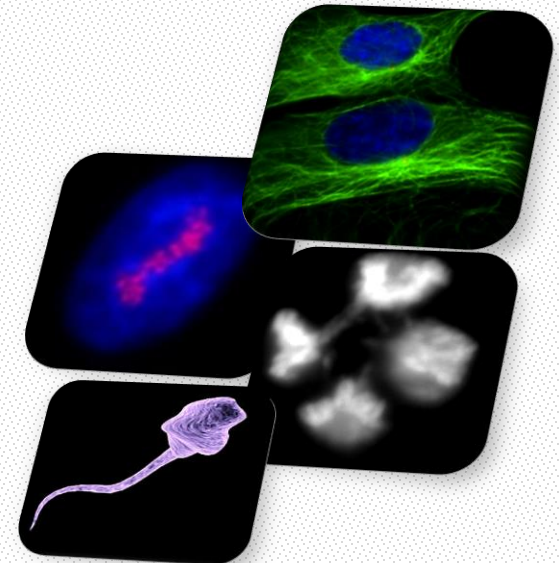
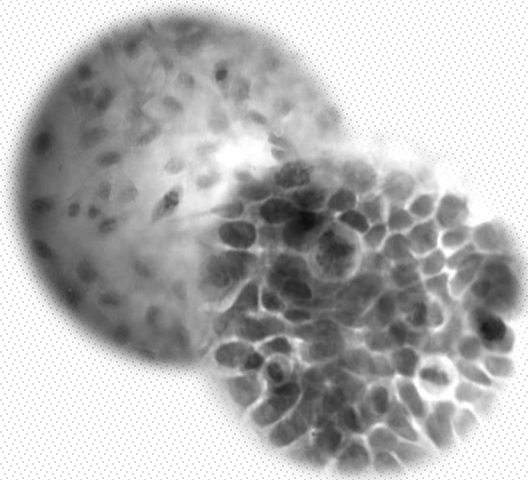




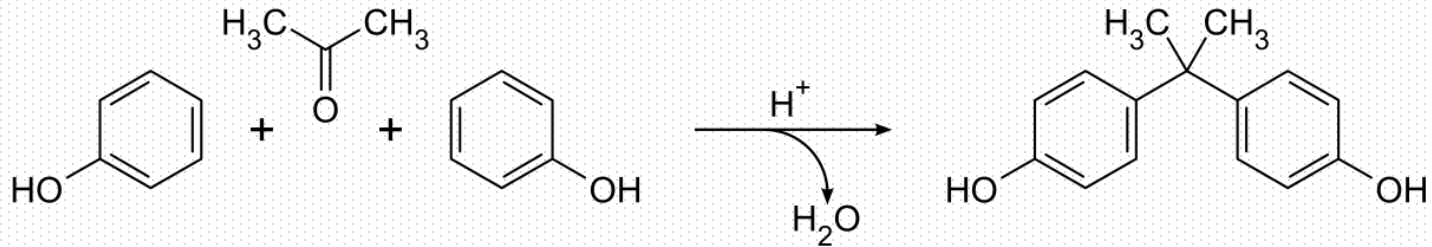
VII Encontro Nacional das Ciências e Tecnologias da Saúde

The paradigmatic Bisphenol A



Bisphenol A

Synthesised by A. P. Dianin in 1891



Two equivalents of phenol with one equivalent of acetone

Widespread production since 1940

Polycarbonate plastics and epoxy resins



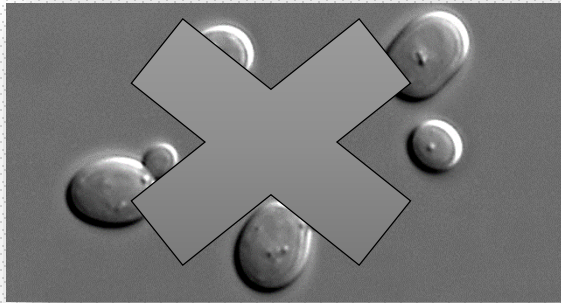
Baby bottles, reusable water bottles, reusable food containers and inner coatings of metallic food and beverage cans.

BPA as an endocrine disruptor chemical

1993 – Krishnan and co-workers

Estrogenic potential of *Saccharomyces cerevisiae*

Grown in culture media prepared using distilled water autoclaved in polycarbonate flasks

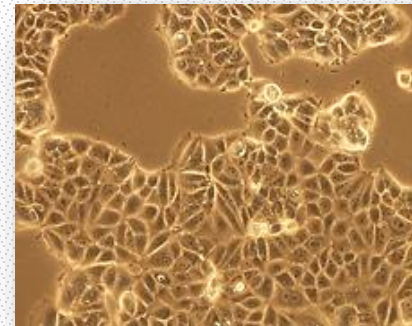


Presence of a substance that competed with estradiol for binding to estrogen receptors (ER)



THE BPA

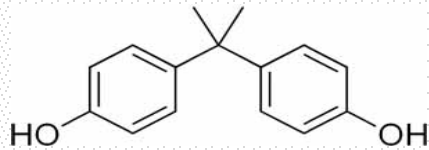
Increased proliferation rate of MCF7 cells



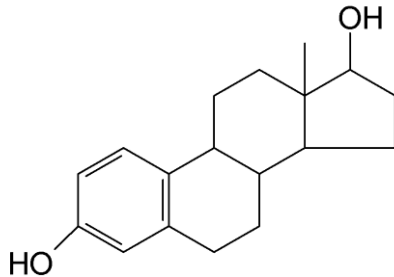
Endocrine disrupting chemicals (EDCs) are exogenous agents that have the capacity to behave as biological signals and interfere with/or mimic estrogenic hormones

BPA as an endocrine disruptor chemical

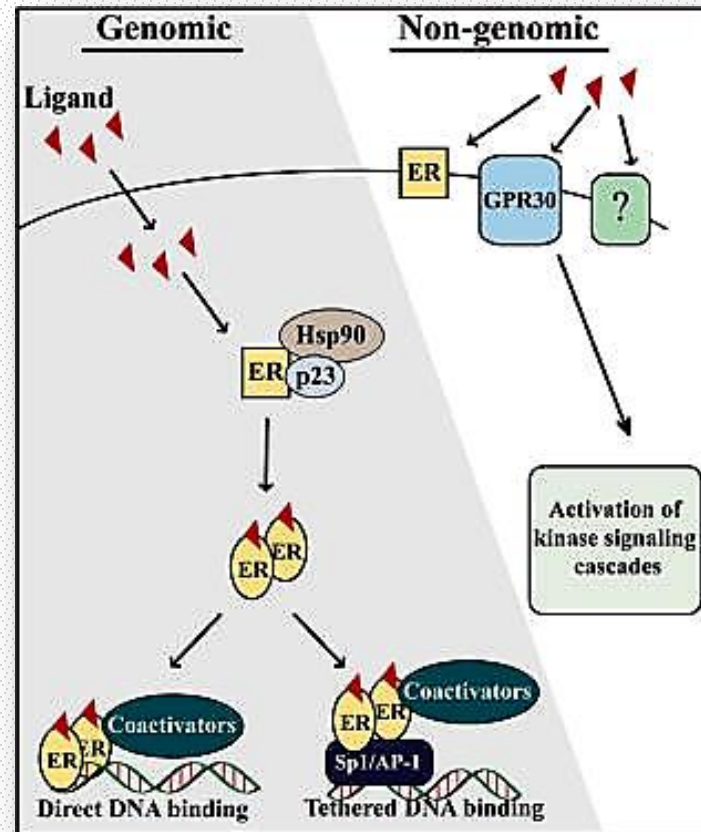
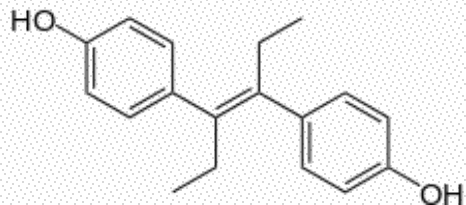
Bisphenol A (BPA)



Estradiol (E2)



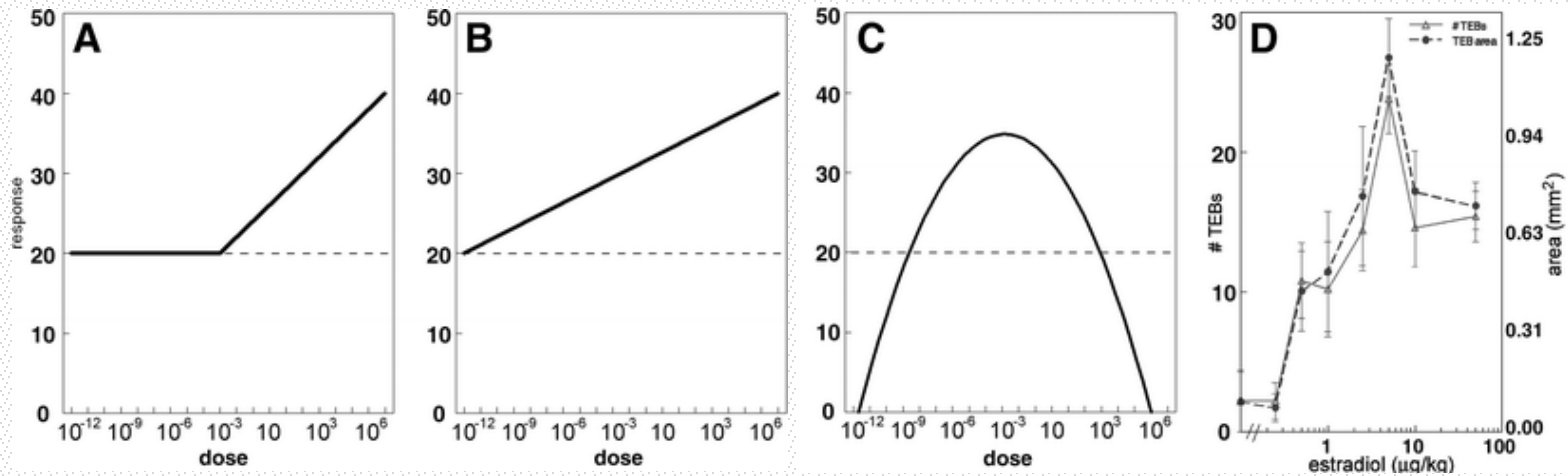
Diethylstilbestrol (DES)



Erin K. Shanle and Wei Xu 2010

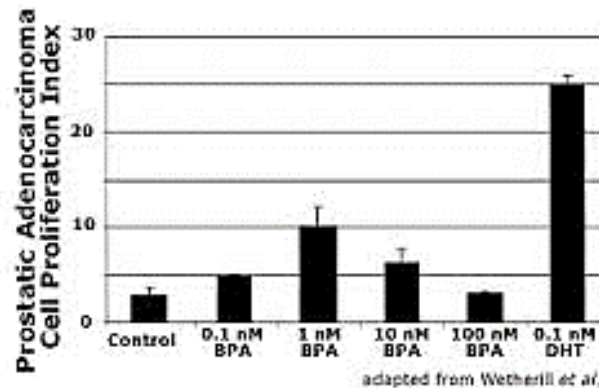
BPA as an endocrine disruptor chemical

Monotonic VS Non-monotonic dose response curves



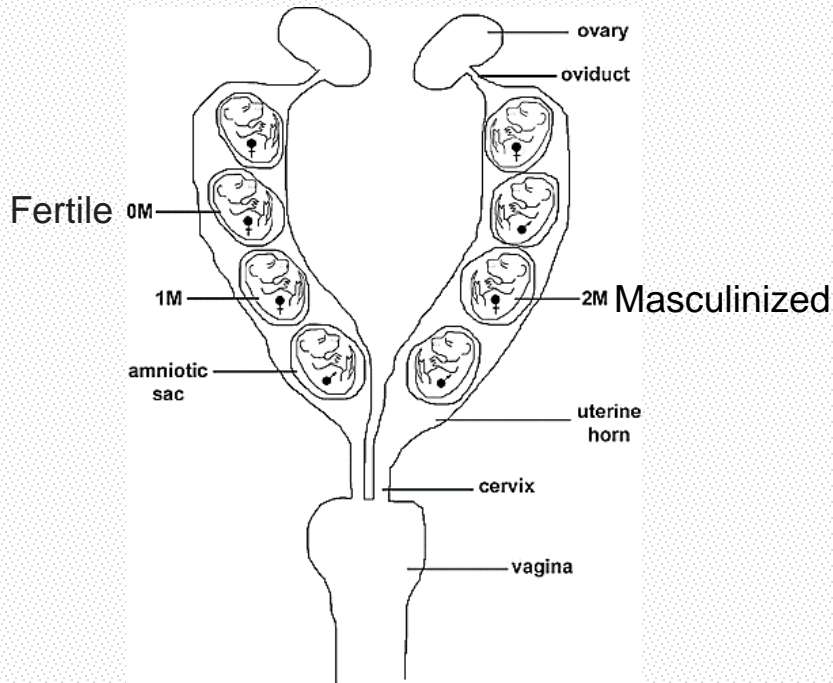
Adapted from Vandenberg L. et al 2009

BPA non-monotonic dose response



The “critical window” of exposure

Bicornate rodent uterus



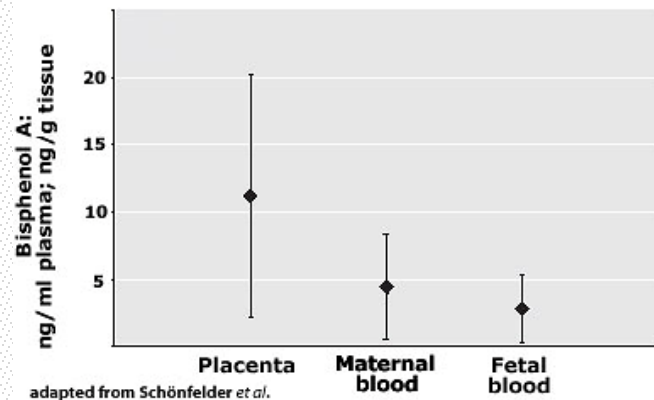
Adapted from Vandenberg L. et al 2009

Testosterone is passively transferred to neighboring fetuses

Physiological, morphological, and behavioral alterations dependent on position in the uterus during gestation.



Schonfelder G. et al 2002

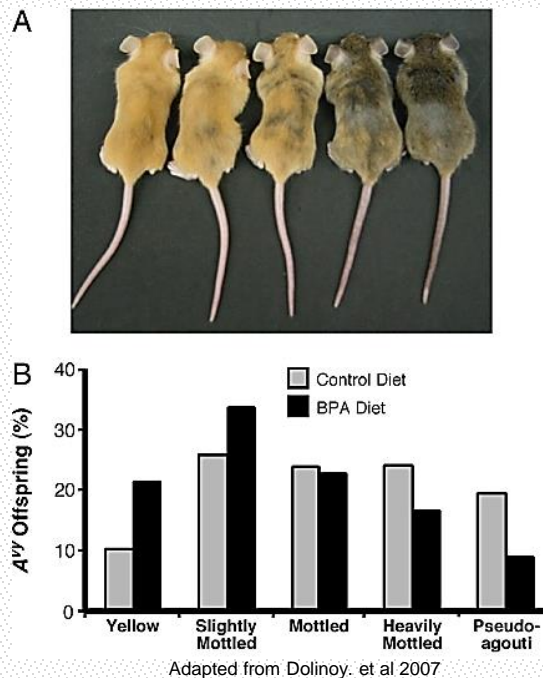


Early developmental exposure to BPA

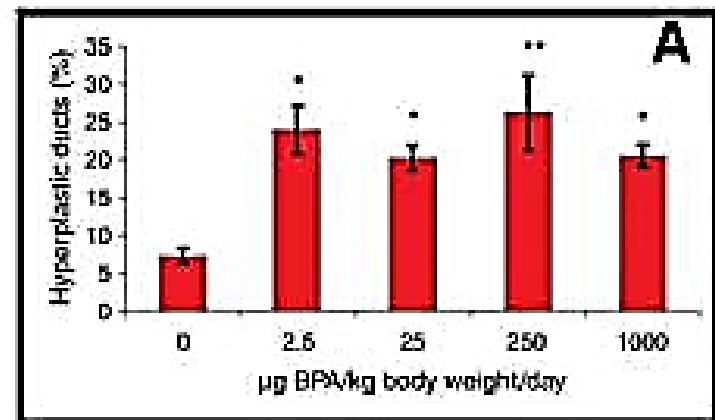
Epidemiological studies revealed that perturbations in the fetal environment such as maternal malnutrition caused diseases and/or organ dysfunctions, such as heart disease, stroke, diabetes and hypertension, that became apparent in adulthood

DES a drug administered between the years 1948 and 1971 to prevent miscarriages is associated with a significant increase of breast cancer incidence.

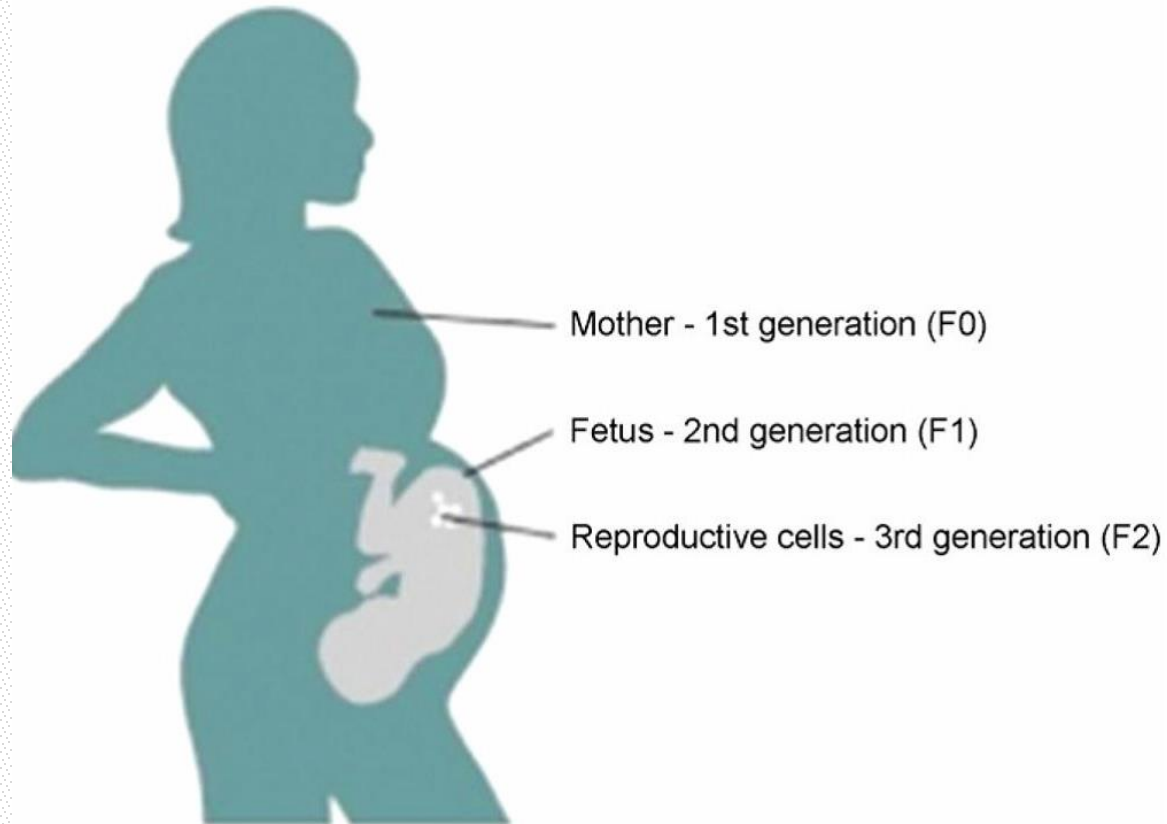
BPA can change offspring phenotype



Fetal exposure to BPA significantly increased the number of pre-neoplastic lesions in puberty and during adult life in mice



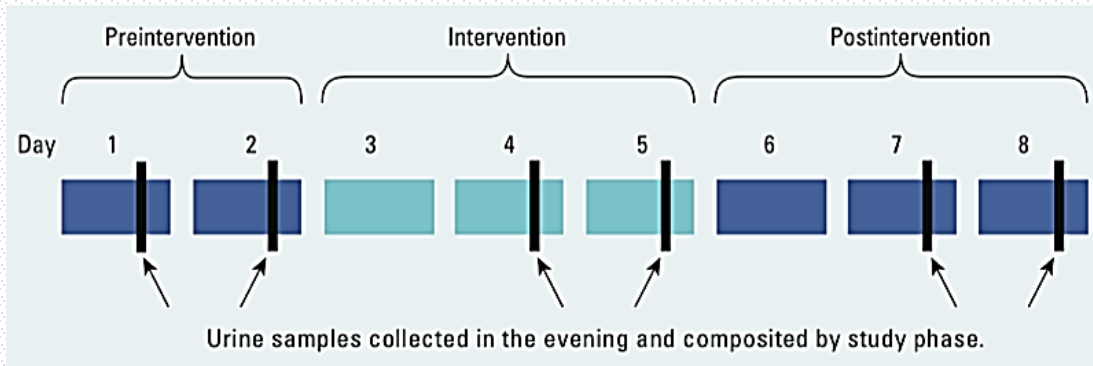
Early developmental exposure to BPA



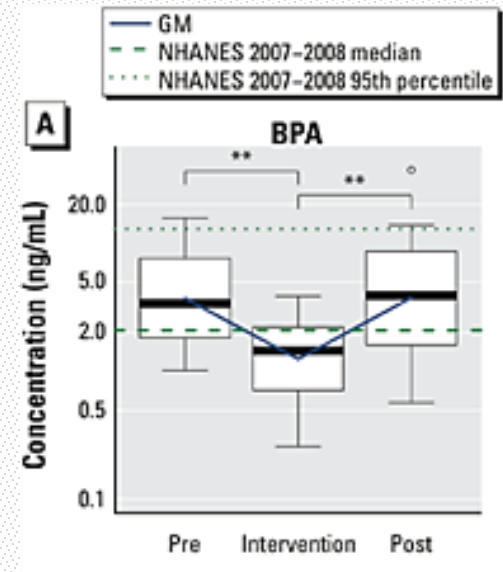
Human exposure to BPA

Ingestion of contaminated food is estimated to contribute with more than **90%** to the overall BPA environmental exposure for all age groups

Dietary intervention study

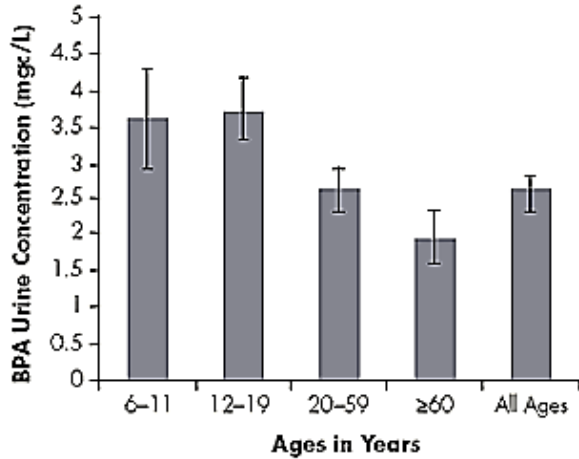


Rudel et al. 2011



The removal of packed food from diet results in a significant reduction of urine BPA levels

Human exposure to BPA



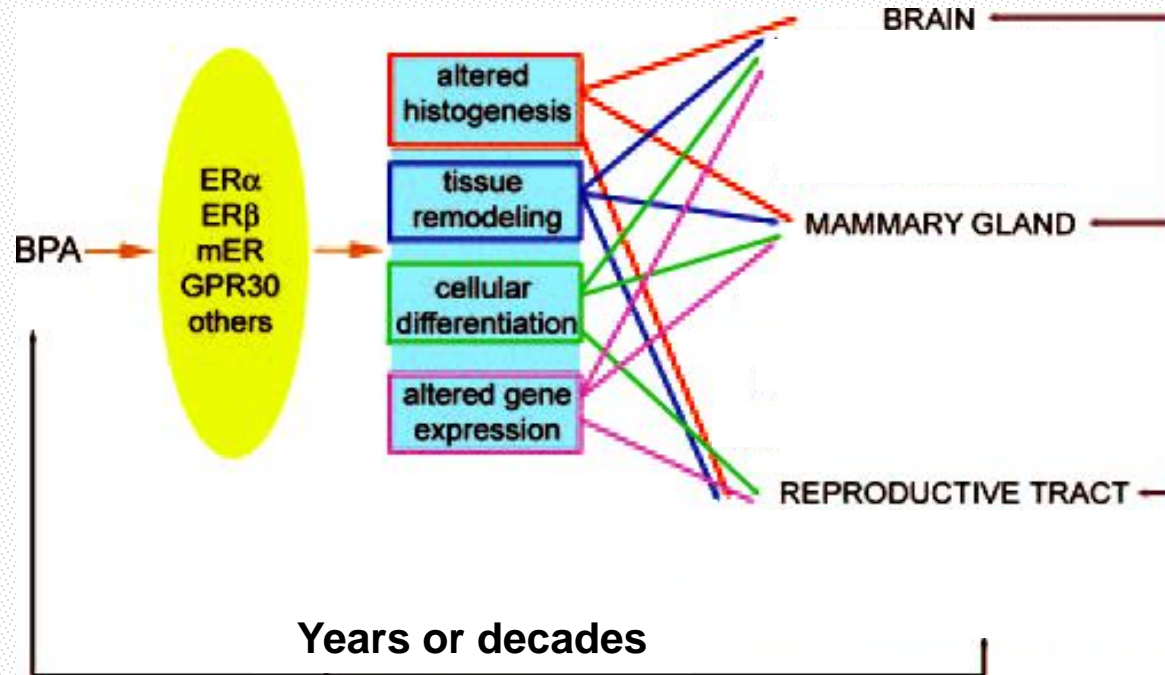
Variables	Breast-fed (n = 30)	Bottle-fed (n = 30)	P value
Age (month)	9.9 ± 3.4	9.9 ± 3.0	0.947
Body weight (kg)	8.9 ± 1.2	9.1 ± 1.1	0.584
Birth weight (kg)	3.29 ± 0.38	3.16 ± 0.39	0.219
Gestational age (week)	39.5 ± 1.0	39.1 ± 1.2	0.145
Bisphenol A (pg/mL)	45.5 ± 34.1	96.6 ± 102.4	0.014

Young-Jun Rhie *et al* 2014

2011 - European Legislation banned the use of BPA in the manufacture of baby bottles



2000-2015 -Worldwide risk assessment of BPA



Adapted from Vandenberg L. et al 2009

Human epidemiological studies

Reproductive malfunctions, Atherosclerosis related diseases, obesity ...

In vitro effects

Cell proliferation and viability, epigenetic modifications, gene transcription ...

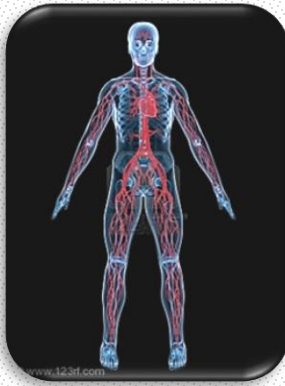
Cellular Models

Vascular system

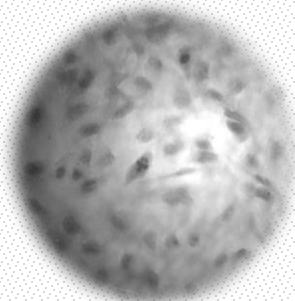
Digestive tract system

Human Umbilical Vein Endothelial Cells

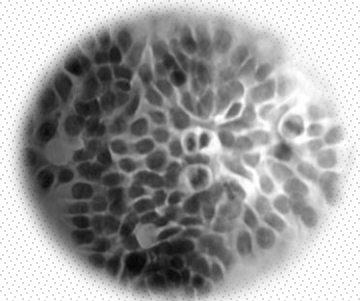
Human Colon Adenocarcinoma Cells



HUVEC



HT29



BPA “low-dose” effects - Biological changes occurring in the concentration range of typical human exposures

BPA exposure:

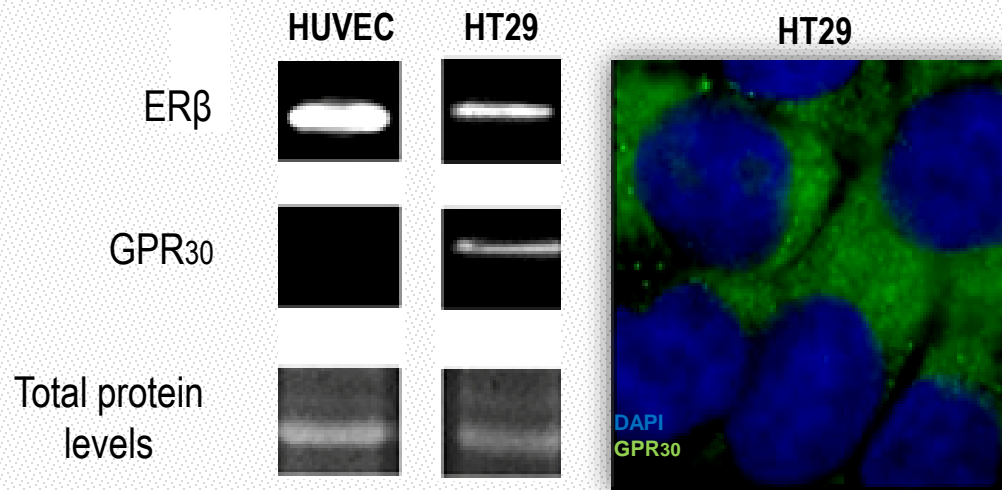
10ng/ml (44nM) (found in human biological samples in environmental exposure)

1µg/ml (4µM)(found in human biological samples in occupational exposure)

Characterization of Estrogen Receptors

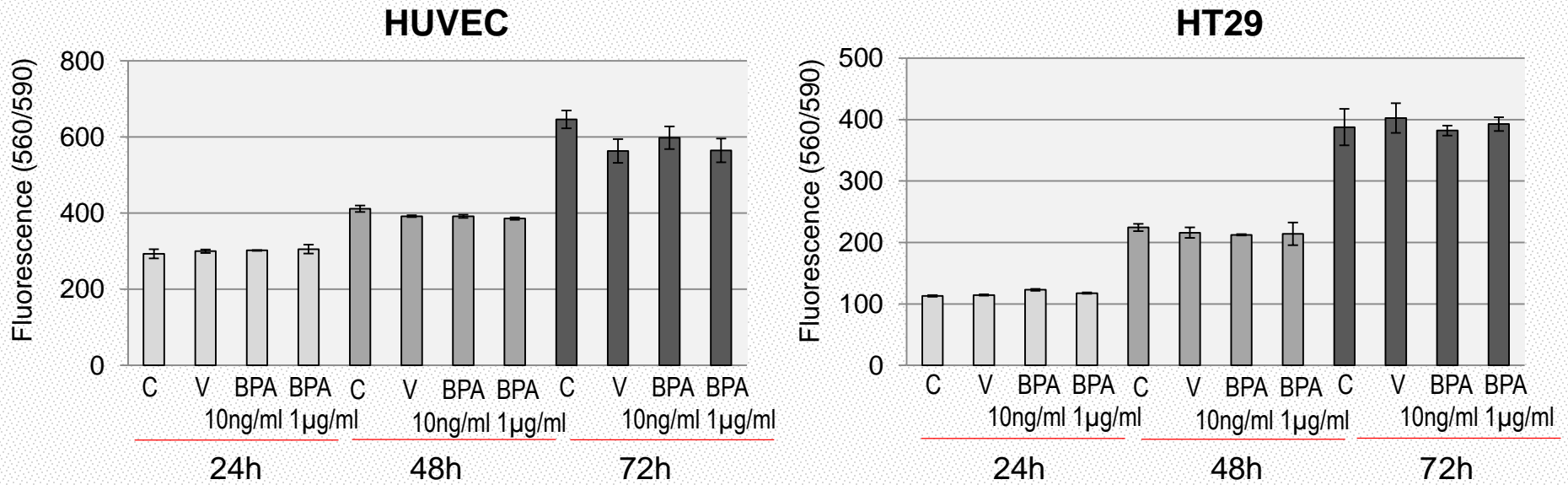
HUVEC and HT29 cell line - Negative for ER α
- Positive for ER β

HUVEC - Negative for GPR30 in culture conditions



HT29 cell line endogenously express GPR30 receptor

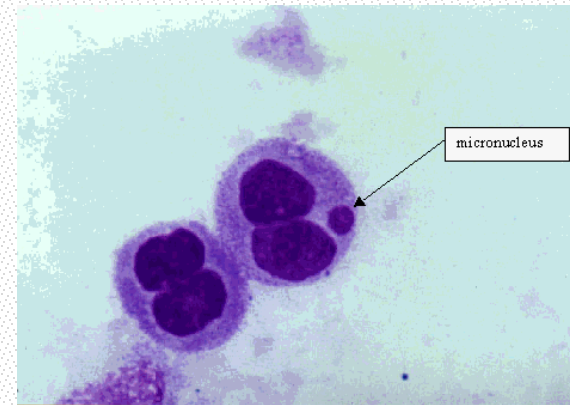
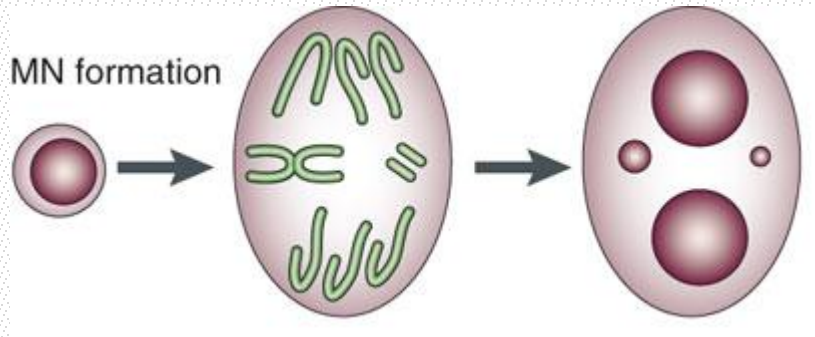
Cell viability and proliferation



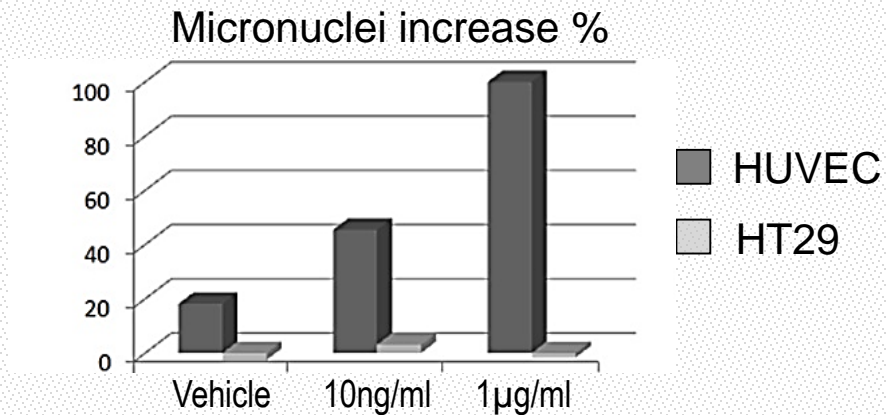
Low BPA concentrations do not affect HUVEC and HT29 cell viabilities or proliferation

Genotoxic effects

Micronuclei formation



Micronucleus formation - chromosome segregation errors and/or chromosome fragmentation

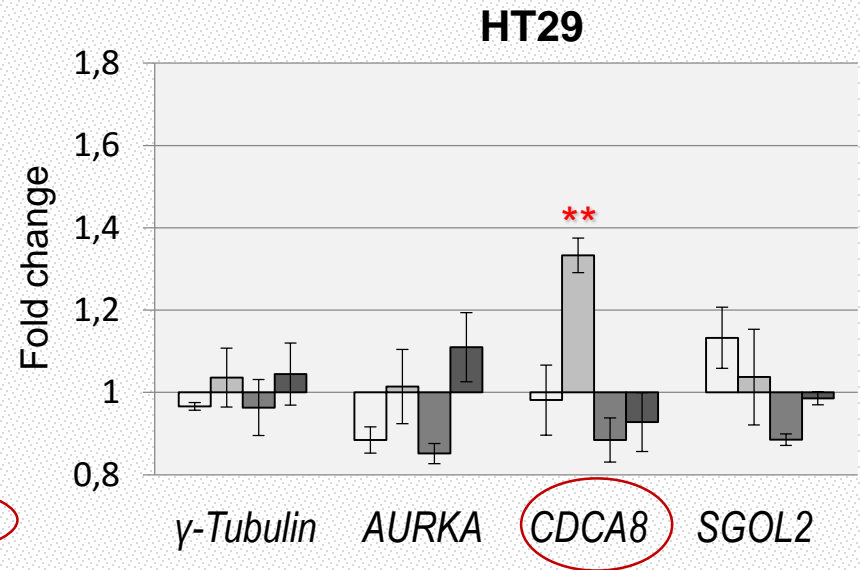
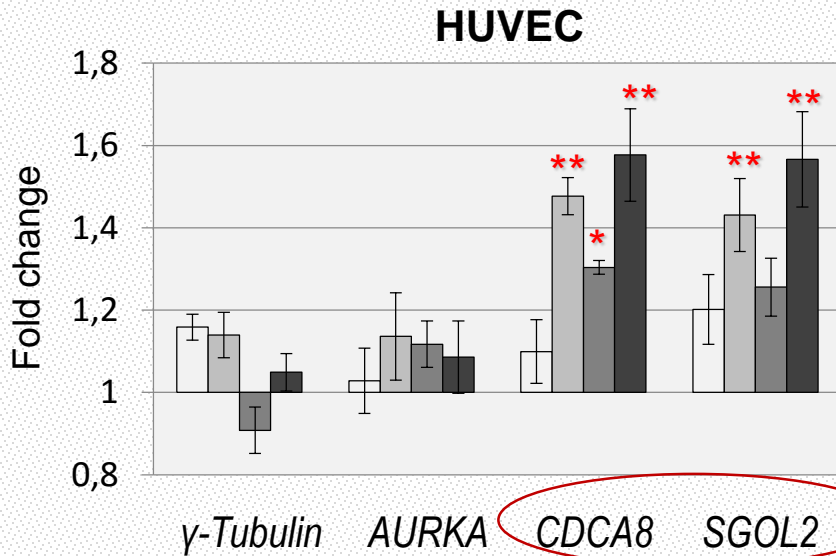


BPA exposure induces micronuclei formation in a dose dependent manner exclusively on HUVEC after 72h of exposure

Transcriptional effects on chromosome segregation related genes

***γ*-tubulin and Aurora A**
Centrosome components

CDCA8* and *SGOL2
Chromatid segregation

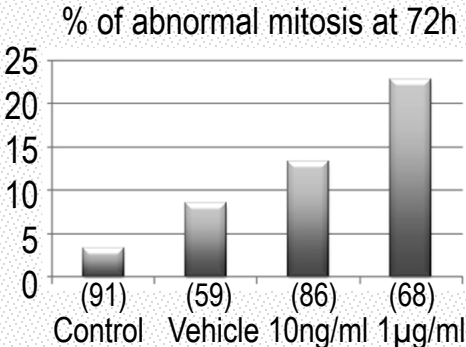
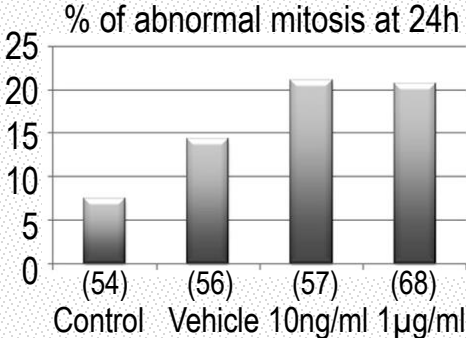
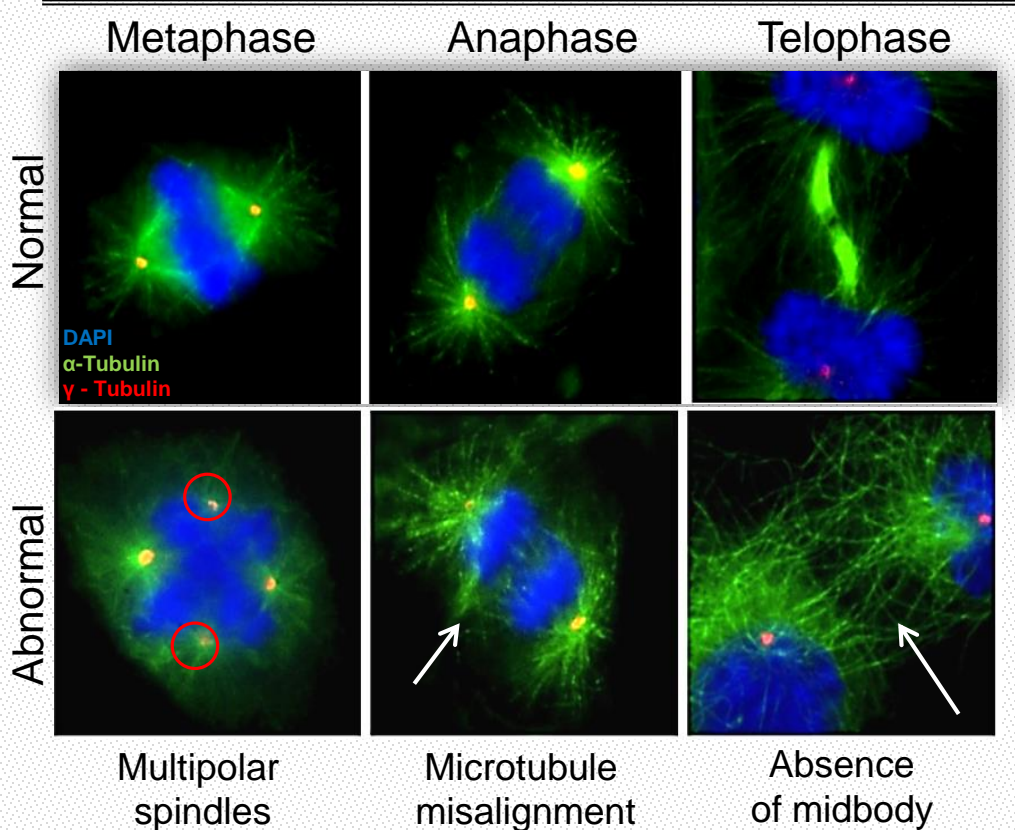


□ 10ng/ml 24h □ 1µg/ml 24h □ 10ng/ml 72h □ 1µg/ml 72h

BPA effects are cell dependent with more pronounced effects on HUVEC

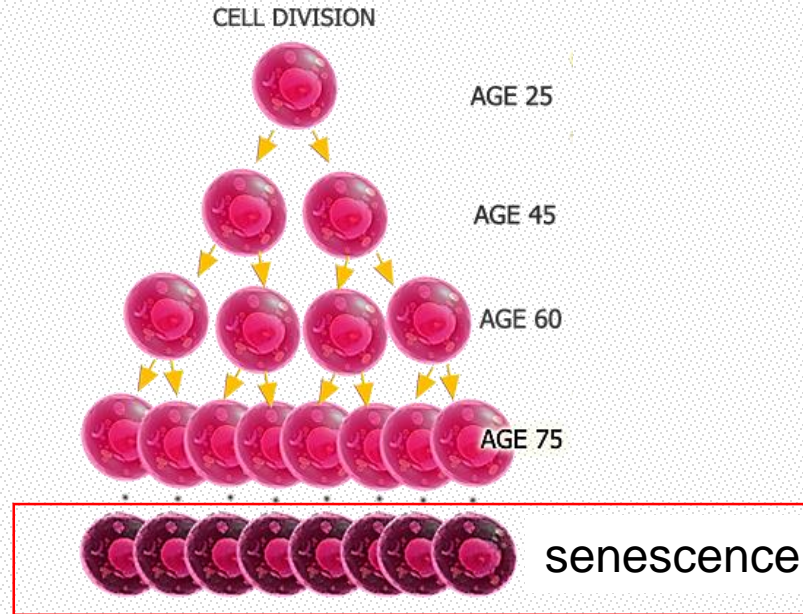
Mitotic stability and spindle organization

HUVEC



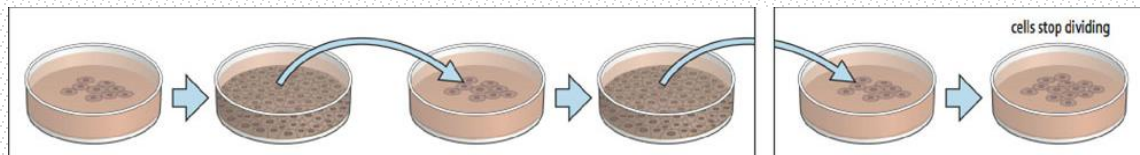
BPA disrupts mitotic microtubules (MT) cytoskeleton with cumulative effects

Cellular Senescence and Aging



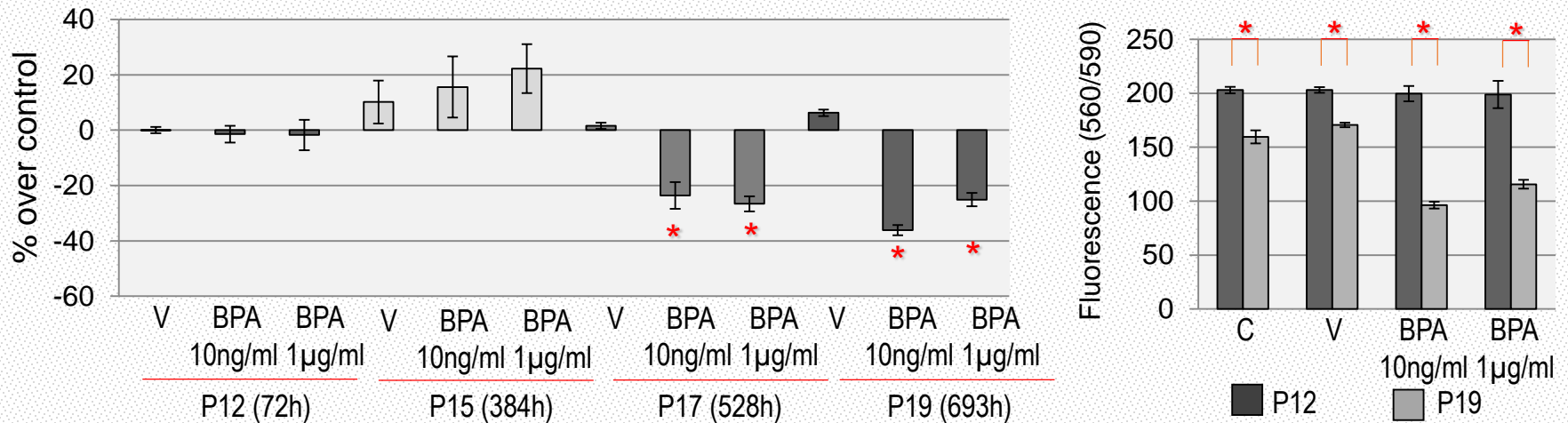
High urinary BPA concentrations have been correlated with pathogenesis of age-related diseases such as coronary and carotid atherosclerosis a form of accelerated arterial senescence

In primary cell cultures (HUVEC) after a number of population doublings actively dividing cell numbers decrease as cells enter replicative senescence



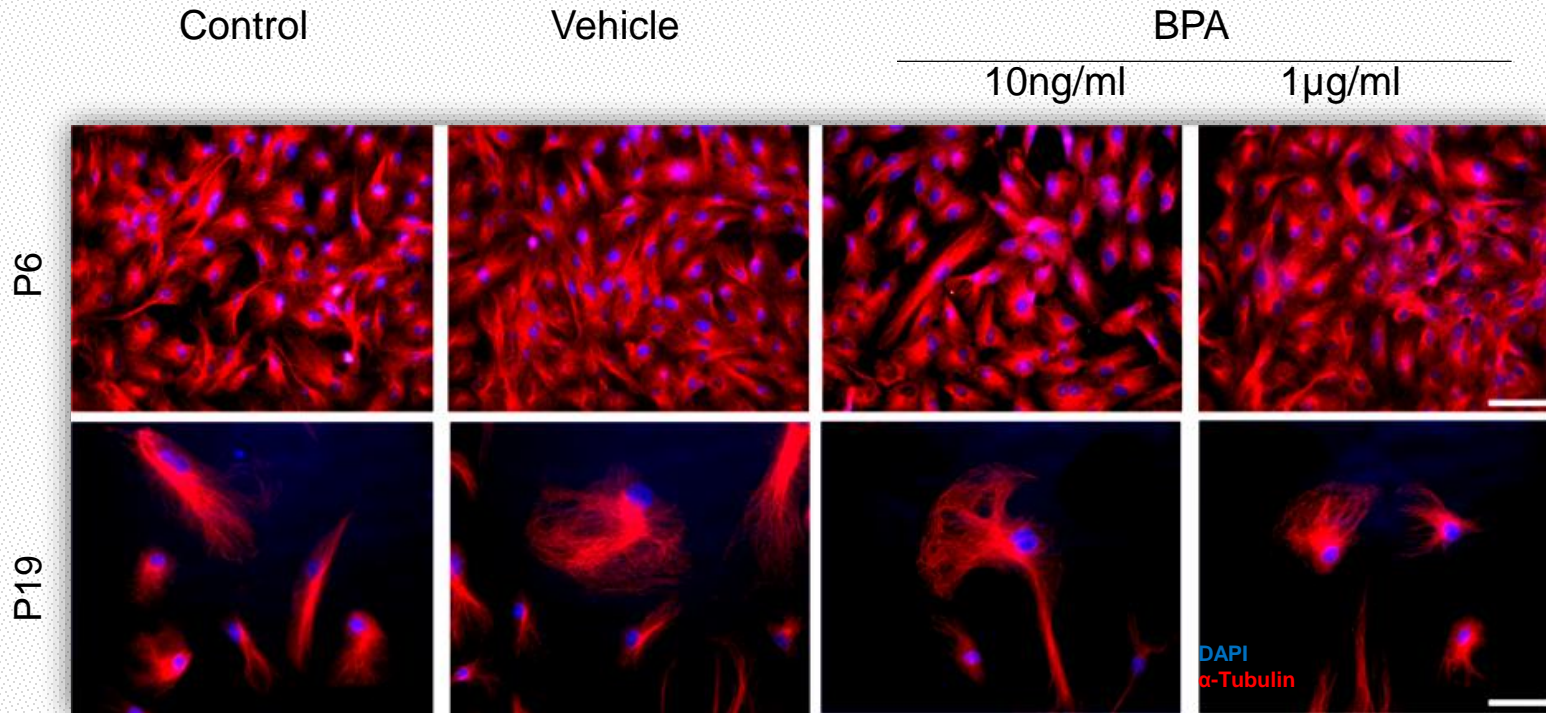
Prolonged exposure effects on aging cells

HUVEC senescent cells viability with continuous BPA exposure



BPA prolonged exposures severely decreases HUVEC viability

Prolonged exposure effects on aging cells



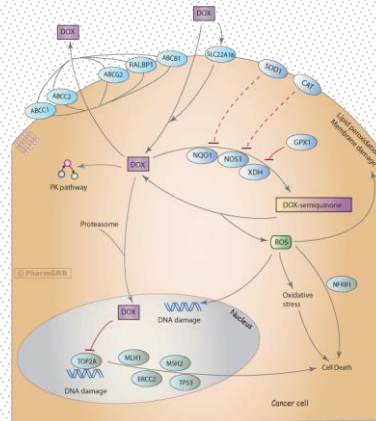
Senescent cells show augmented surface area, enlarged nuclear size, stellate outlines and microtubules enrichment

BPA did no affect cytoskeleton organization on aging HUVEC

BPA interactions with an antineoplastic drug

Cancer treatments combine chemicals that act by different mechanisms to improve efficacy

Doxorubicin (DOX)



www.pharmgkb.org/views/pathway/PA165292163.png

Chemoresistance

Combine effects of DOX and BPA

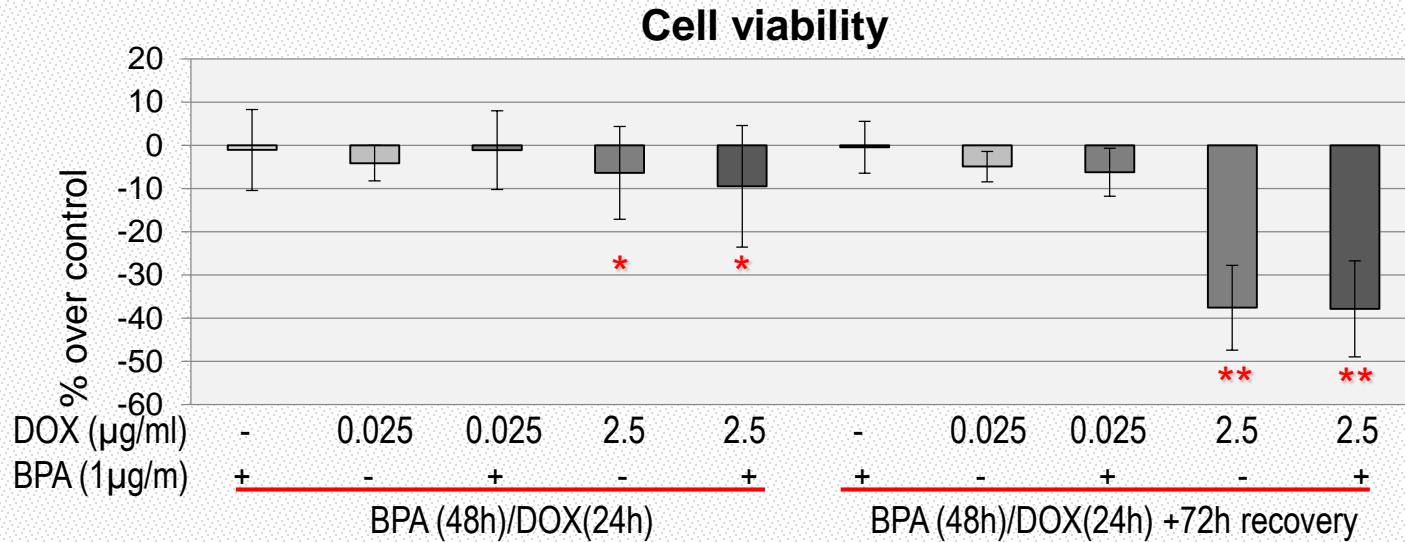


In T47D and MDA-MB-468 cells
10ng/ml BPA confers chemoresistance to DOX



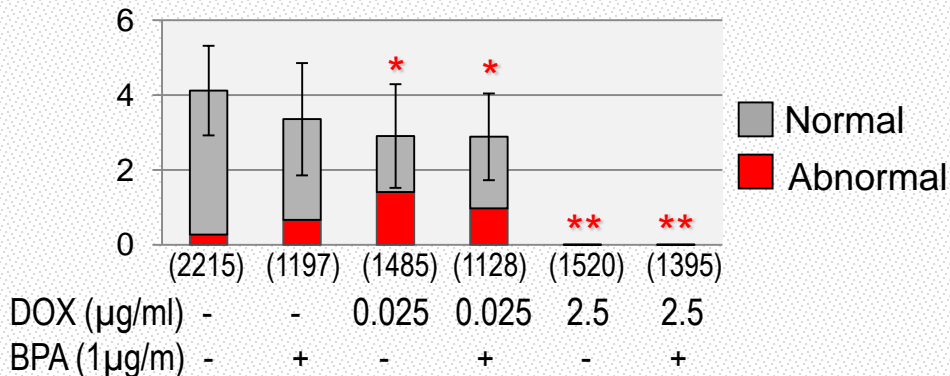
Associated to increased expression of antiapoptotic proteins
Independent of ER α or ER β receptors

BPA interactions with Doxorubicin (DOX)

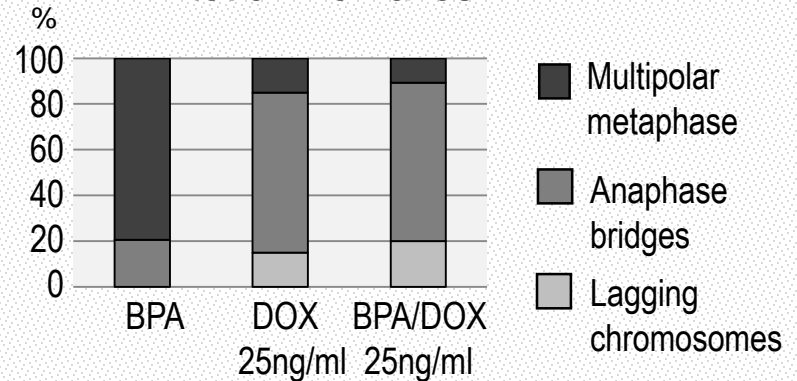


DOX effects on cell viability are not altered by BPA co-exposure

Mitotic Index



Mitotic Anomalies



BPA does not affect mitotic disruption induced by DOX sub-therapeutic dose

BPA interactions with Doxorubicin sub-therapeutic dose

Transcriptional effects on cancer related genes

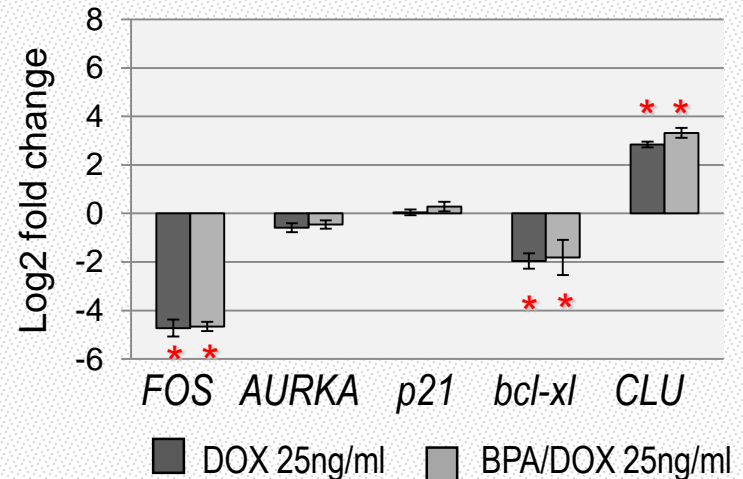
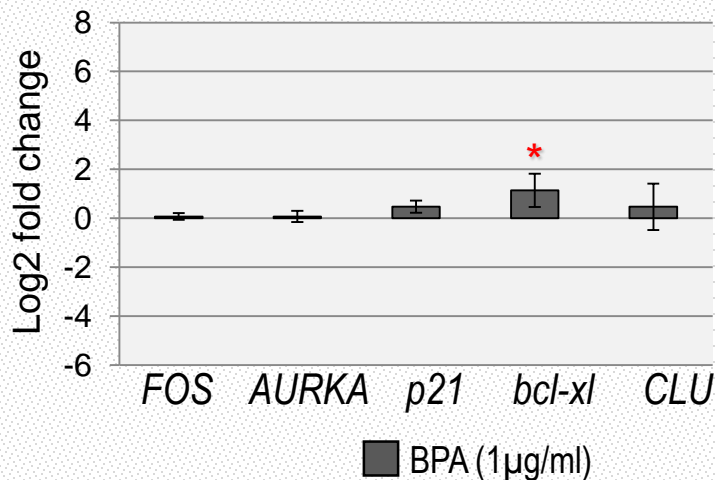
FOS - AP-1 transcription factor required for accurate regulation of several genes associated with cell proliferation, differentiation, apoptosis and oncogenic transformation

AURKA - a mitotic key regulatory protein associated to cancer aggressiveness

p21 - cyclin-dependent kinase inhibitor tightly connected with cell cycle progression and cancer outcome

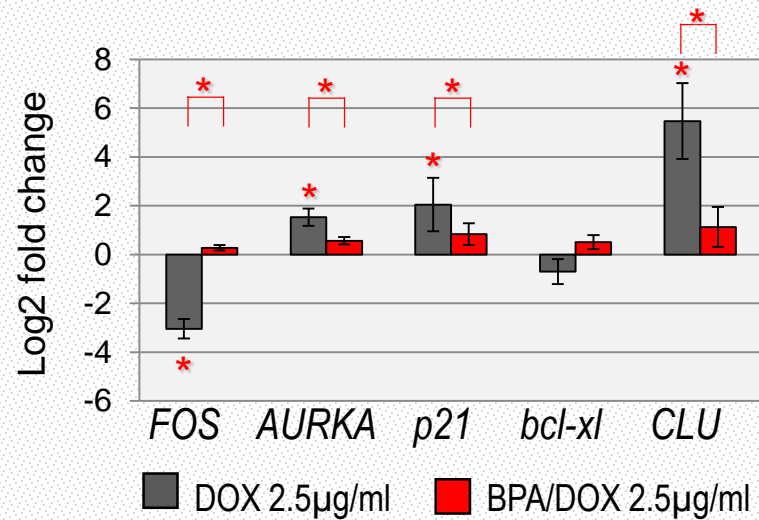
bcl-xL – Anti-apoptotic, pro-survival

CLU – Multifunctional protein that has been implicated both in promotion of carcinogenesis as well as in tumor suppression

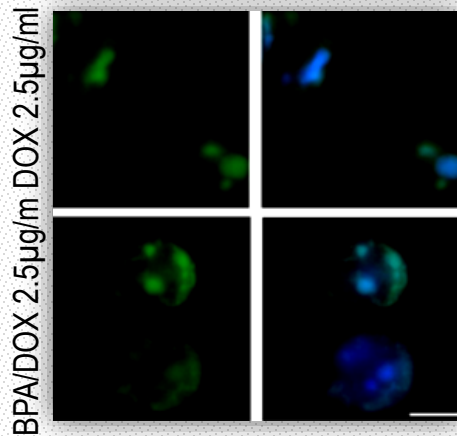


BPA interactions with Doxorubicin therapeutic dose

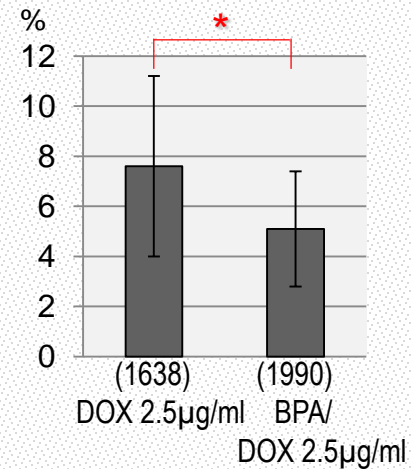
Transcriptional effects on cancer related genes



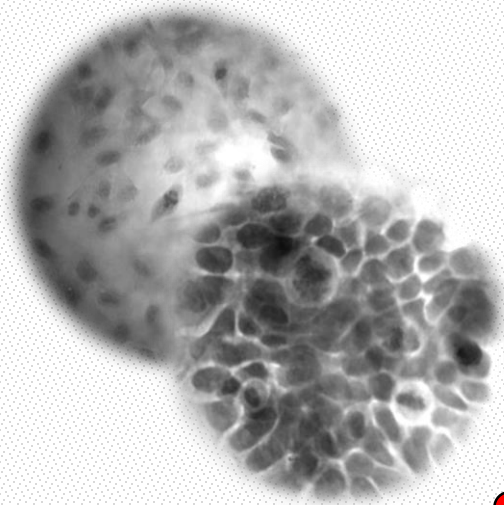
TUNEL



Apoptotic bodies



BPA co-exposure antagonises the effects of DOX therapeutic concentration



BPA?

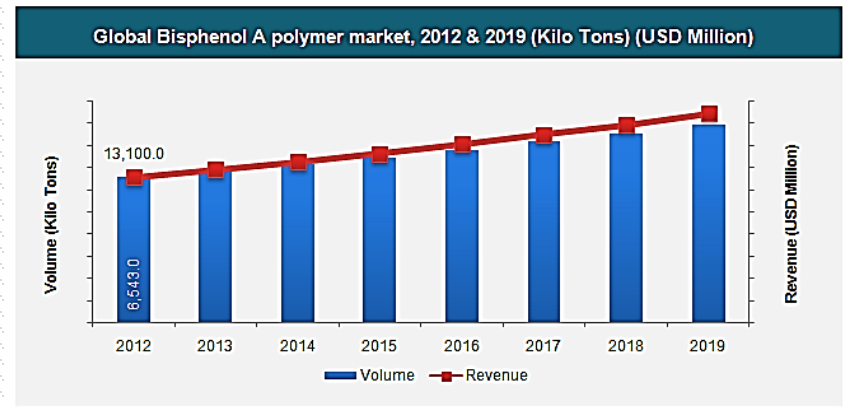
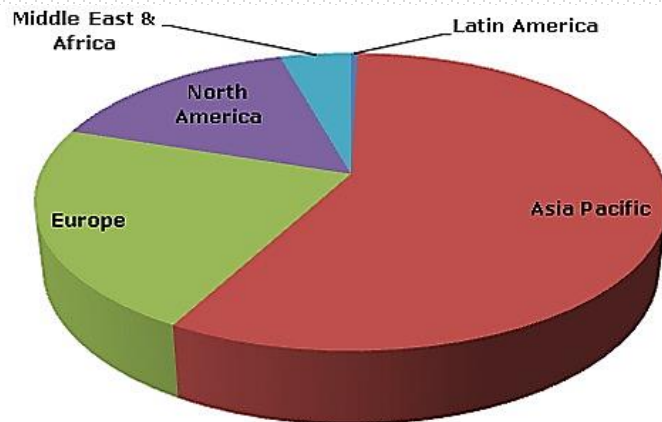


- BPA effects are cell specific
- BPA can interfere with cell aging process
- BPA is able to interact with the effect of other chemicals

Importance of BPA risk assessment in the context of aging related diseases and chemotherapy

Occupational exposure to BPA

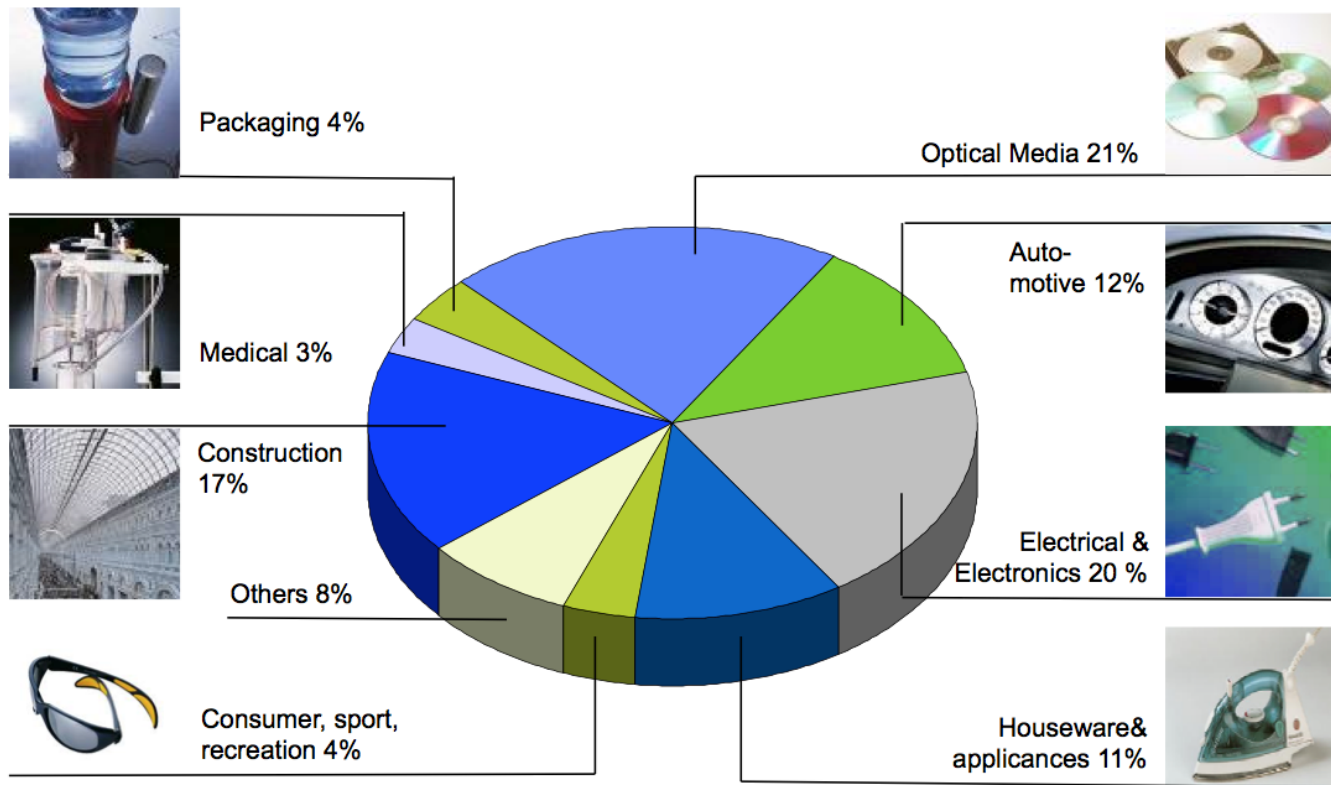
Asia is the dominant BPA manufacturer with nearly 53% share of the overall production volume, which is equal to slightly over 2.4 million tonnes. It is followed by Europe (25%) and North America (18%).



Source: ICIS, Dow Chemicals, PlasticsEurope, Primary Interviews, Transparency Market Research

Occupational exposure to BPA

Global polycarbonate demand 2010:
approx. 3.4 million tons



Occupational exposure to BPA



Nasal cavity
(63.37%)

Trachea-bronchus
(5.93%)



Alveolus
(30.7%)

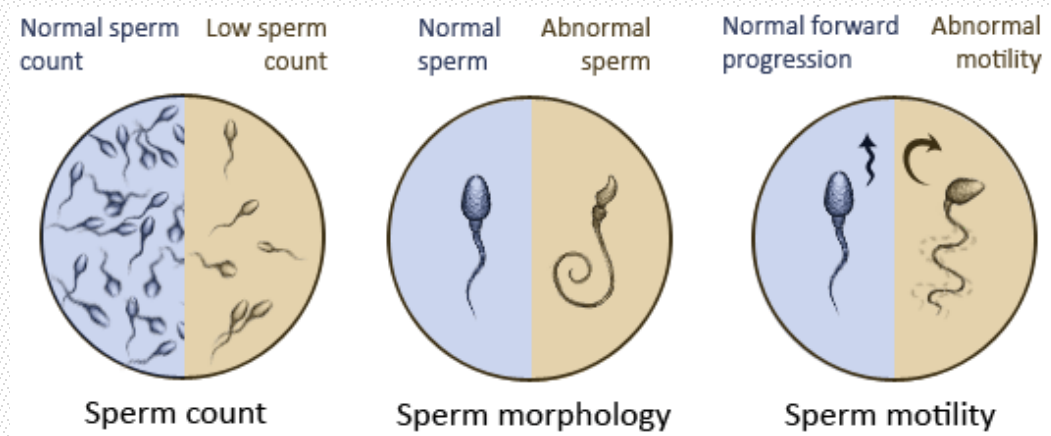
Occupational exposure to BPA



Occupational exposure to BPA: Effects

BPA-exposed male workers

Decreased semen quality and male fertility



Alterations of relevant laboratory parameters that may contribute to male infertility: **prolactin, estradiol, sex hormone-binding globulin (SHBG) and androstenedione (AD)**

Occupational exposure to BPA: Effects

BPA-exposed female workers

Alterations of reproductive hormones:

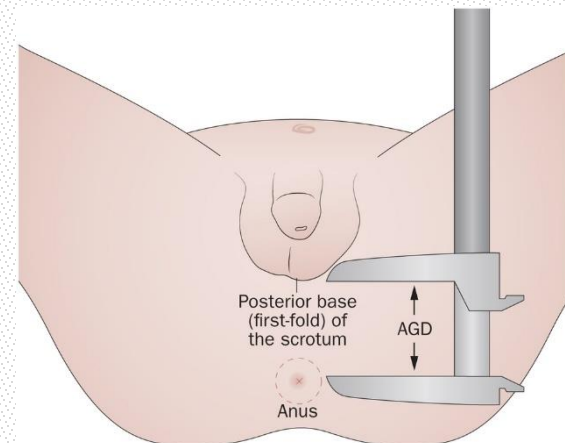
Increased prolactin, progesterone and estradiol levels

BPA exposure in the workplace during pregnancy

Decreased birth weight of offspring



Shortened anogenital distance in male offspring

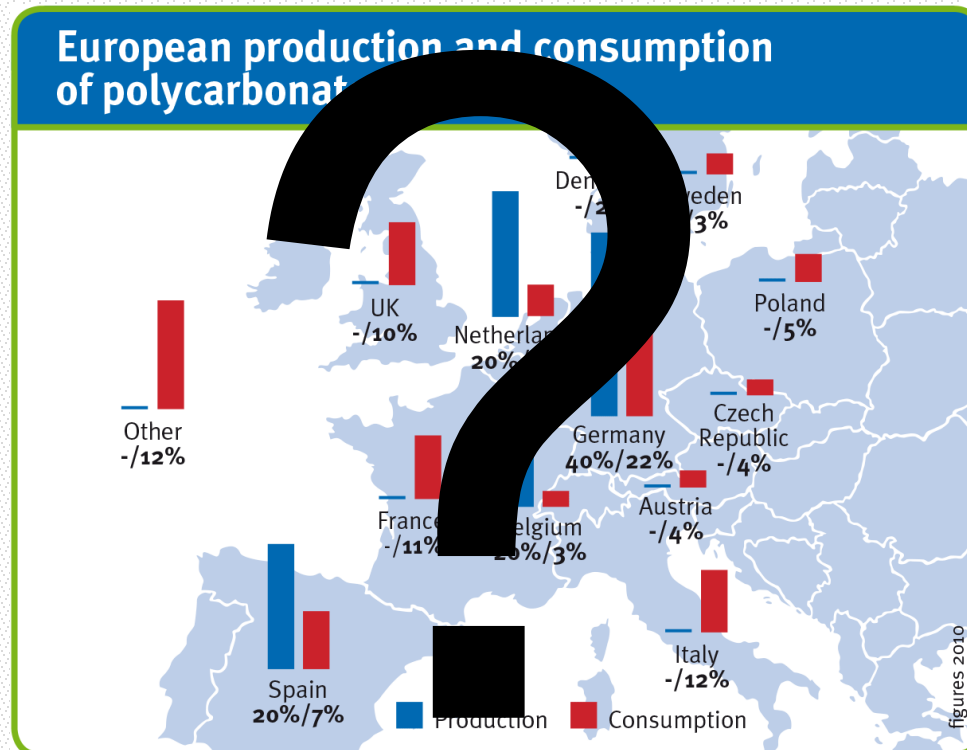


Occupational exposure to BPA: Effects in Europe?

1.45 million people work in about 60,000 PVC companies

2.3 million jobs in Epoxy-based coating technology

110,000 jobs in Epoxy processing machinery





**Importance of BPA risk assessment in the context of
environmental and occupational exposures**

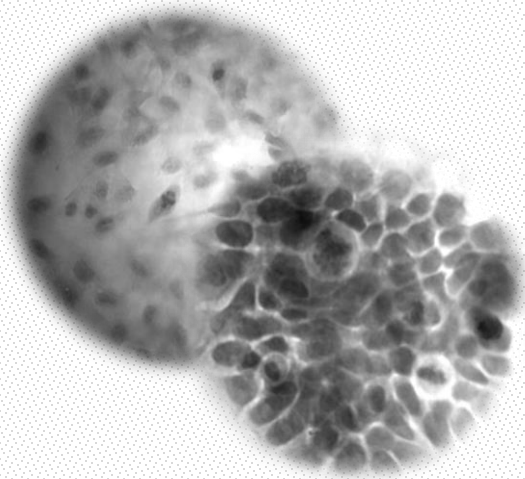


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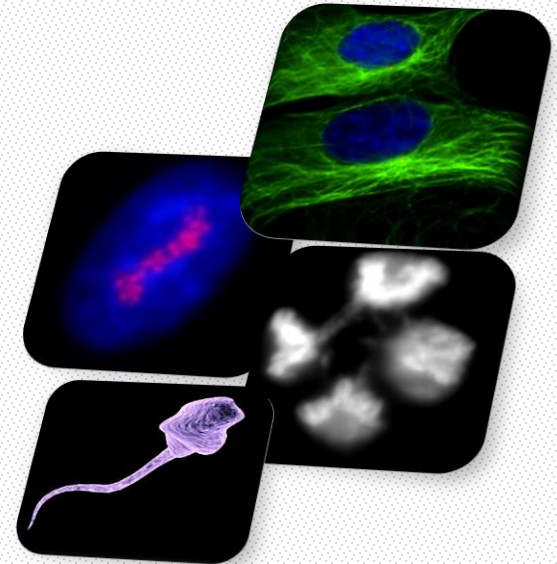
35
anos



The paradigmatic Bisphenol A



BPA?



Edna Ribeiro Varandas PhD.