Endocrine Disrupters

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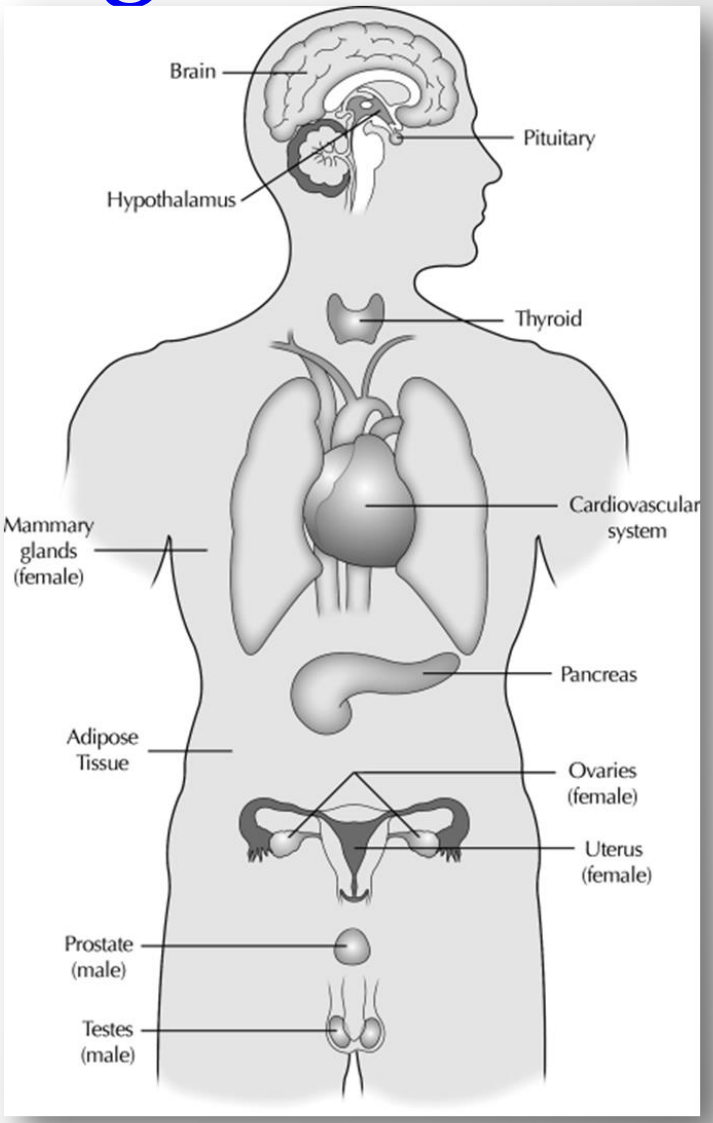
Outline of the talk

- Endocrine disrupters (EDs)
  - Sources,
  - Exposures
  - Mechanisms
- Endocrine disrupters (EDs)
  - Effects

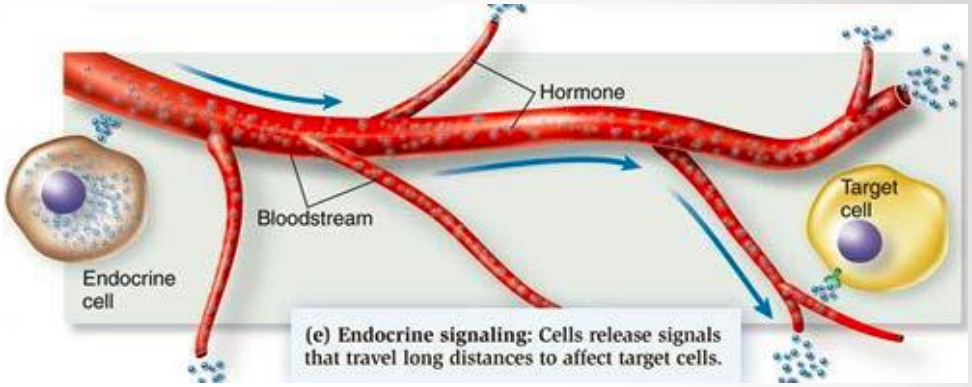
The Endocrine systems are targets of endocrine disrupting chemicals

- The endocrine system consists of hormone producing glands.
- The hormones are released into the bloodstream and are involved in basic body functions:
- Growth, metabolism, reproduction, sexual development and behavior

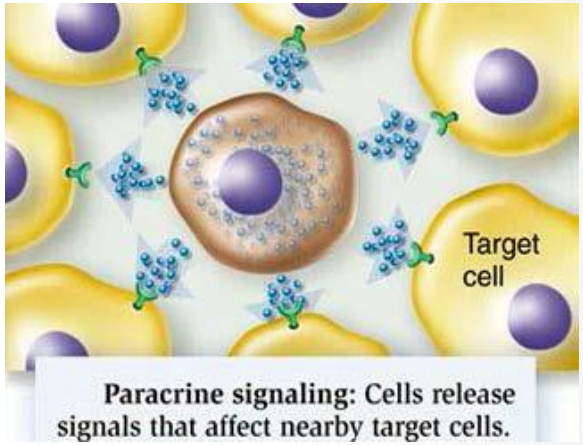


From: Endocr Rev. Jun 2009; 30(4): 293–342. doi: 10.1210/er.2009-0002

Signal pathways between cells

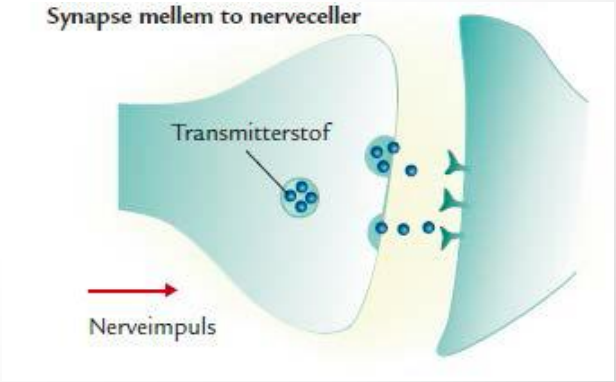


- Endocrine signaling: hormones produced by the hormone glands travel long distance via the bloodstream



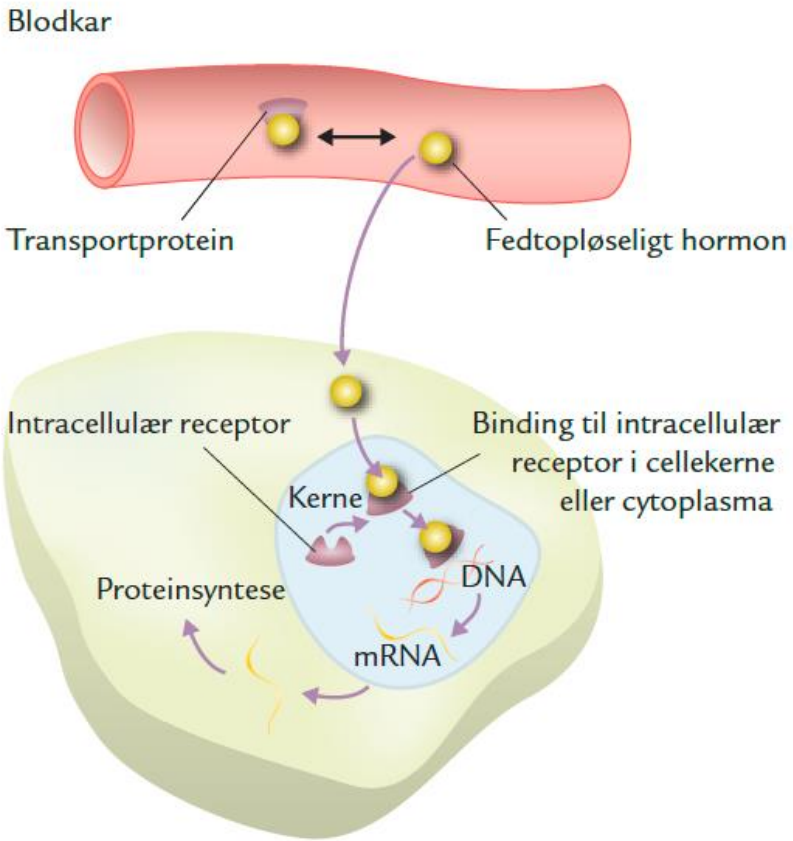
- Paracrine signaling: local, affecting nearby cells

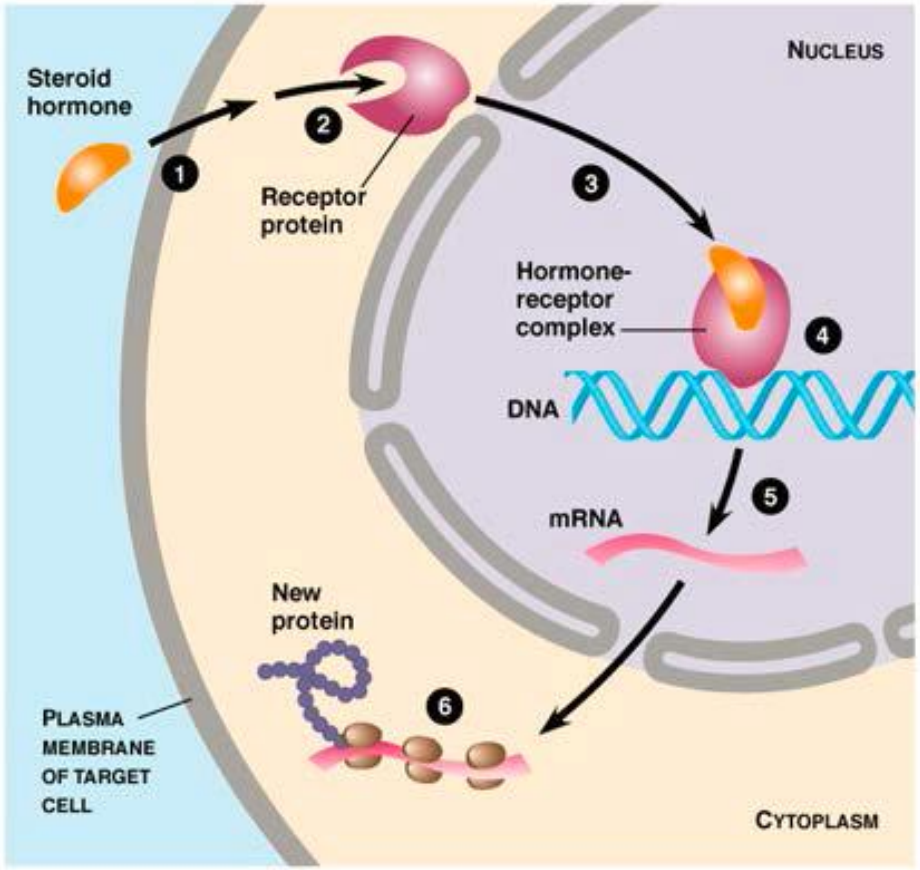
- Neuronal signaling / synaptic: via nerves, fast signals, neurotransmitter binds to receptors



Steroid Hormones

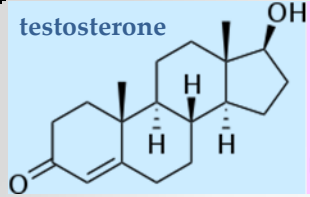
Fat-soluble hormones (steroids) binds to transport proteins and are transported via the blood stream to the organ/tissue to act where they binds to intracellular receptors and affect the cell's protein synthesis

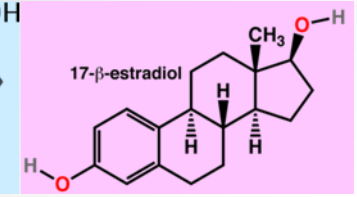


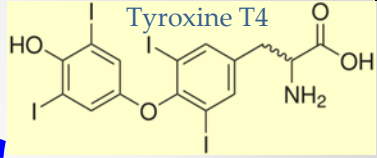


What is Endocrine Disrupting Chemicals EDCs

- Normal hormones are responsible for the maintenance of homeostasis, growth, reproduction, development and behavior
- EDCs are chemicals that can mimic and interfere with normal hormones
  - Synthesis, secretion, transport, binding, function (activity), or elimination from the body
  - thereby, block and / or interfere with hormone-related functions
- EDCs can be found everywhere, water, air, food, and dust
  - So we are exposed to them daily through our environment and food
- Several EDs are resistant to degradation in the environment and the organisms (animals- human).
- Thus they are persistent and therefore bio-accumulate in body tissues and / or adipose tissue.

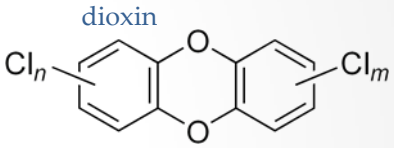


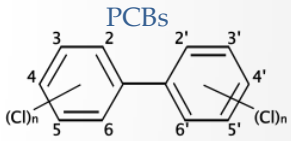




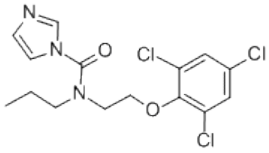
Examples of EDCs

- **Persistent** organochlor/brom/fluor compounds **(POPs)** bio-accumulate
- Primarily in fat
  - Dioxins and Polychlorinated Biphenyl's(PCBs)
  - Pesticides, organochlor pest.(DDT/DDE),
  - Brominated flame retardants
- Primarily in organs
  - Perfluorinated compounds (PFCs)

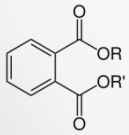




- **Non-Persistent**
  - An array of currently used pesticides
  - Plastic Components

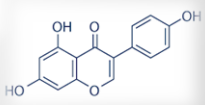


prochloraz



    - Bisfenol A (xeno-estrogen) (plastic lining in food cans, dental composite fillings, baby bottles)
    - Phthalater (xeno-androgens)(Plastic softener in e.g. PVC, cosmetics, paints, glue)
    - Octylphenols (degradation product of alkylphenol ethoxylates)
      - washing powder / cosmetics / paint / pesticides

  - Phytoestrogens (xeno-estrogen) (e.g. soy products genistein and daidzein)



Bio-accumulation up through the food chain

Lipophilic POPs:

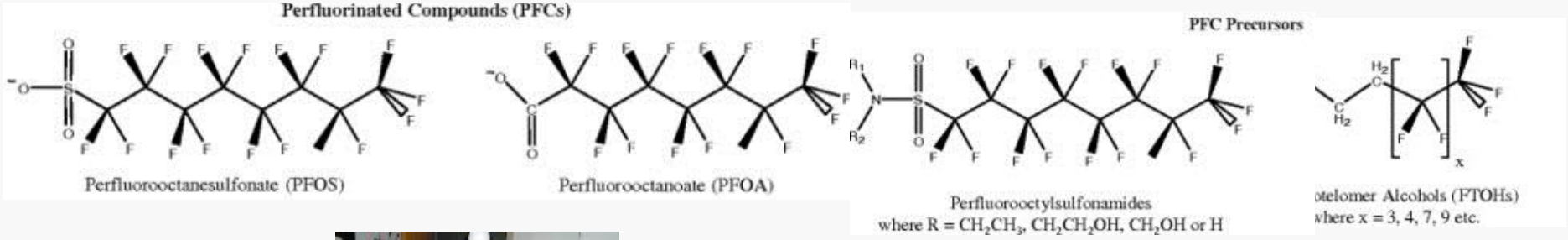
PCBs (**P**oly**C**hlorinated **B**iphenyls), dioxins **(T1/2 op til 10 år)**
Organochlor-pesticides (**OCP**) e.g. DDT/DDE (**T1/2** op til 5 år).

De ”amphiphillic” – both vater and fat repellent

Per**F**louro**a**lkyl **C**ompounds (**PFACs**) **(T1/2 4-8 år):**

Perfluoroalkylated Substances (PFAS)

Bio-accumulate in animals (T1/2: 4t-150 days) and humans (T1/2: 4-8 years)

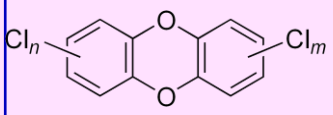
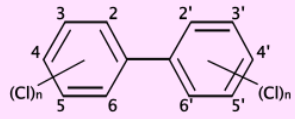
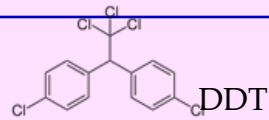
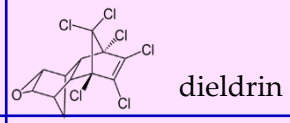
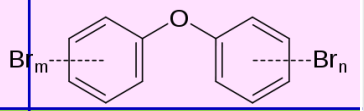


Suspected health effects of POPs / PFASs:

Studies in animals: Cancer, decreased immune defense, general organ toxicity, obesity.

Human studies: Disruption of ***i.*** fetal development e.g. CNS, ***ii.*** fertility, ***iii.*** decreased immune defense, ***iv.*** increased risk of breast cancer

Persistent Endocrine Disrupters (EDCs)

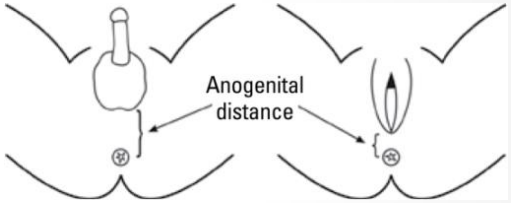
EDCs	Characteristic	Source	Effects	Structure
Dioxins fx 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)	Lipophilic Food Inhalation	By-products of combustion; paper production, PVC plastic production; preservative wood, textiles, paint, glue and others.	Immune suppressive acute toxic Affects CNS and sexual development / de-maskulination Disturbs thyroid hormone Anti-estrogen carcinogen	
PCB	Lipophilic Food Inhalation	Lubricants, coolant, transformer oil, adhesives, plasticizers, sealants	Reduced IQ (learning) Immune suppressive Reduced birth weight (Anti)-estrogen / androgen Carcinogen	
Organochlor pesticides: DDT, endosulfan, dieldrin, endrin, aldrin, mirex, heptachlor, kepone, dicofol, chlordane, pentachlorophenol	Lipophilic (both /and) Food Inhalation Skin	Insecticides and fungicides: Agriculture, horticulture, wood products, etc.	Reproductive and Developmental Disorder Acute toxic Diabetes Carcinogen	 DDT  dieldrin
Brominated Flame retardant	Lipophilic Food Inhalation	Electronics, plastics, textiles, furniture	Reproductive and Developmental Disorder	
Perfluorinated Compounds	Amphiphilic: Organs Water, dust, food, textiles	Impregnating products: textiles, shoes, pots and pans, food packaging etc.	Decreased fertility Reduced birth weight Immune suppressive Carcinogen	
Metaller: arsenic, cadmium, lead, mercury	Cd Food, Smoking	Industry / mining, electronics / agriculture / Waste Incineration	Neurotoxic, Reproductive Disorders Neurotoxic / Reduced IQ	As, Cd, Pb, Hg

Important differences between normal hormones and endocrine disruptors

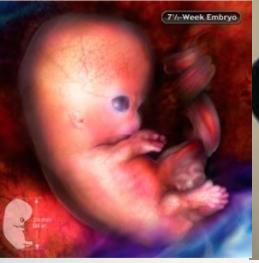
- Normal sex steroid hormones
  - is bound to the steroid hormone binding globulin (SHBG) in an inert stage
- Environmental xeno-estrogens e.g. POPs
  - accumulate in the food chain and tissue / fatty tissue of humans and animals
  - since they generally do not bind to SHBG they achieve a higher bio-available concentration
- Phytoestrogens are
  - rapidly degraded, have often anti-estrogen effect, can induces SHBG production and thus improve hormone homeostasis

In Utero Exposure to Dioxins and Dioxin-like Compounds and Anogenital Distance (AGD) in Newborns and Infants

- **Aim:** Assessment whether *in utero* exposure to dioxins and dioxin-like compounds adversely influences AGD in newborns and young children (median age, 16 months; range, 1–31 months).
- **Method:** Measurement of the AGD (AGD; anus to upper penis / back vagina) among participants of the "Rhea" mother–child cohort study in Crete and Barcelona. Plasma dioxin-like activity in maternal blood at delivery



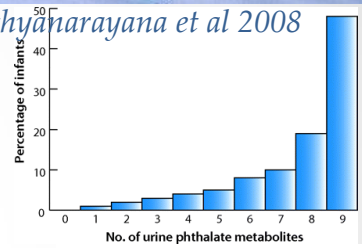
- **Results**
  - AGD distances were sexually dimorphic, being longer in males than females.
  - Plasma dioxin-like activity was negatively associated with AGD in male newborns.
  - The estimated adjusted change in AGD per 10 pg CALUX®–toxic equivalent/g lipid increase was –0.44 mm (95% CI: –0.80, –0.08).
  - Negative non-significant associations were observed for AGD in young boys.
  - No associations were found in girls.
- **Conclusion: Male infants may be susceptible to endocrine-disrupting effects of dioxins** (Marina Vafeiadi et al. 2013)



Plastic Materials / phthalates

- Drinking water and dust
- Contaminated foodstuff
- Inhalation
- Intravenous tubing equipment used for newborns / children
- Toys, for example. vinyl products
- Personal care products
- Medicine - pills
  - Some medical pills for oral ingestion are coated with phthalates to control pill solution
  - Phthalate esters used in bags for storage of packed red blood cells can cause exposure
  - Shown to activate the release of pro-inflammatory cytokines (Leonard et al. 2009)

Sathyanarayana et al 2008

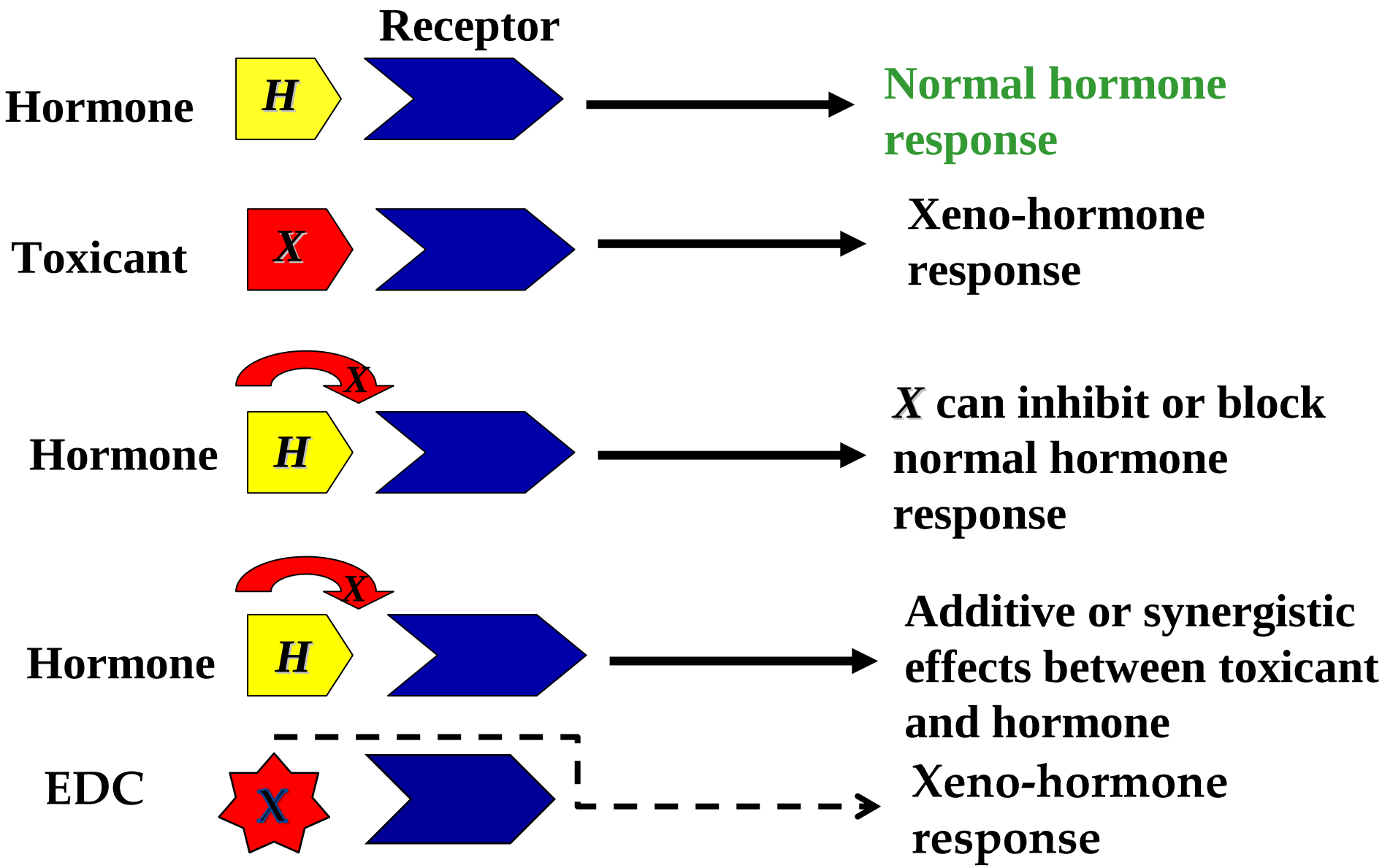


Parent	Metabolite
BBzP	MBzP, MBP
DBP	MBP, MCPP
DiBP	MiBP
DEP	MEP
DEHP	MEHP, MEHHP, MEOHP
DnOP	MCPP
DMP	MMP

Phthalates: In utero and lactation Reproductive effects in rats

- Plasticisers:
  - *Dietylhexyl- (DEHP)>, butylbenzyl- (BBP)>, dibutyl- (DBP)>, diisoninyl phthalate (DINP)*
- Dosis-related effects
  - (375-1,500mg/kg/dag per os)
- Abnormal sexual development of male rat
  - Anti-androgen
  - Reduces testosterone to female rat level
  - Decreases central nerve system development
  - Feminization
  - Hypospadias, cryptorchidism, abnormal prostate development
  - Reduced birth weight and testes weight

Effect mechanisms of xeno-hormones



The pesticide Prochloraz can down regulate the expression of ERa and ERb mRNAs and the ERa protein level

- Background: Exposure to endocrine disrupters such as dioxins, PCBs and certain pesticides are suspected to affect human reproductive health.
- Method: on-line RT-PCR quantification of the ERa and ERb mRNA levels in the human breast cancer cell line, MCF7-BUS. Western blot analyses
- Results: Exposure with E2 or prochloraz decreased ERa and ERb mRNAs after 48 h of treatment. Co-treatment with the ER antagonist ICI 182,780 abolished these mRNA down regulations.
- A decreased ER protein level after 3 h of exposure with prochloraz was found but after 24 h the ERa protein level had recovered to basal level

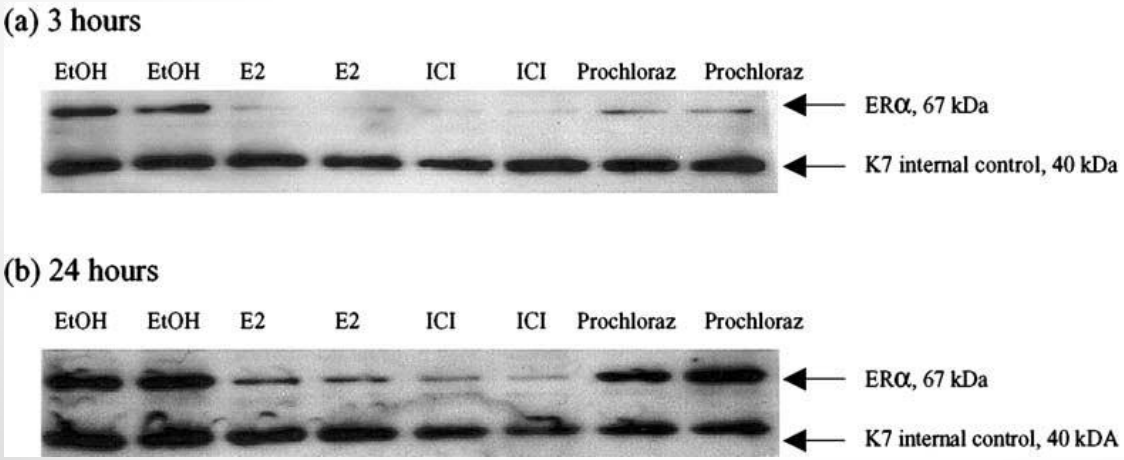
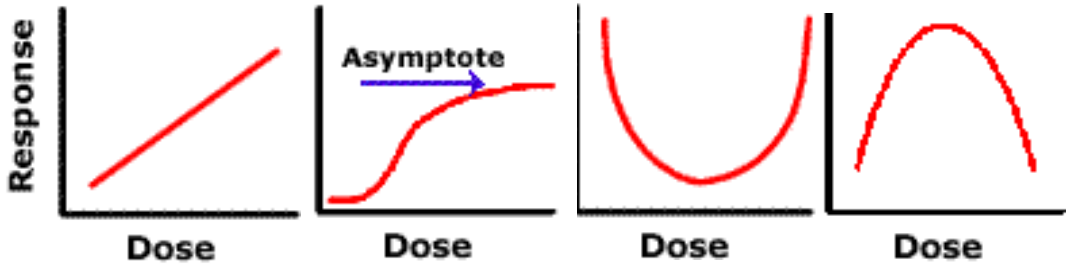


Fig.2. Western blot analyses of the ERa protein (67 kDa) level in MCF7-BUS cells after treatment with E2 (10 nM), ICI 182,780 (ICI) (100 nM) and prochloraz (10 lM). ER a protein level after exposure with chemicals for (a) 3 h and (b) 24 h. Keratin no.7 (K7) (40 kDa) was used as an internal control.

Hofmeister & Bonefeld-Jørgensen. Toxicology in Vitro 18 (2004) 427–433

Exposure to EDCs and risk assessment

- Exposure concentrations, the dose-response reactions may be non-monotonic and thereby may result in low doses is more potent than higher doses vice versa

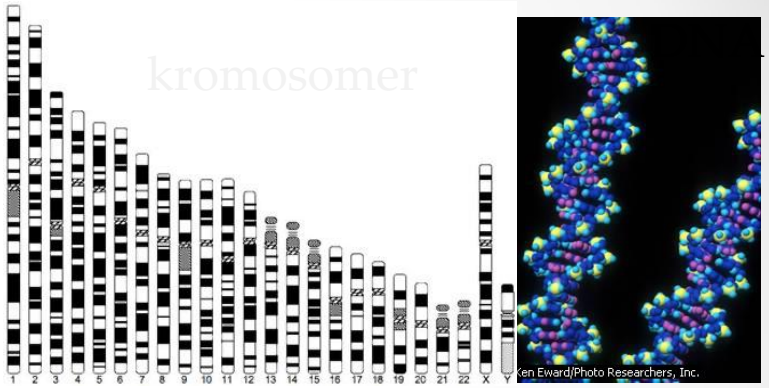


- No-adverse-effect-level (NOAEL) assumes monotonous dose-response
- Exposure to individual substances vs. complex mixtures:
  - Single compound principle in exposure are used today in risk assessment, although the illustrated cocktail effects of mixtures of many EDCs (more than additive or "synergism"
- Latency between exposure and adverse health outcomes
  - Exposure in fetal life can cause diseases / effects later in life
- Preventive strategies

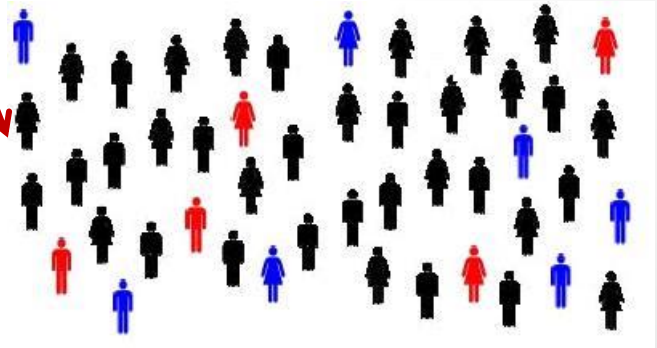
Individual sensitivity

- The genetically background

Is different between individuals

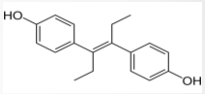


- This cause individual differences in sensitivity to exposure and disease

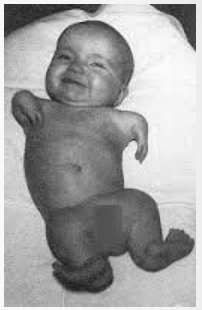


➢ Genes, age, nutrition, lifestyle

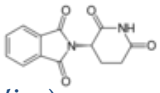
Examples of where traditional risk assessment has not worked

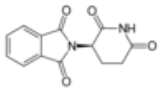


- Diethylstilbestrol (DES), *pregnant women* (1940 to 1970);
  - 1st. Breast cancer, 1st., 2nd. and 3rd endocrine disruption, decreased fertility, vagina cancer in offspring

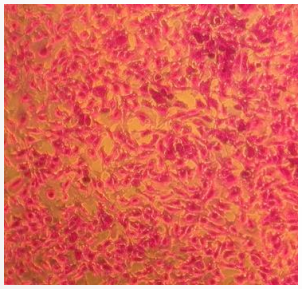


- Thalidomide, *pregnant women* (1960^{ies}; morning sickness, no effects in rat experiments)
  - structural malformations in the baby

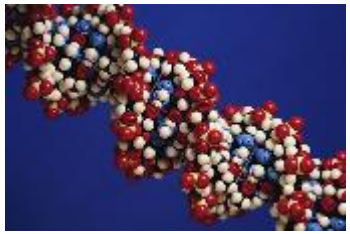




- Perfluorinated compounds (PFCs), (1950'ies)
  - Reproductive disorders, organ toxicity, carcinogenesis



Celle kultur

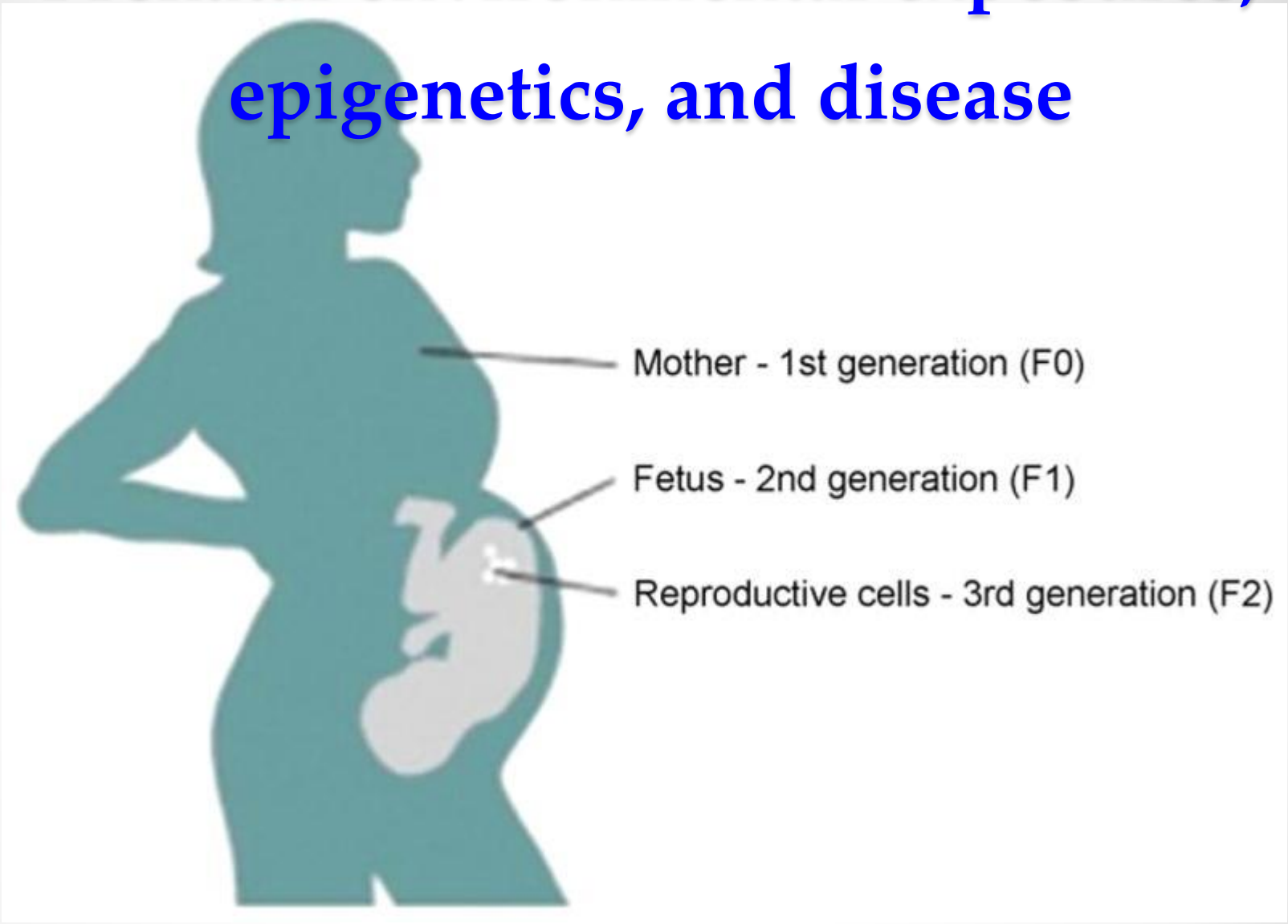


Gene og protein ekspression, oxidative stress

Rat exposure ADME

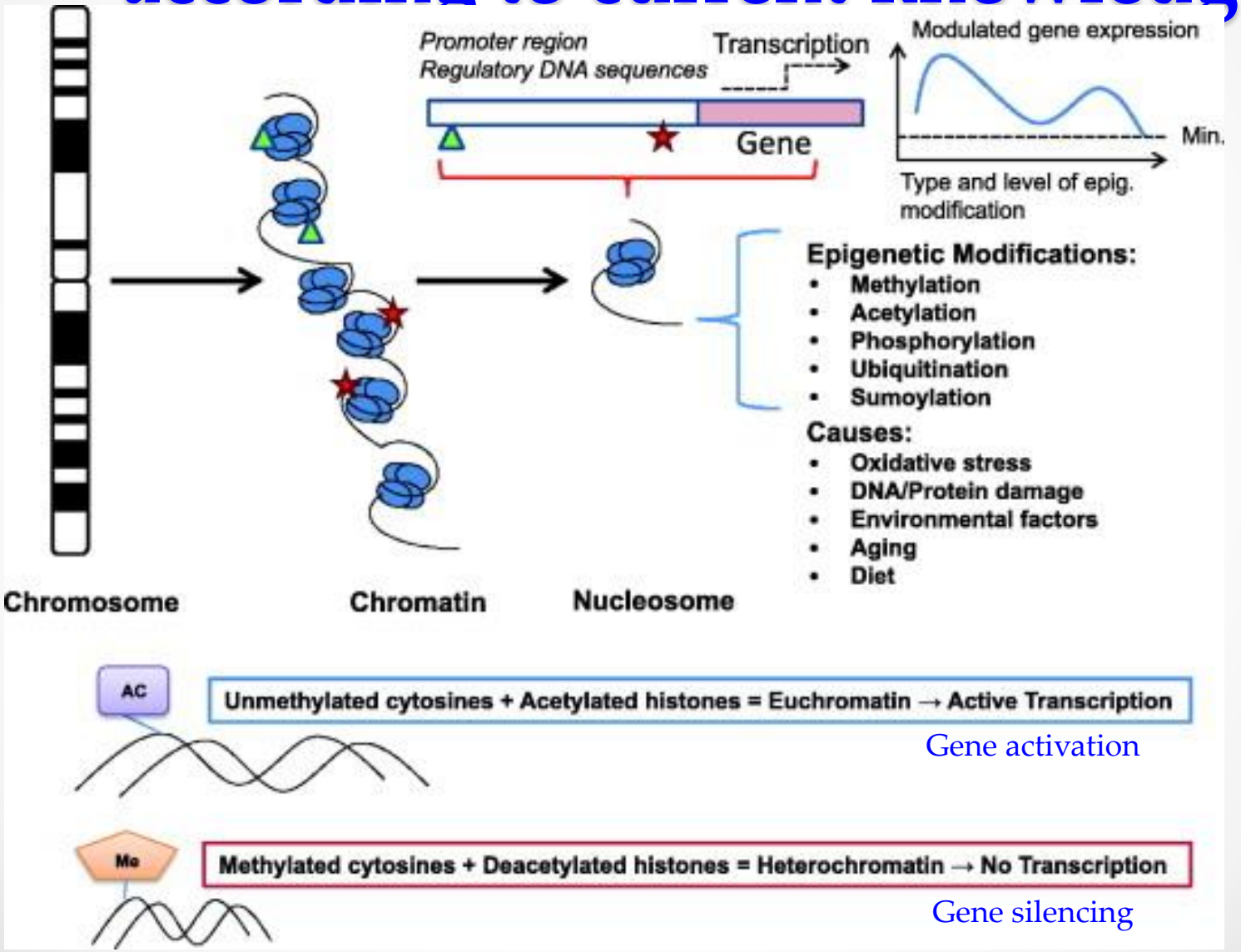
ADME: Adsorption, Distribution, Metabolism, Excretion

Prenatal environmental exposures, epigenetics, and disease

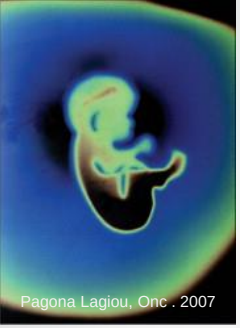


Three generations at once are exposed to the some environmental conditions (diet, toxics, hormones, etc.). In order to provide a convincing case for epigenetic inheritance, an epigenetic change must be observed in the 4th generation. Pedera and Herbstman, Reprod toxicol 2011 (31,3)

Model of epigenetic modifications according to current knowledge



Nowsheen et al. Cancer Letters, Vol.342 (2) 2014.

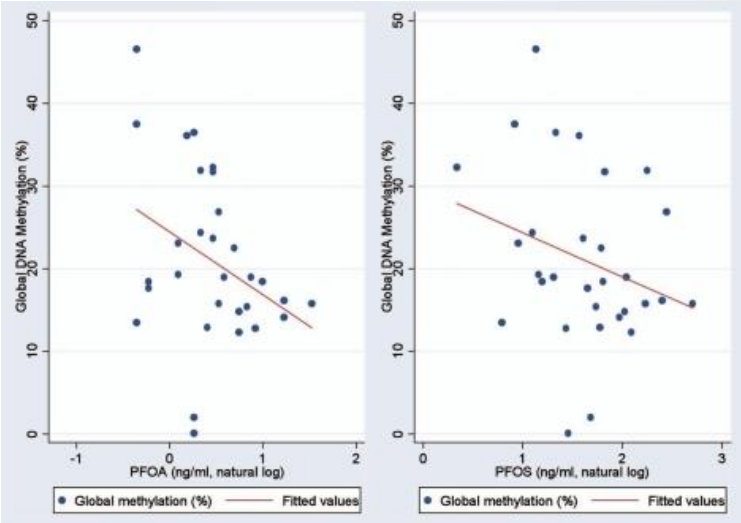


What is Genomic Imprinting?

- Genomic imprinting (GI) is an epigenetic process resulting in parent-of-origin specific mono-allelic expression
- The majority of GI genes (~80%) are linked in a cluster meant to facilitate coordinated regulation
- A single genetic / epigenetic change in an imprinting control region can consequently result in disruption of many genes
- Deregulation of GI is associated with pre-natal lethality in mice and many pathologies in humans ranging from behavioral disorders to cancer
- Epigenetic modifications are inherited at the somatic-cell division level and can be found in subsequent generations

DNA methylation, has been associated with cancer initiation and progression.
POPs decrease DNA methylation in humans

- *In utero* exposure to xeno-estrogens, assed by isolated lipPOPs from placentas, decrease global DNA methylation of repetitive elements for placentas of boys but not for girls.(Vilahur et al.2014; Env, Internal.)



- *In utero (cord blood)* exposure to PFOA, to less degree PFOS, was inversely correlated with Global DNA methylation. (Guerrero-Preston et al 2010; Epigenitics)

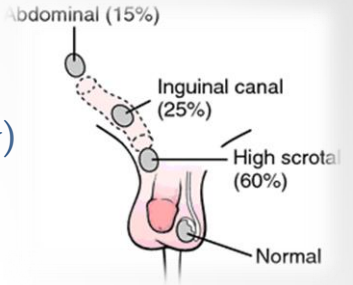
- In high exposed adult Inuit the level of serum PCB and persistent organochlor-pesticides (OC) was significantly inverse correlated to global methylation *Rusiecki et al. Environ Health Perspect* (2008).

- In low-dose exposed adult Koreans the level of serum OC pesticides and to some degree PCB and PBDE, was inverse associated with global DNA methylation.(Kim et al. *Environ Health Perspect* . 2010).

POP: persistent organic pollutants

Suspected effects of endocrine-disrupting pollutants on human health especially by exposure during fetal life

- Reproduction:
  - Reduced fertility (decreased sperm count, increased TTP (time to pregnancy)
  - Birth defects (cryptorchidism, hypospadias, changes in AGD, reduced birth weight)
  - Increased risk of testicular and breast cancer
  - Puberty and Menstrual Disorders
- Immune system defects
  - Reduced vaccination response, frequent infections
- Central nerve system
  - Thyroid hormone disruption / Decreased IQ
  - Behavioral abnormalities (Autism, ADD / ADHD, reduced learning)
- Change in metabolism (*carcinogen induction*)
- Endometriosis vs. reproduction
- Diabetes type 2?
- Parkinson desease ?



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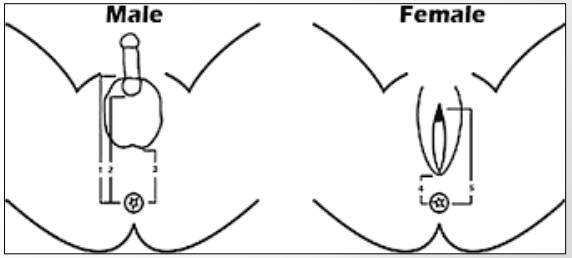

AGD: anogenital distance

Question

What is an endocrine disruptor?