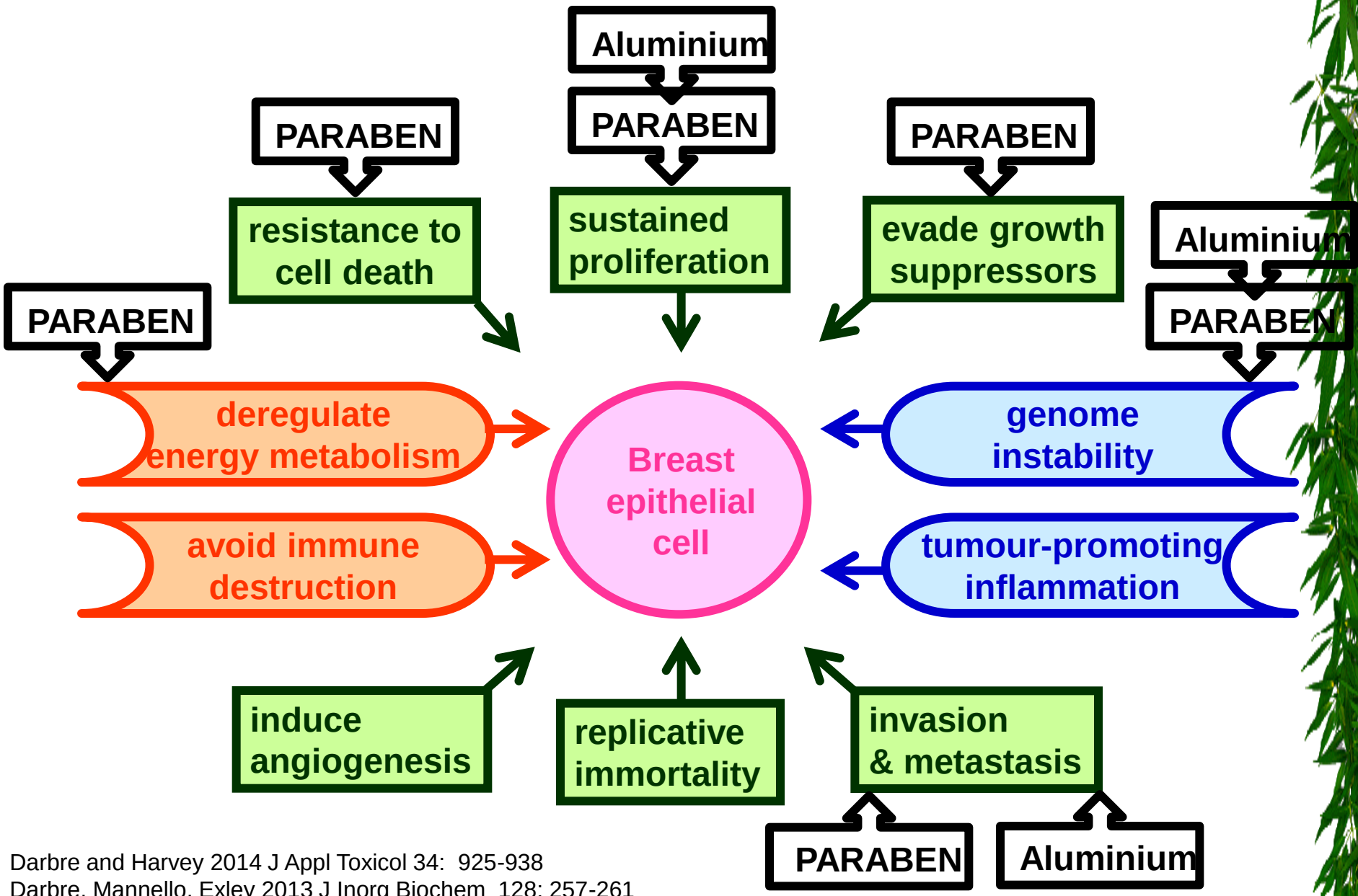


Farasani & Darbre. J Inorg. Biochem (in press)

CONCLUSIONS - ALUMINIUM

- The human breast is exposed to aluminium
- Aluminium has been measured in human breast tissue structures
- Aluminium can enable multiple hallmarks of cancer
  - Aluminium can cause DNA double strand breaks and turn human breast epithelial cells into a transformed phenotype in culture
  - Long-term exposure to aluminium results in loss of BRCA1
  - Aluminium can influence migratory and invasive properties of human breast cancer cells in culture

PARABENS and ALUMINIUM ENABLE MULTIPLE HALLMARKS OF CANCER



Darbre and Harvey 2014 J Appl Toxicol 34: 925-938
Darbre, Mannello, Exley 2013 J Inorg Biochem 128: 257-261

FINAL CONCLUSIONS

- Many environmental chemicals are found in the human breast
  - may be because the breast is a fatty organ
- Some environmental chemicals possess oestrogenic activity
  - which can enable proliferation of human breast cancer cells
- Some environmental chemicals are genotoxic
  - act to damage DNA
  - enable growth in suspension (characteristic of transformed phenotype)
  - reduce BRCA1 (or other DNA repair capability)
- Some environmental chemicals can increase cell motility or invasion
- Since so many environmental chemicals have been measured in the human breast, there is the potential for
  - Long-term exposure
  - Mixtures of chemicals each present at low doses
- Reduction in exposure may offer a new option for prevention of breast cancer

The Halifax Project (June 2015) Carcinogenesis vol 36, pages S1-S296.
11 review papers linking low dose chemical exposures
to each of the hallmarks of cancer