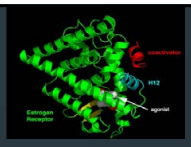


# 'Legacy' endocrine disruptors: the convergence between basic biology, (eco)toxicology and epidemiology

## *Perturbateurs endocriniens (1) : La convergence entre biologie fondamentale, (éco)toxicologie et l'épidémiologie*

**Rémy Slama**

Collège de France & Inserm

The relations between human health and the environment in the Anthropocene

Lecture #5 – 11 May 2022

COLLÈGE DE FRANCE 1530

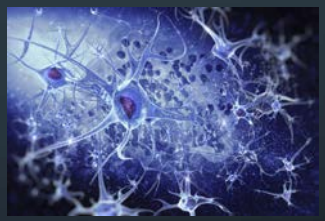
1

## Disruption of 3 essential (communication) systems of the body

Genes

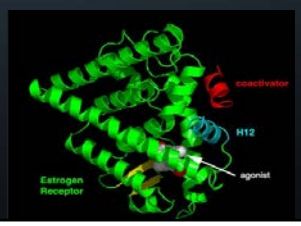
Genotoxicity

Nervous system



Neurotoxicity

Endocrine system



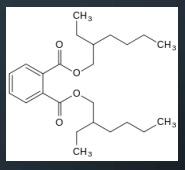

Endocrine disruption

2

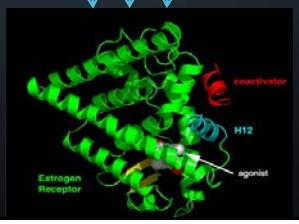
## Endocrine disruption originated at the convergence of several research streams during the 2nd half of the 20th Century

Health effects of in utero exposure to **synthetic estrogens** (Diethylstilbestrol)(1971-)
(Herbst, *NEJM*, 1971; Swan, *APMIS*, 2000)


Effects of Persistent Organic Pollutants (POPs) on wildlife (1950s-)



Effects of chemicals with **anti-androgenic** properties (e.g., **DEHP, a phthalate, and also a DDT metabolite**) on male reproductive parameters
(Skakkebaek, *Hum Reprod*, 2001; Conley, *Env Int*, 2021)




Endocrine disruption

3

## Lecture overview

Lecture #5

A. Introduction: classifying toxicants
B. Endocrine disruption – Generic considerations
C. Disrupting the estrogenic axis: DES, DDT
D. Disrupting the thyroid axis: PCBs, PFASs
E. Characterizing effects of non-persistent compounds in humans
F. Triclosan and bisphenols
G. Social inequalities in exposure
H. Health and societal impact
I. Evaluation of risk management options
J. Risk management

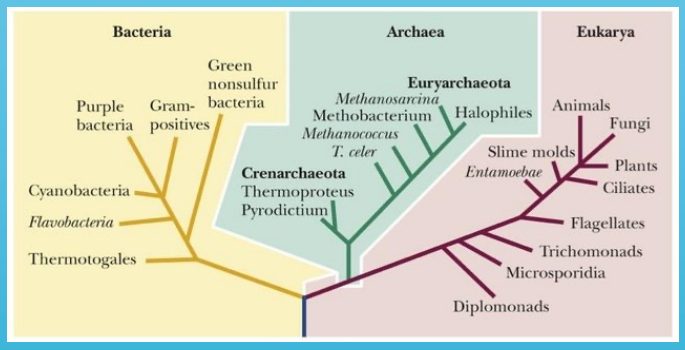
Lecture #6

Interactions with nuclear receptors *(seminar of W. Bourguet)*

Mixture effects *(seminar of Pr. A. Kortenkamp)*

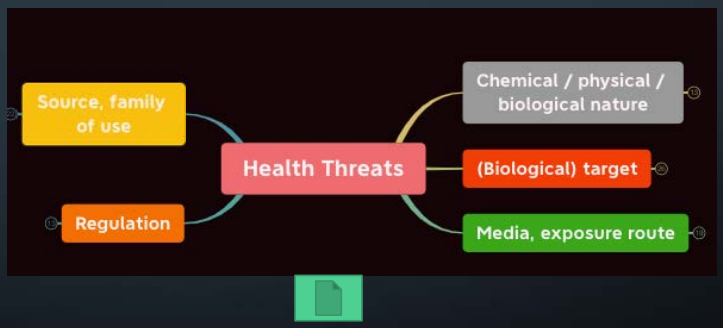
4

# A. Introduction : classifying toxicants

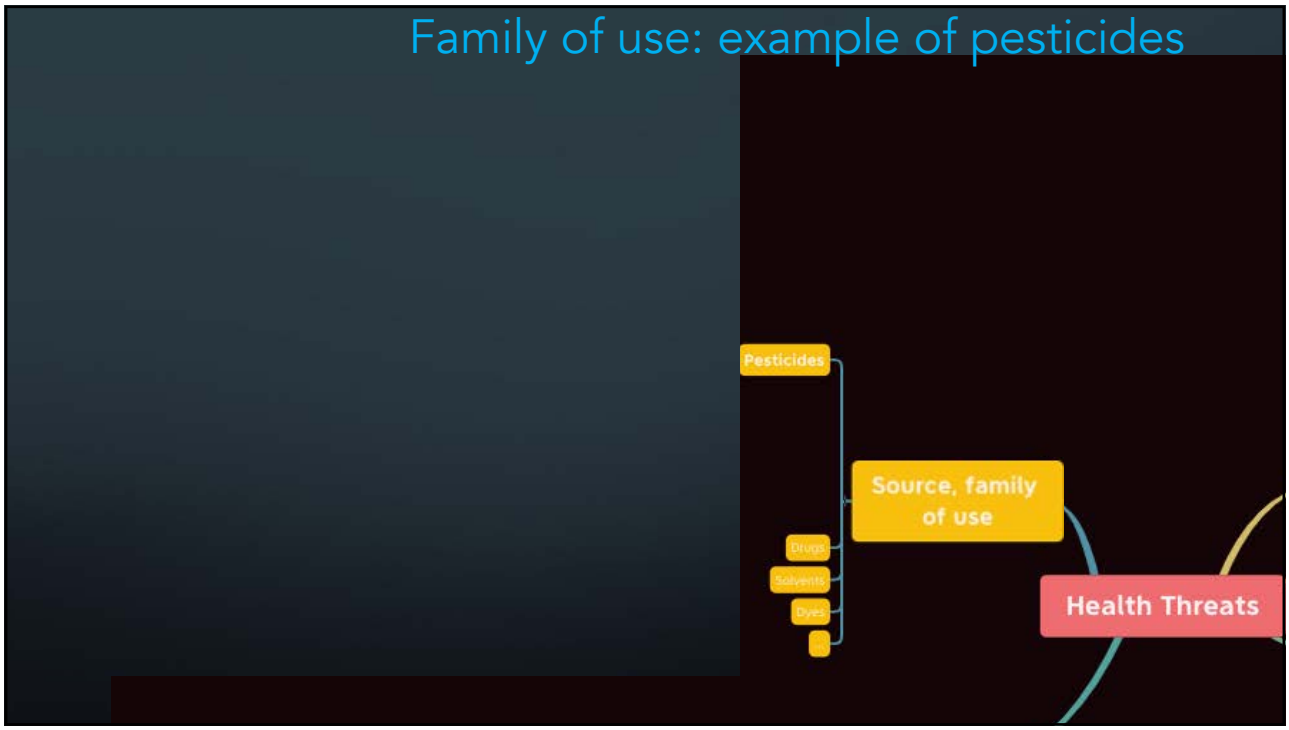



5

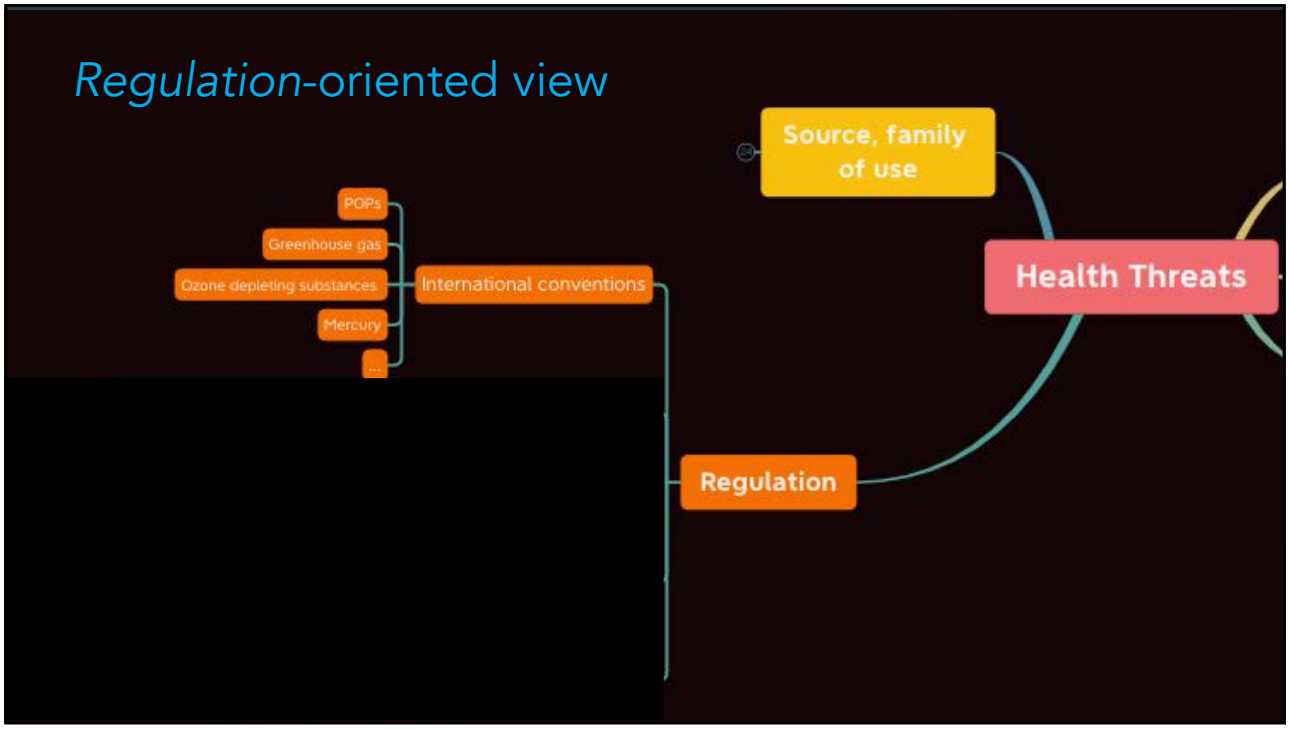
# Health threats can be qualified according to several overlapping dimensions



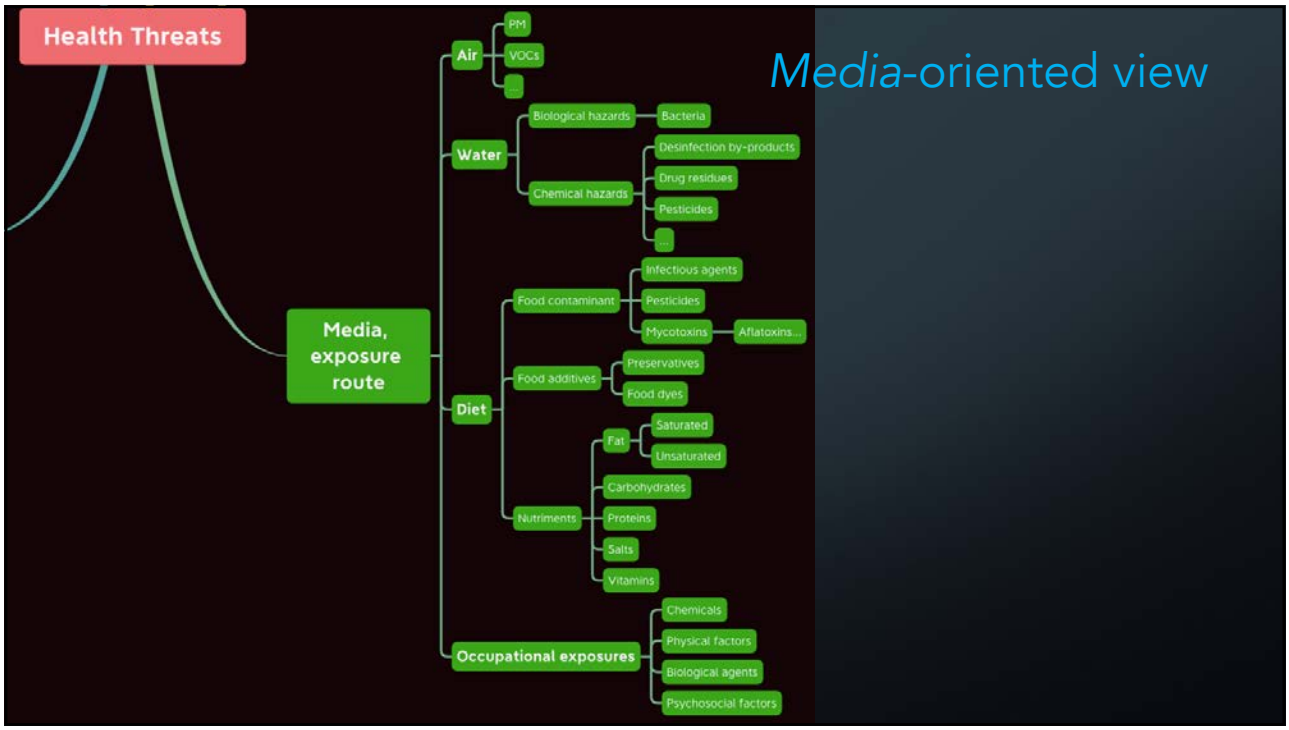

6


Family of use: example of pesticides
Pesticides
Source, family of use
Drugs
Solvents
Dyes
...
Health Threats

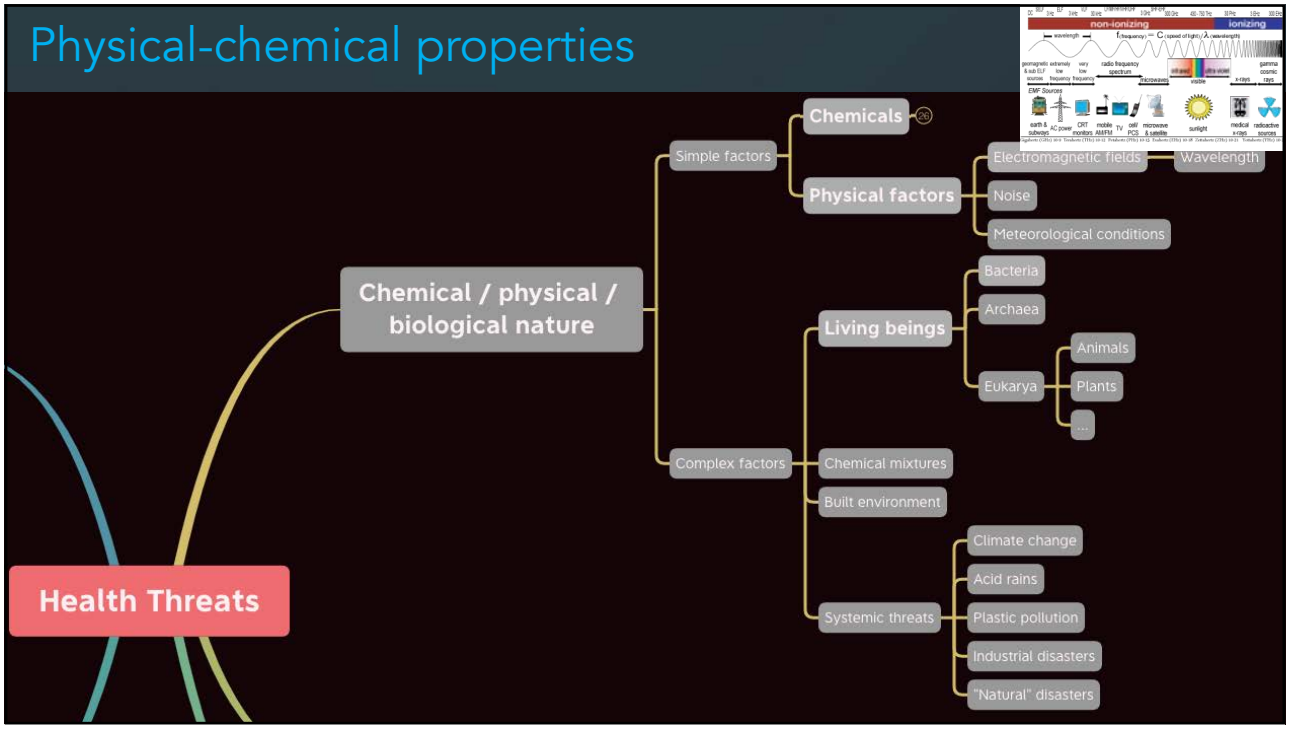
7


*Regulation*-oriented view
Source, family of use
POPs
Greenhouse gas
Ozone depleting substances
Mercury
...
International conventions
Health Threats
Regulation

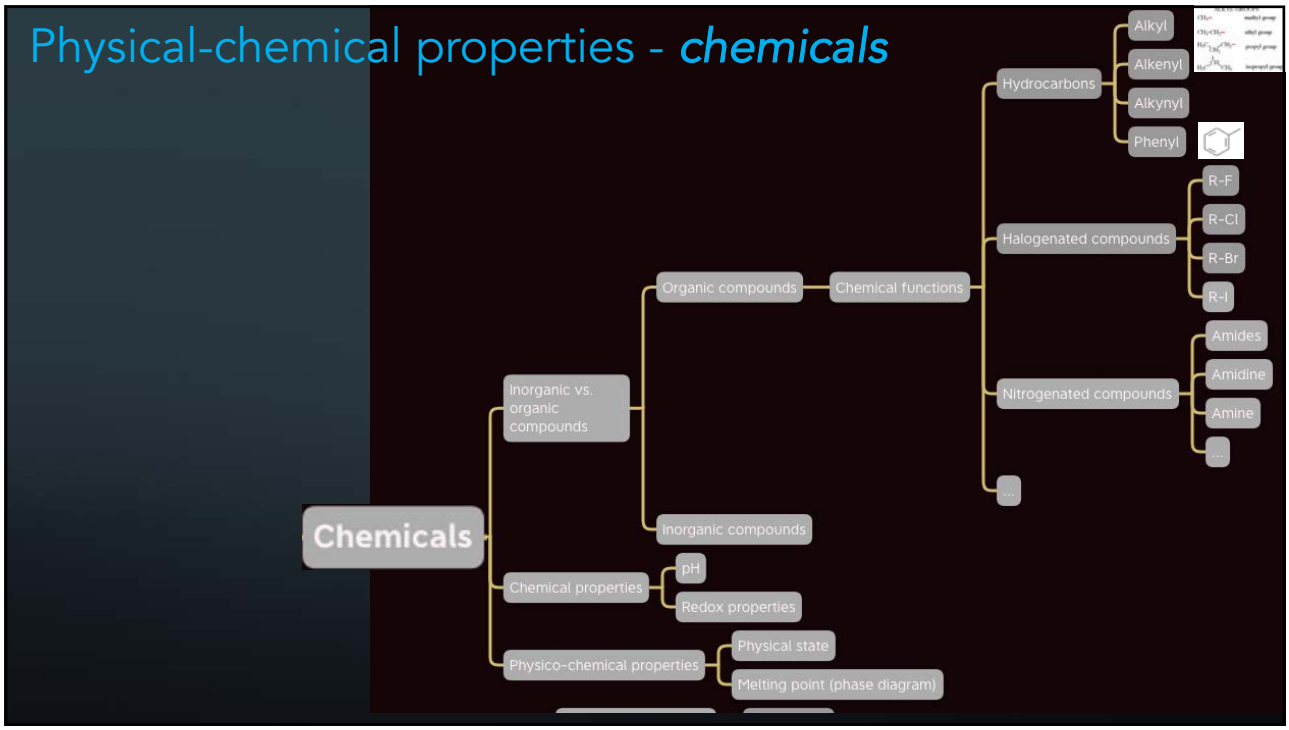
8


Health Threats
Media, exposure route
Air
PM
VOCs
...
Water
Biological hazards
Bacteria
Chemical hazards
Desinfection by-products
Drug residues
Pesticides
...
Diet
Food contaminant
Infectious agents
Pesticides
Mycotoxins
Aflatoxins...
Food additives
Preservatives
Food dyes
Nutriments
Fat
Saturated
Unsaturated
Carbohydrates
Proteins
Salts
Vitamins
Occupational exposures
Chemicals
Physical factors
Biological agents
Psychosocial factors
Media-oriented view

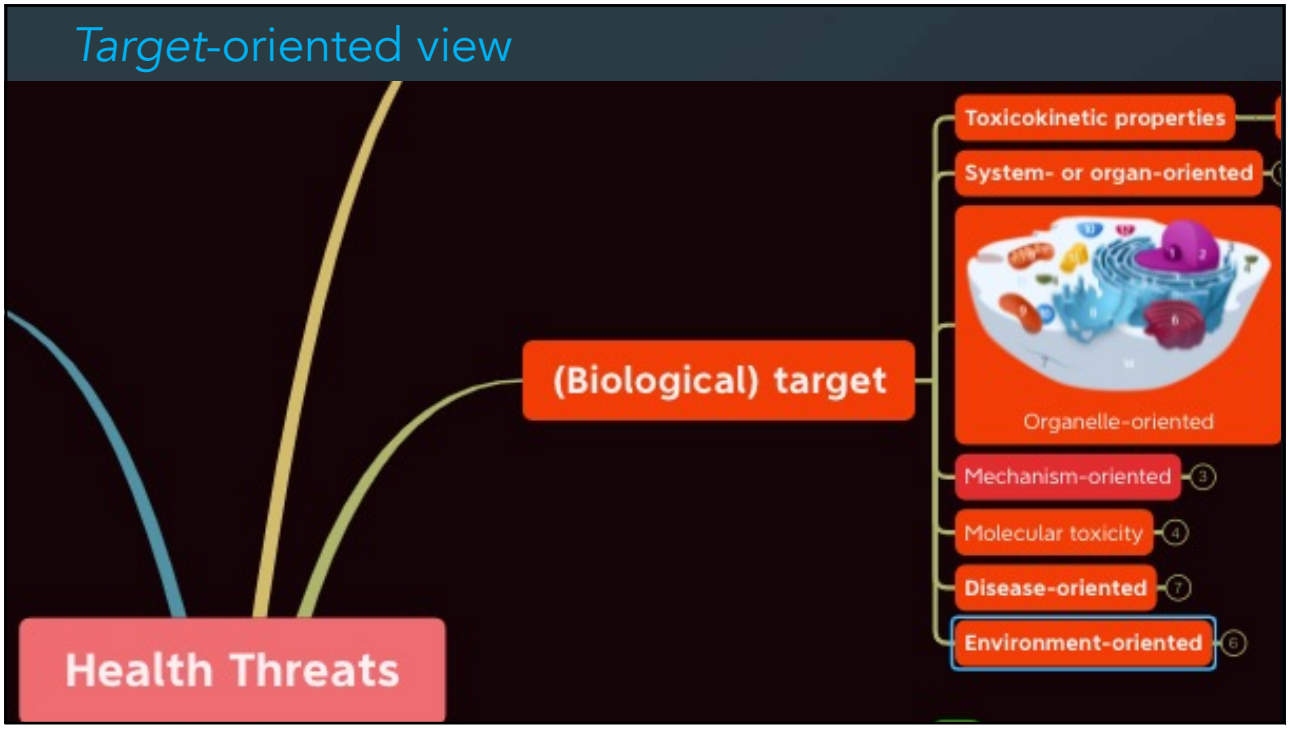
9


Physical-chemical properties
Health Threats
Chemical / physical / biological nature
Simple factors
Chemicals
Physical factors
Electromagnetic fields
Wavelength
Noise
Meteorological conditions
Complex factors
Living beings
Bacteria
Archaea
Eukarya
Animals
Plants
...
Chemical mixtures
Built environment
Systemic threats
Climate change
Acid rains
Plastic pollution
Industrial disasters
"Natural" disasters

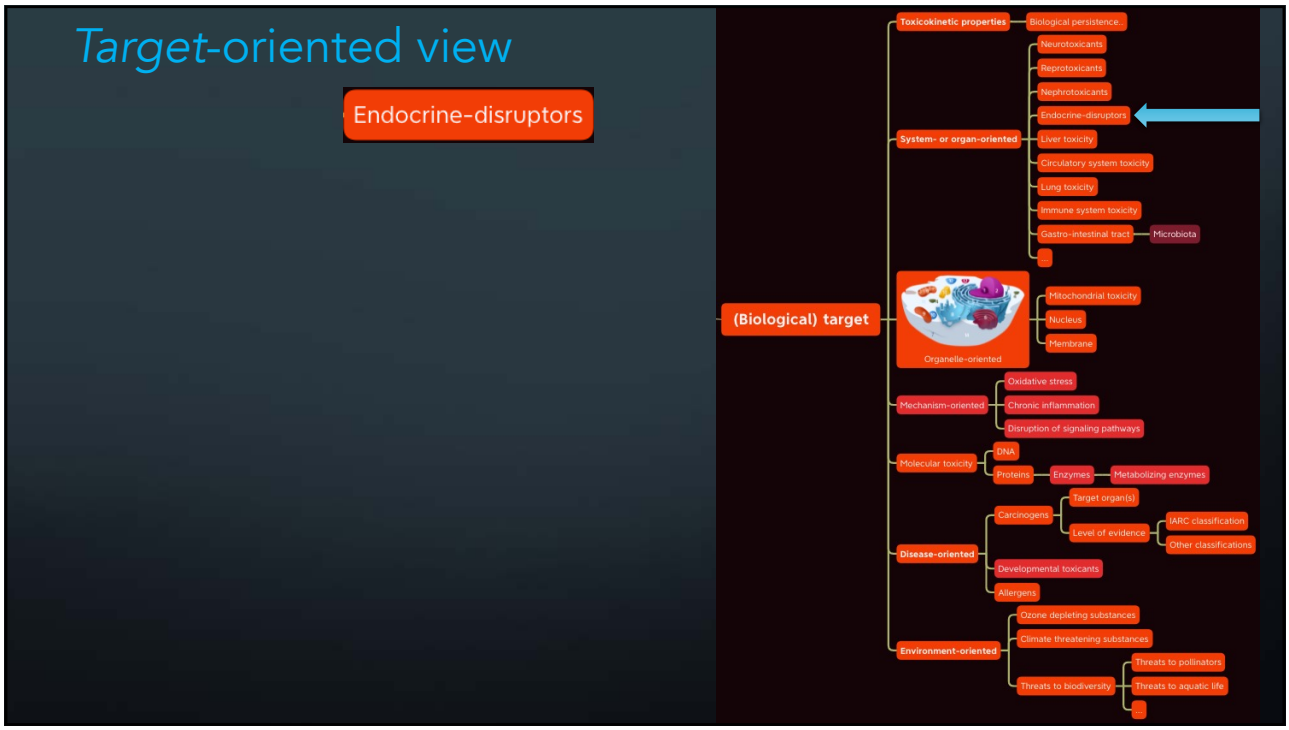
10


Physical-chemical properties - *chemicals*
Chemicals
Inorganic vs. organic compounds
Organic compounds
Chemical functions
Hydrocarbons
Alkyl
Alkenyl
Alkynyl
Phenyl
Halogenated compounds
R-F
R-Cl
R-Br
R-I
Nitrogenated compounds
Amides
Amidine
Amine
Inorganic compounds
Chemical properties
pH
Redox properties
Physico-chemical properties
Physical state
Melting point (phase diagram)

11


*Target*-oriented view
(Biological) target
Health Threats
Toxicokinetic properties
System- or organ-oriented
Organelle-oriented
Mechanism-oriented
Molecular toxicity
Disease-oriented
Environment-oriented

12




13

B. Endocrine-disruptors

Some generic considerations

14

## Definition of endocrine disruptors (EDs)

An exogenous substance or mixture that alters the functioning of the endocrine system and consequently causes adverse effects in an intact organism or its progeny or (sub) populations.

(WHO and IPCS, 2002)

Clinical component ← Mechanistic component

a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences

15

## Definitions of endocrine disruptors (EDs)

An exogenous substance or mixture that alters the functioning of the endocrine system and consequently causes adverse effects in an intact organism or its progeny or (sub) populations.

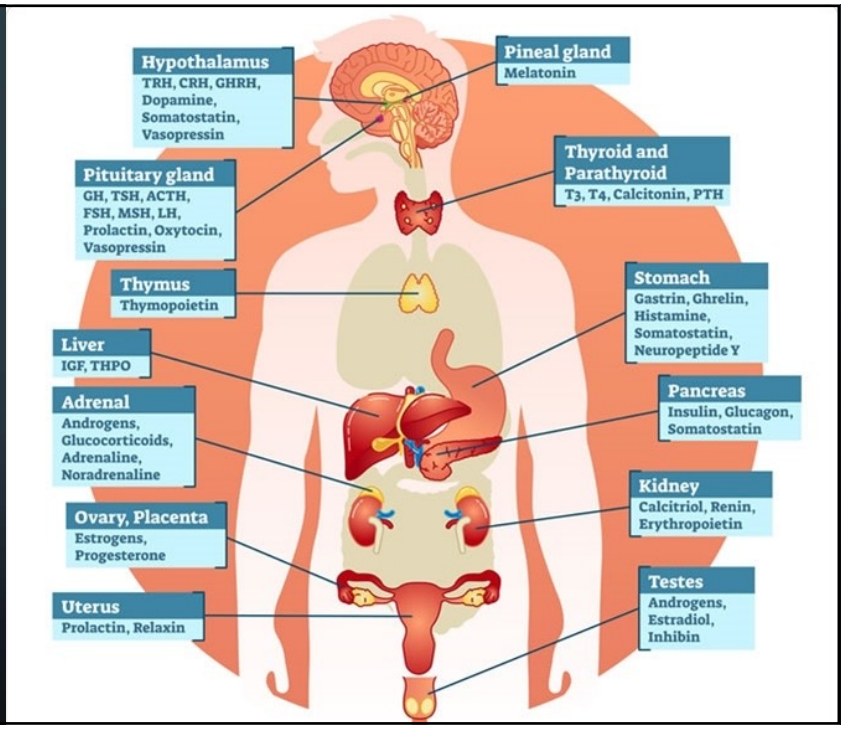
(WHO and IPCS, 2002)

*More general and simpler (non official) definition:*

EDs are chemicals that interfere with the endocrine system.

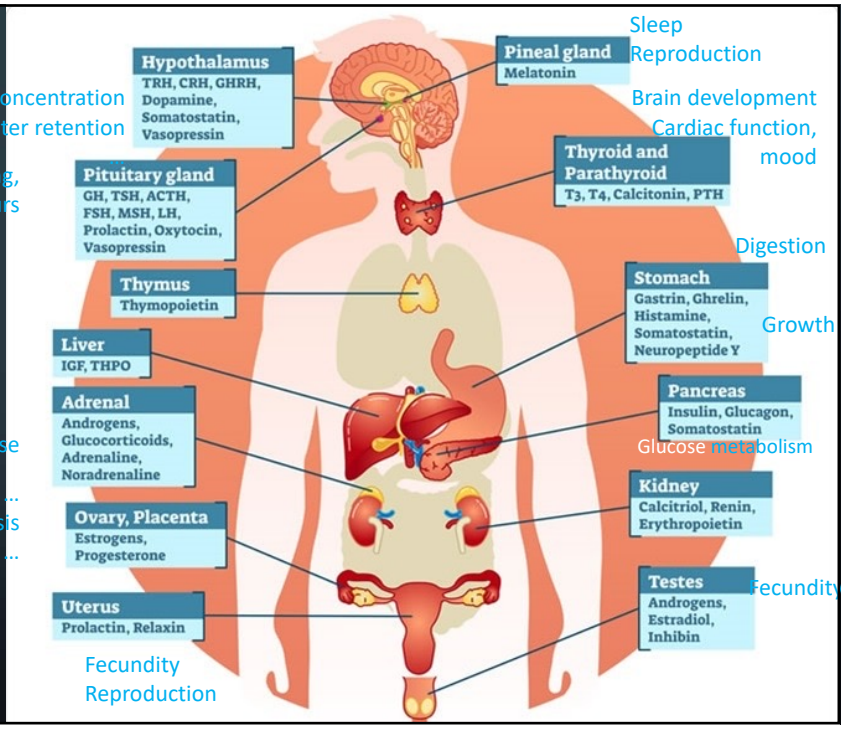
16

# Organs of the endocrine system



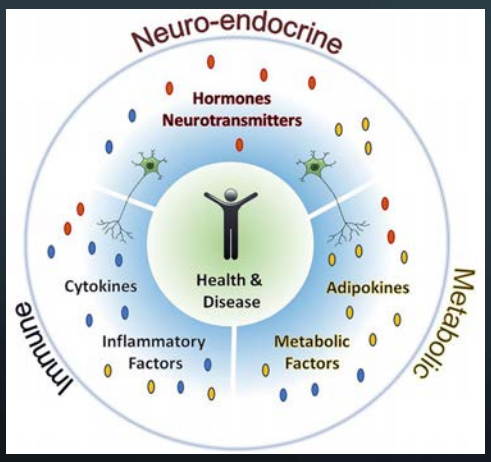

17

# Functions



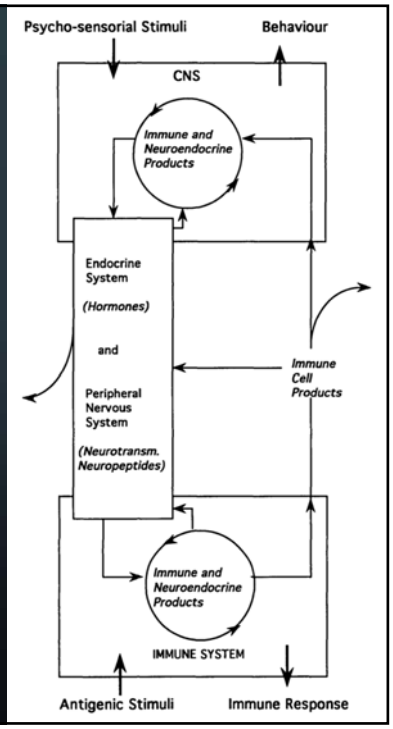

18

# Interactions of the endocrine system with other systems:
## the neuro-endocrine-immune connection




Gene expression

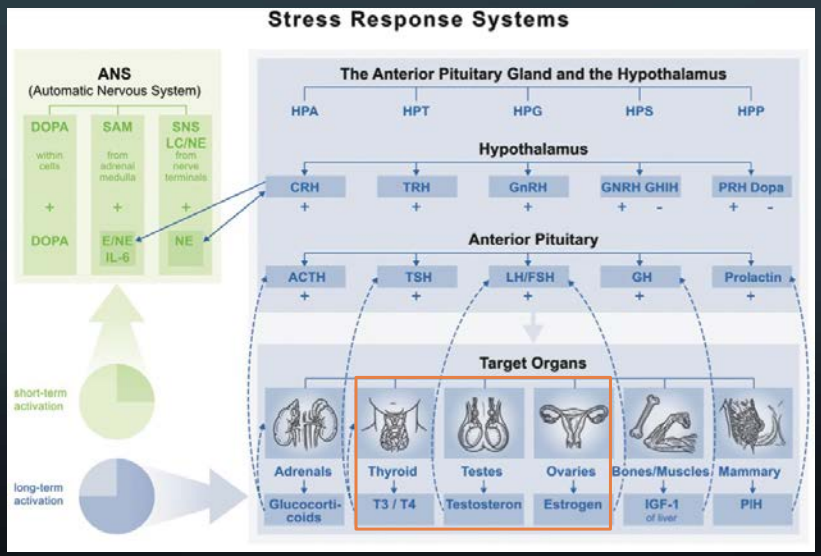
(Perez, *Front Endocrinol*, 2021)




(Besedovsky, *Endocrin Rev*, 1996)

19

# Endocrine modalities

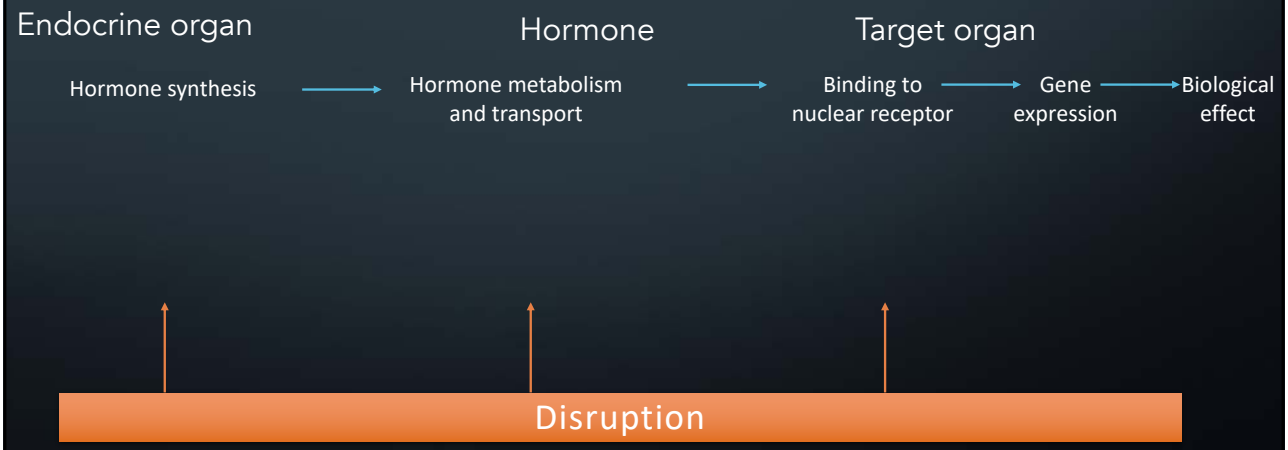



HPA, hypothalamic–pituitary–adrenal;
HPT, hypothalamic–pituitary–thyroid;
HPG, hypothalamic–pituitary–gonadal;
HPS, hypothalamic–pituitary–somatotropic;
HPP, hypothalamic–pituitary–prolactin;
CRH, corticotropin-releasing hormone;
TRH, thyrotropin-releasing hormone;
GnRH, gonadotropin-releasing hormone;
GHRH, growth hormone-releasing hormone;
GHIH, growth hormone-inhibiting hormone;
DOPA, dopamine; ACTH, adrenocorticotropic hormone; TSH, thyroid-stimulating hormone;
LH, luteinizing hormone;
FSH, follicle-stimulating hormone;
GH, growth hormone
IGF-1, insulin-like growth factor 1
PIH: Prolactin inhibiting hormone.

(Wipert, *Front Endocrinol*, 2017)

20

## The endocrine function: *a dynamic view*



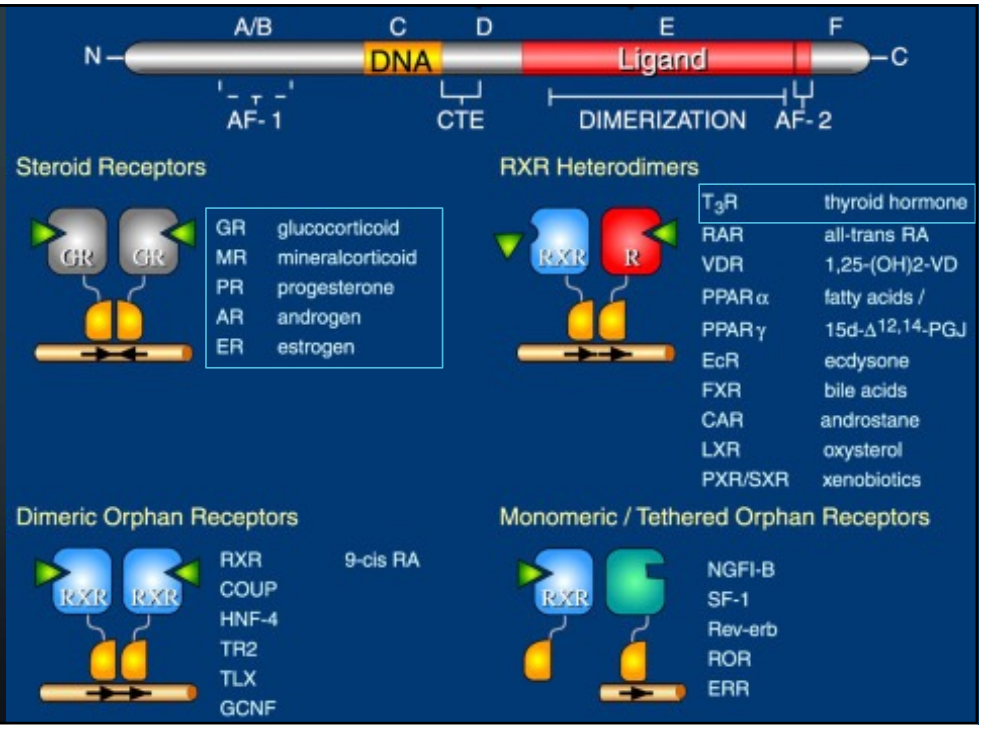

21

## Families of nuclear receptors

Biologically speaking, nuclear receptors are transcription factors that bind to DNA

Only some nuclear receptors have hormonal ligands (the other NRs are termed *orphan* NR)

(Olefsky, *J Biol Chem*, 2001)




22

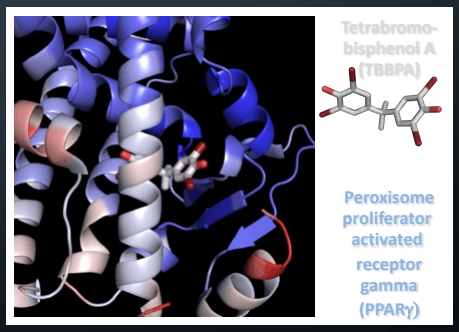
## Nuclear receptors as key targets of EDs

(Natural) hormone

Nuclear receptor

Endocrine disruptor

**Interaction of a brominated derivative of Bisphenol A with PPARγ nuclear receptor**




(W. Bourguet, P. Balaguer, Inserm, CNRS, personal communication)

23

## Some consequences of the definition of EDs

- "Complexity" of biological or clinical effects of some EDs
  - In terms e.g. of dose response functions (non-monotonicity of the effects of many hormones)
  - In relation to the complexity of effects of hormones on the body
- Complexity to firmly establish a compound as an ED
  - In relation to the complexity of the definition of EDs
- Expected variety of EDs in terms of sources and chemical nature
  - Because the definition is *mechanism-oriented* rather than *source-oriented*
- Expected variety of possible clinical effects of EDs
  - due to the diversity of functions of the endocrine system
- Some EDs may exert effects at very low doses (in particular those interacting with nuclear receptors (because hormones also act at very low doses)

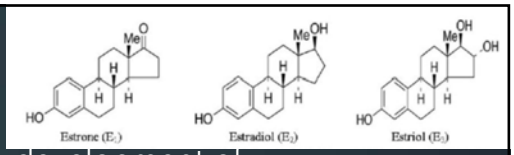
24

## C. Oestrogen axis disruption: Diethylstribestrol (DES) and DDT



25

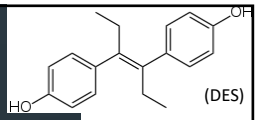
## The estrogen hormones




- Steroid hormones with essential roles in the development of secondary sexual characteristics (breast), menstrual cycle, sperm maturation, sexuality
  - Also implied in metabolism, fat storage, body weight homeostasis, bone formation, water retention
- Bind to estrogen receptors (ERs) and also rapid-signaling membrane receptors (e.g., GPR30)
- The ER-estrogen complex possibly binds to over 80 DNA sequences
- 4 major oestrogen hormones: estrone (E1), **estradiol (E2)**, estriol (E3) and estetrol (E4, produced only during pregnancy)
- Synthesized from androgens by aromatase enzyme
- Estrone was first purified in 1929. Estrogen-like compounds were shortly after used as drugs (estradiol benzoate, ethinyl estradiol, DES…)

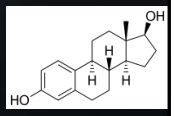
26

# DES (Diethylstilbestrol), a potent synthetic estrogen



(DES)


- Synthetic estrogenic compound. Synthesized in 1938 (C. Dodds). First hormone to be synthesized
- At this time, spontaneous abortions were assumed by gynaecologists to originate from a lack of estrogens
- Authorized in 1941 by the FDA (USA) and in the mid 1940s in France
- A randomized control trial among 1600 women demonstrates a lack of protection against the risk of spontaneous abortion (Dieckmann, *Am J Obst Gyn*, 1953)
- Number of pregnant women exposed to DES: USA, 1-2 million; France, 200,000
- Use demonstrated to be associated with a strongly increased risk of clear cell adenocarcinoma of vagina and cervix in young women (Herbst, *NEJM*, 1971)
- Not authorized anymore during pregnancy in the USA (1971) and France (1977)
- Also used as a growth hormone in cattle and chickens (banned in 1973 in the USA)



Oestradiol

(Swan, *APMIS*, 2001; Fillion & Torny, *La Recherche*, 2013)

28


FEED YOUR CATTLE FUL-O-PEP PRO-SWEET WITH

Stilbestrol

TO BOOST GAINS AND FEEDING EFFICIENCY!

Cattle fed stilbestrol need added nutritional strength

Stilbestrol is a hormone-like, gain-boosting ingredient – not a nutrient. As a result, cattle need a *quality ration containing extra food values* to get full benefit of the stilbestrol. That's why it's important that you feed your cattle Ful-O-Pep Pro-Sweet with stilbestrol. For Ful-O-Pep Pro-Sweet is highly fortified with *added nutritional strength* cattle need for fast gain and feeding efficiency. Yes, these extra nutrients help promote smooth, profitable finish, eye-catching bloom and high-yielding carcasses . . . help you get full feeding value from your grain and roughage.

Steers fed Ful-O-Pep gain ½ lb. more per steer, per day!

Yes, recent tests at the Ful-O-Pep Research Farm show that steers fed Ful-O-Pep Pro-Sweet containing stilbestrol gained almost *½ pound more per steer, per day* . . . ate 86 pounds less feed per 100 pounds of gain . . . were appraised at an average of 50¢ more per 100 pounds. What's more, *twice as many of the carcasses graded prime* as compared to steers fed the same feed without stilbestrol. So for top feeding efficiency . . . top gains, feed Ful-O-Pep Pro-Sweet containing stilbestrol.

FUL-O-PEP PRO-SWEET SUPPLEMENT

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SUCCESSFUL FARMING, OCTOBER, 1955 69

(1955)


"Really?"

Yes . . .

**desPLEX®**

to prevent ABORTION, MISCARRIAGE and PREMATURE LABOR

*recommended for routine prophylaxis in ALL pregnancies . .*

96 per cent live delivery with desPLEX in one series of 1200 patients[4]— — bigger and stronger babies, too.[1, 3]

No gastric or other side effects with desPLEX — in either high or low dosage[3,4,5]

(Each desPLEX tablet starts with 25 mg. of diethylstilbestrol, U.S.P., which is then ultramicronized to smooth and accelerate absorption and activity. A portion of this ultramicronized diethylstilbestrol is even included in the tablet coating to assure prompt help in emergencies. desPLEX tablets also contain vitamin C and certain members of the vitamin B complex to aid detoxification in pregnancy and the effectuation of estrogen.)

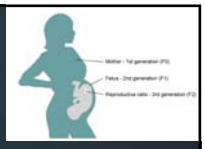
For further data and a generous trial supply of desPLEX, write to: Medical Director

REFERENCES

1. Canaria, E. M., et al.: Am. J. Obst. & Gynec. 65:1298, 1953.
2. Gitman, L., and Koplowitz, A.: N. Y. St. J. Med. 50:2823, 1950.
3. Karnaky, K. J.: South. M. J. 45:1166, 1952.
4. Peña, E. F.: Med. Times 82:921, 1954; Am. J. Surg. 87:93, 1954.
5. Rose, J. W.: J. Nat. M. A. 43:20, 1951; 43:223, 1953.

GRANT CHEMICAL COMPANY, INC., Brooklyn 26, N.Y.

29



## DES (Diethylstilbestrol): Absorption, distribution, metabolism and excretion (ADME)

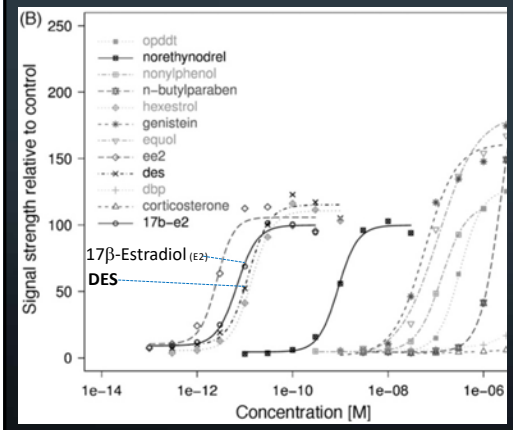
- Non persistent compound.
  Biphasic depletion curve with initial half life at 17 hr and a later half-life at 5.5 days (cattle)
- Distributes in the whole organism. Reaches the fetus and tends to accumulate in the fetal genital tract (mice).
- DES is metabolized, in particular by cytochrome enzymes; some metabolites are able to bind to DNA.
- Eliminated through biliary excretion in the intestine and faeces (traces can be found in urine)

Reviewed in (IARC, monography 100A, 2012)

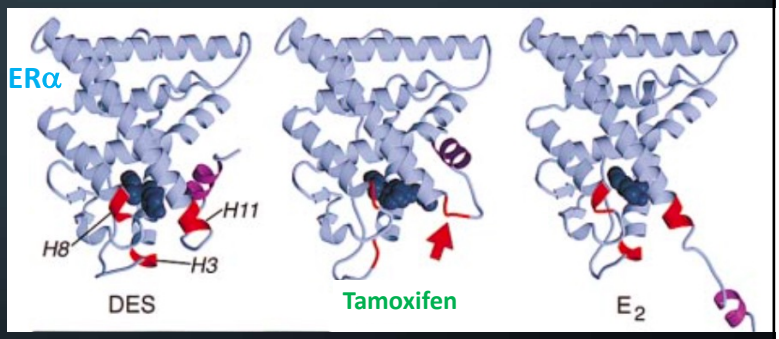
30

## Molecular scale: DES interacts with ERα estrogen receptor

ERα agonism CALUX reporter gene assay



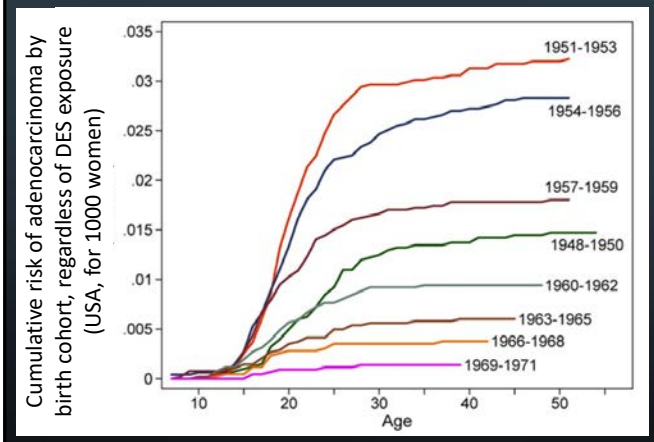

(van der Burg, *Reprod Tox*, 2010)




(Shiau, *Cell*, 1998)

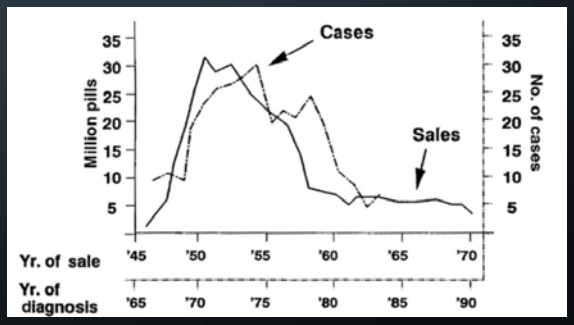
31

# Clear cell adenocarcinoma of vagina and cervix – ecological data (USA)


(Huo, *Gyn Oncol*, 2017)

DES sales and cases of clear cell adenocarcinoma 20 years later (USA)




(Swan, *APMIS*, 2001)

32

878 THE NEW ENGLAND JOURNAL OF MEDICINE Apr. 22, 1971

## ADENOCARCINOMA OF THE VAGINA*

### Association of Maternal Stilbestrol Therapy with Tumor Appearance in Young Women

ARTHUR L. HERBST, M.D., HOWARD ULFELDER, M.D., AND DAVID C. POSKANZER, M.D.

**Abstract** Adenocarcinoma of the vagina in young women had been recorded rarely before the report of several cases treated at the Vincent Memorial Hospital between 1966 and 1969. The unusual occurrence of this tumor in eight patients born in New England hospitals between 1946 and 1951 led us to conduct a retrospective investigation in search of factors that might be associated with tumor appearance. Four matched controls were established for each patient; data were obtained by personal interview. Results show maternal bleeding during the current pregnancy and previous pregnancy loss were more common in the study group. Most significantly, seven of the eight mothers of patients with carcinoma had been treated with diethylstilbestrol started during the first trimester. None in the control group were so treated (p less than 0.00001). Maternal ingestion of stilbestrol during early pregnancy appears to have enhanced the risk of vaginal adenocarcinoma developing years later in the offspring exposed.

CANCER of the vagina is rare, occurring usually as epidermoid carcinoma in women over the age of 50 years.[1] Between 1966 and 1969, however, these patients and their families with an appropriate control group to uncover factors that might be associated with the sudden appearance of these tumors.

A. Herbst

(Herbst, *N Eng J Med*, 1971)

33

# Clear cell adenocarcinoma – case-controls and cohort studies

In case-controls studies, DES in-utero exposure was the only factor discriminating clear cell adenocarcinoma cases from controls (Herbst, NEJM, 1971; IARC, monography 100A, 2012).

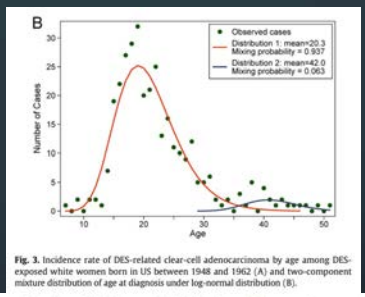



Fig. 3. Incidence rate of DES-related clear-cell adenocarcinoma by age among DES-exposed white women born in US between 1948 and 1962 (A) and two-component mixture distribution of age at diagnosis under log-normal distribution (B).

From cohort studies, the lifetime cumulative risk of clear cell adenocarcinoma of the vagina or cervix in women exposed to DES in-utero is about 1 to 2 per 1000 women. This corresponds to a 30-40 fold increased risk (IARC, monography 100A, 2012)

(Huo, *Gyn Oncol*, 2017)

**TABLE II. Standardized Incidence Ratios (SIR) and 95% Confidence Intervals (CI) for Total and Site-specific Cancer in Prenatally DES-exposed and Unexposed Daughters, 1978–2013**

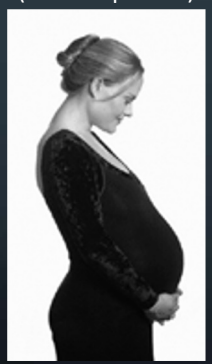
| | DES Exposed | | | Unexposed | | |
|---|---|---|---|---|---|---|
| Cancer Site[a] | Observed | Expected | SIR[b] (95% CI) | Observed | Expected | SIR[b] (95% CI) |
| All Cancer (first primaries) | 335 | 328 | 1.02 (0.91–1.14) | 159 | 154 | 1.03 (0.88–1.21) |
| All Cancer (multiple primaries) | 361 | 342 | 1.06 (0.95–1.17) | 167 | 159 | 1.05 (0.89–1.22) |
| Breast | 178 | 152 | 1.17 (1.01–1.36) | 74 | 69.6 | 1.06 (0.83–1.33) |
| Clear Cell Adenocarcinoma | 4 | 0.15 | 27.6 (7.51–70.6) | 0 | 0.06 | 0 (0–66.3) |

(Troisi, *Env Mol Mutag*, 2019)

34

# DES and breast cancer: human evidence

Pregnant woman
(adult exposure)



Increased breast cancer risk:
RR = 1.3 [1.1-1.5]

(Titus-Ernstoff, *Br J Canc*, 2001)

1st generation
exposed *in utero*


Slightly increased breast cancer risk: SIR: 1.2

| Characteristics of DES Exposure | Person-years | Breast Cancer # of Cases | HR | 95% CI |
|---|---|---|---|---|
| DES Dose[b] | | | | |
| Low | 50300 | 65 | 1.00 | — |
| High | 78700 | 107 | 1.18 | 0.86-1.62 |
| Gestational Week at First DES Exposure[c] | | | | |
| ≤ 7 | 31400 | 31 | 1.00 | — |
| 8–10 | 26900 | 35 | 1.20 | 0.73-1.96 |
| 11–14 | 19300 | 25 | 1.14 | 0.67-1.95 |
| ≥ 15 | 24100 | 35 | 1.14 | 0.70-1.87 |

« ...DES effects on established risk factors may explain much of the excess in breast cancer risk [in in-utero exposed women] compared with the general population. »

(Troisi, *Env Mol Mutag*, 2019)

35

# In-utero DES exposure and cancers: human evidence

**TABLE II. Standardized Incidence Ratios (SIR) and 95% Confidence Intervals (CI) for Total and Site-specific Cancer in Prenatally DES-exposed and Unexposed Daughters, 1978–2013**

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| Clear Cell Adenocarcinoma | 4 | 0.15 | 27.6 (7.51–70.6) | 0 | 0.06 | 0 (0–66.3) |
| Ovary | 18 | 15.0 | 1.20 (0.71–1.90) | 9 | 6.79 | 1.32 (0.61–2.51) |
| Endometrium | 16 | 26.4 | 0.61 (0.35–0.98) | 20 | 12.9 | 1.55 (0.95–2.40) |
| Thyroid | 16 | 22.5 | 0.71 (0.41–1.16) | 6 | 9.05 | 0.66 (0.24–1.44) |
| Non Hodgkin's Lymphoma | 22 | 12.8 | 1.72 (1.08–2.61) | 8 | 5.98 | 1.34 (0.58–2.64) |
| Leukemia | 7 | 6.92 | 1.01 (0.41–2.09) | 7 | 3.19 | 2.19 (0.88–4.51) |
| Colorectal | 15 | 21.4 | 0.70 (0.39–1.15) | 13 | 10.5 | 1.24 (0.66–2.12) |
| Pancreas | 11 | 4.53 | 2.43 (1.21–4.34) | 1 | 2.36 | 0.42 (0.01–2.36) |
| Lung & Bronchus | 20 | 25.0 | 0.80 (0.49–1.23) | 11 | 13.46 | 0.82 (0.41–1.46) |
| Other[c] | 54 | 55.3 | 0.98 (0.75–1.27) | 18 | 25.6 | 0.70 (0.44–1.12) |

Increased risk of pancreatic cancer in in-utero exposed women has been confirmed in a later follow-up of the cohort (Troisi, *J DOHaD*, 2020)

No strong evidence of an increased risk of testicular cancer in in-utero exposed boys (Swan, *APMIS*, 2001)

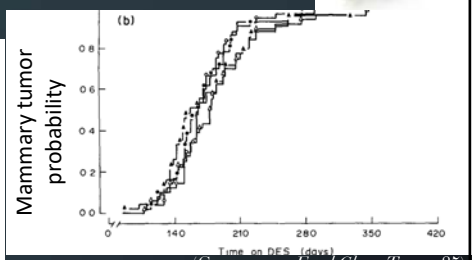
(Troisi, *Env Mol Mutag*, 2019)

36

# DES and (breast) cancer: experimental evidence regarding tumorigenesis

| | | | | | |
|---|---|---|---|---|---|
| Boylan and Calhoon 1979 [16] | Rat/DMBA | 1.2 µg | Week 2 G | Not studied | Multiplicity↑ |
| | | 1.2 µg | Week 3 G | | Multiplicity↑ |
| Boylan and Calhoon 1983 [18] | Rat/DMBA | 0.6 + 0.6 µg | 15 + 18 G | Not studied | Incidence + multiplicity↑ |
| Rothschild *et al.* 1987 [19] | ACI rat | 0.4 + 0.4 µg | 15 + 18 G | Not studied | No change |
| | | 4 + 4 µg | 15 + 18 G | | Incidence↑ |
| Ninomiya *et al.* 2007 [20] | Rat/DMBA | 0.1 µg | Birth (one dose) | Normal cycle, 40% CL | Multiplicity↑ |
| | | 1 µg | Birth (one dose) | 19% PE, 50% CL, U w↓ | Incidence + multiplicity↑ |
| | | 10 µg | Birth (one dose) | 77% PE, 92% CL, U w↓ | Multiplicity↑ |
| | | 100 µg | Birth (one dose) | 100% PE, 100% CL, O + U w↓ | No change |
| Yoshikawa *et al.* 2008 [22] | Rat/DMBA | 14 x 1 µg | 0-14 Postnatal | 100% PE, no CL, O + U w↓, E2 and P↓ | Incidence↓ |
| | | 5 x 1 µg | 0-5 | PE, no CL, O + U w↓, E2 and P↓ | Incidence↓ |
| | | 9 x 1 µg | 6-14 | PE, no CL, O + U w↓ | No change |
| Kawaguchi *et al.* 2009 [21] | Rat/DMBA | 0.1 ppm | 0-21 G | Few surviving pups | |
| | | 1 ppm | 0-21 G | No surviving pups | |
| | | 10 ppm | 0-21 G | No surviving pups | (Assessed 10 weeks after |
| | | 100 ppm | 0-21 G | No surviving pups | DMBA exposure): |
| | | 0.1 ppm | 13-21 G | 11% no CL | Incidence + multiplicity↑ |
| | | 1 ppm | 13-21 G | 30% no CL | Incidence + multiplicity↑ |
| | | 10 ppm | 13-21 G | Very few surviving pups | Incidence↑ |
| | | 100 ppm | 13-21 G | No surviving pups | |

[a]In control mice, corpora lutea (CL) is present in about 31 to 36% of adults, whilst in control rats it is present in 100% of adult animals. DES, diethylstilbestrol; DMBA, dimethylbenz[a]antracene; E2, estradiol; F[1], F1 generation; G, gestation; HAN, hyperplastic alveolar nodule; O, ovary; P, progesterone; PE, persistent estrus; U, uterus; w, weight.




(Greenman, *Food Chem Tox*, 1985)

Overall, oral DES exposure can induce tumors of the ovary, endometrium and cervix and mammary adenocarcinoma (IARC, monography 100A, 2012)

[a] In control mice, corpora lutea (CL) is present in about 31 to 36% of adults, whilst in control rats it is present in 100% of adult animals. DES, diethylstilbestrol; DMBA, dimethylbenz[a]antracene; E2, estradiol; F1, F1 generation; G, gestation; HAN, hyperplastic alveolar nodule; O, ovary; P, progesterone; PE, persistent estrus; U, uterus; w, weight.

(Hilakivi-Clarke, *Br Canc Res*, 2014)

37

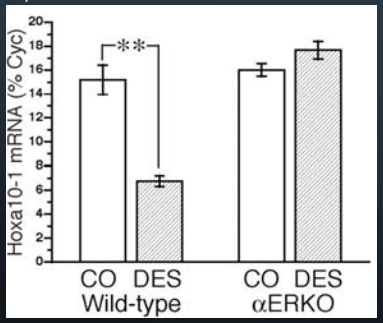
# DES and cancer: possible underlying mechanisms

- DES was shown to disrupt NF-κB signalling pathway, which is linked to breast cancer progression and anti-estrogen resistance
- DES can also induce epigenetic changes, including in areas coding for DNMTs (DNA methyl transferases)
- Some DES metabolites (DES-quinone) may be mutagenic
- Some of the tumorigenic effects of DES were not observed in ERα KO animals (Couse, *Dev Biol*, 2001)
- Overall:
  - several partly connected pathways are implied in the carcinogenicity of DES, including estrogen dependent and estrogen independent pathways
  - the implication of estrogen receptor (ERα) in DES effects is deemed very likely (IARC, monography 100A, 2012)

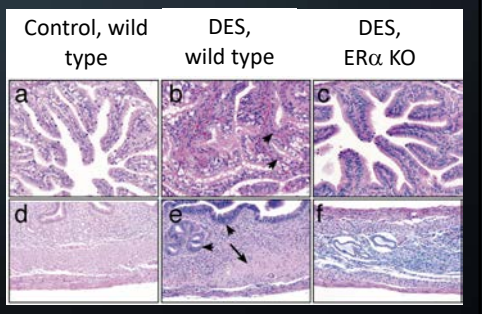
38

# Demonstrating the implication of estrogen related pathways comparing wild-type to ERα knock-out mice

DES decreases Hoxa-10 gene expression in wild-type but not ERα KO mice.
(Hoxa-10 is a gene possibly implied in the reproductive tract anomalies induced by DES)




Characteristic morphological effects of neonatal DES exposure occur in the reproductive tract tissues (oviduct) of adult wild-type but not ERα KO mice




(Couse, *Dev Biol*, 2001)

39

# Carcinogenic effects of DES from the molecular to the population scales

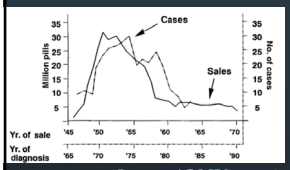
Population scale (ecological studies)

Individual scale (cohorts)

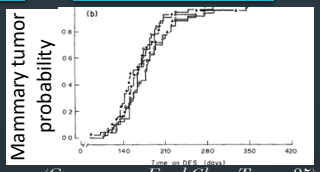
Individual scale (in vivo toxicology)

Cellular scale (in vitro toxicology)

Molecular scale

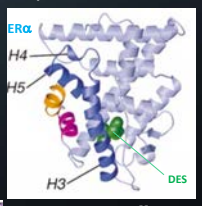



(Swan, *APMIS*, 2001)




(Greenman, *Food Chem Tox*, 1985)

High affinity of DES for estrogen receptor ERα




(Shiau, *Cell*, 1998)

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|---|---|---|---|
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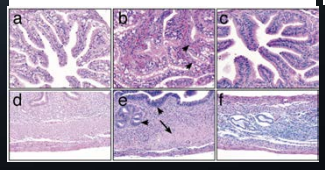
| Clear Cell Adenocarcinoma | 4 | 0.15 | 27.6 (7.51–70.6) |

(Troisi, *Env Mol Mutag*, 2019)

Control, wild type | DES, wild type | DES, ERα KO




(Couse, *Dev Biol*, 2001)

40

# Non cancerous effects of DES in in-utero exposed women

TABLE 4. *Incidence of adverse pregnancy outcomes in DES daughters and estimates of their relative risk*[1]

| Outcome | Incidence in controls | Incidence in DES daughters abnormal DES[3] | | All DES | Estimate of relative risk[2] (95% confidence interval) | |
|---|---|---|---|---|---|---|
| | | Vagina-cervix | Uterus | | Abnormal DES[3] | All DES |
| Ectopic pregnancy | 0.005 | 0.063 | 0.076 | 0.044 | 13.5 (2.1–84.7) | 8.6 (3.4–21.9) |
| Premature live birth | 0.02 | 0.75 | 0.38 | 0.13 | 9.6 (4.0–23.4) | 4.7 (2.8–7.9) |
| Spontaneous abortion | 0.13 | 0.19 | 0.36 | 0.23 | 2.6 (1.8–3.8) | 1.8 (1.5–2.2) |
| Not a full-term birth[4] | 0.15 | 0.67 | | 0.41 | 4.9 (3.1–7.7) | 2.7 (2.2–3.0) |

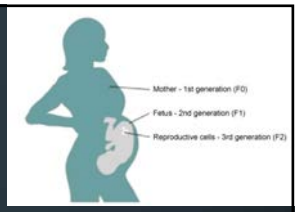
[1] Adapted from (40).
[2] Mantel-Haenzel estimate of relative risk; Robins-Greenland estimate of 95% confidence interval.
[3] DES-associated abnormality.
[4] Includes ectopic pregnancy, premature birth, and spontaneous abortion.

- Infecundity, adverse pregnancy outcomes
- Menstrual cycle disorders

(Swan, *APMIS*, 2001)

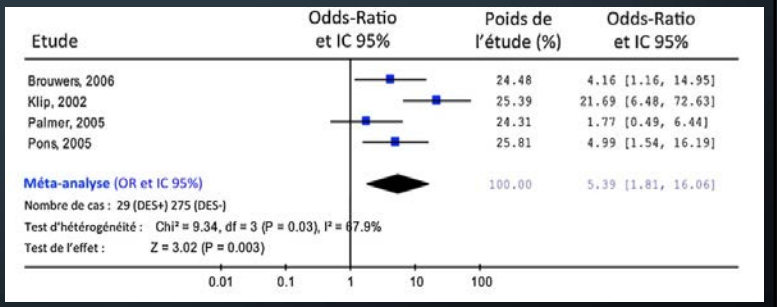
41


# Risk of congenital malformations of the male genitalia (hypospadias) in the sons of women exposed in-utero to DES




Records from Port-Royal maternity (Paris), 1993 to 2002 (boys only).

| In utero DES exposure | Hypospadias | |
|---|---|---|
| | No | Yes |
| No | 17,349 | 44 (0.025%) |
| Yes | 237 | 3 (1.23%) |

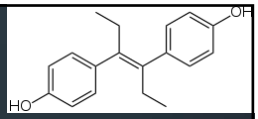



(Pons, *Prenat Diag*, 2005)

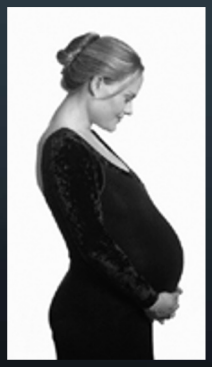
(Slama & Cordier, *J Gyn Obs Biol Reprod*, 2013)

42

# DES (Diethylstilbestrol), a potent synthetic estrogen: *Adverse effects*




**Pregnant woman**
(adult exposure)



Increased breast cancer risk

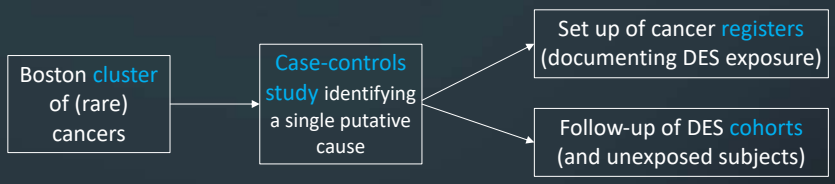
**1st generation**
(exposed *in utero*)


**2nd generation**

Probable increase in the risk of male congenital malformations of genitaliae (hypospadias)

43

## Some lessons from the DES drama regarding the detection of adverse effects of drugs




- "The first 7 cases of clear cell adenocarcinoma were seen by Herbst and colleagues at Massachusetts General Hospital, where the prevalence of use of DES by pregnant women was high and the DES doses were among the highest in the USA.
- This high prevalence resulted from the fact that George and Olive Smith, ardent proponents of DES in the 1940's for the ''treatment of threatened and habitual abortion'' practiced at this hospital.
- Thus, the identification of this potent carcinogen required two unlikely events:
  - an unusually high prevalence of use of the drug in a small area,
  - and recognition by alert clinicians of this highly unusual cancer cluster.
- Without this connection, no DES cohorts would have been established, no screening for DES would have occurred and the teratogenic reproductive effects in these women may have gone unrecognized."

➔ Relying only on *clusters* to trigger larger studies cannot be considered as an efficient approach to identify the effects of toxicants, in particular those with less specific effects than DES. More systematic screening and research tools are required. (Swan, *APMIS*, 2001)

44

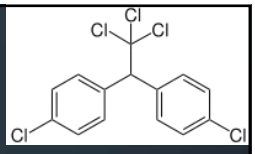
## 6 years to cross the Atlantic: why did France react with delay to ban DES pregnancy use?


- FDA banned DES pregnancy use in the USA in 1971 (the year of the first case-controls study linking it to adenocarcinoma).
- DES pregnancy prescriptions continued until 1977 in France, 1980 in Spain, 1981 in Italy and 1983 in Hungary.
- A. Herbst presented his findings in France in 1972 without strong immediate reaction. The 1st case of clear cell adenocarcinoma in a young woman following DES exposure was identified in France in 1974.
- When DES use during pregnancy was banned in France, a simple information was added in the list of authorized drugs (Vidal) without public announcement nor effort to follow-up persons exposed during pregnancy or in-utero

(Fillion & Torny, *La Recherche*, 2013; *Sc Soc & Santé*, 2016)

45

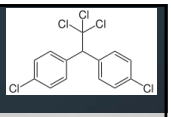

# 1940-1970s: Organochlorinated pesticides

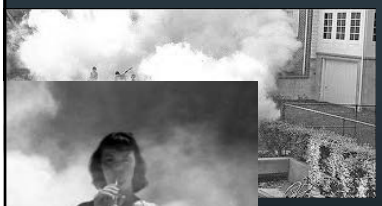



- *1939:* Paul Müller discovers the insecticide properties of DDT
- *World war 2:* Development of the pesticides industry
  - DDT used to fight against typhus in the army
  - DDT used to fight against malaria (Italy, Southern America…)
  - Paul Müller received the physiology Nobel prize in 1948
- *After world war 2*, aerial spreading of pesticides to fight against mosquitoes and ants develops in the USA

46

# Uses of DDT (1940s-70s)



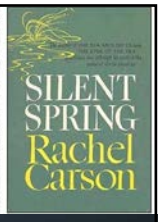
DDT sprayed from a TIFA (Todd Insecticidal Fog Applicator) around model Kay Heffernon to supposedly demonstrate it will not contaminate her food, Jones Beach, New York (1948).

48


https://envisioningtheamericandream.com/2014/04/01/decorating-with-ddt/

49

## DDT: long half-life, short shelf-life…




- 1948: Nobel prize awarded to P. Müller
- 1945-60: Discovery of the environmental persistence of DDT and demonstration of adverse effects on wildlife (e.g. bald eagle)

- 1962: Rachel Carson's Silent Spring book published
- Banned in specific countries (1972: US, W Germany) and internationally (Stockholm POPs convention, 2004)

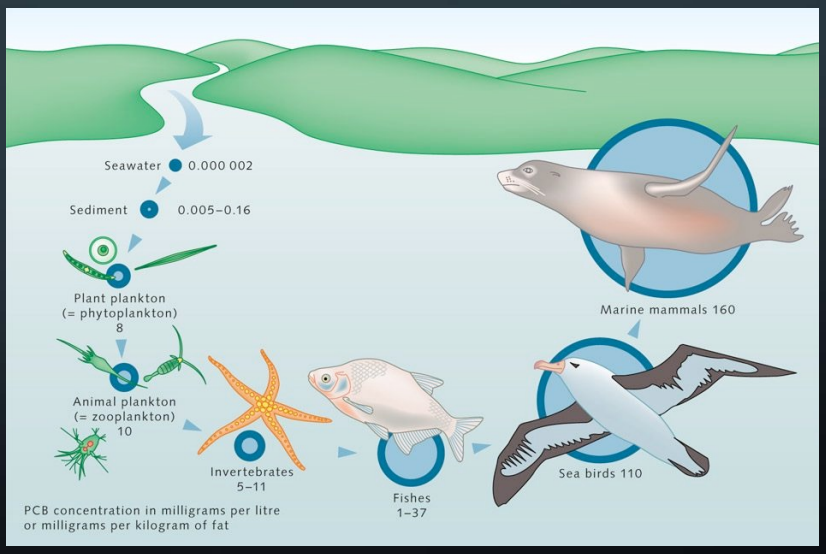
50

## DDT – Absorption, distribution, metabolism

- The commercial DDT was actually a mixture of p,p'-DDT, o,p'-DDT and p,p'-DDD
- Lipophilic compound
- Biodegradation half-life of DDT in soil: 2 to 15 years
- Human half-life: 10 years. Mostly stored in fat tissues
- Main metabolites: DDE and p,p'-DDT
- DDT and its metabolites can cross the placenta
- Excretion in breast milk, resulting in offspring exposure

51

## Biomagnification of DDT and other organochlorine compounds

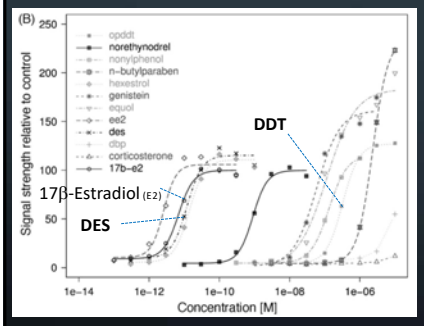



52

# Molecular scale: interactions of DDT and its metabolites with endocrine modalities

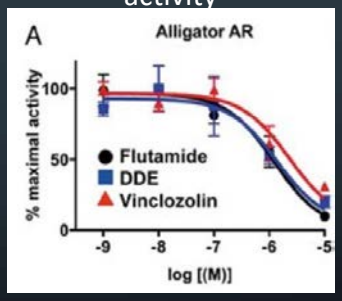
*Note:* Binding affinities of a specific compound are species dependent (Matthews, *J Ster Biochem Mol Biol*, 2000)

Weak estrogenic activity



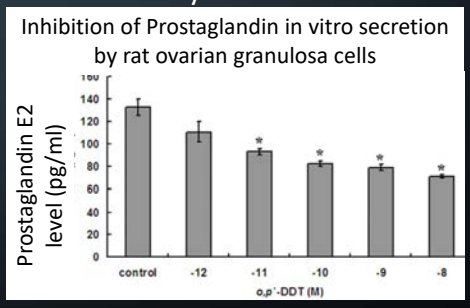

(van der Burg, *Reprod Tox*, 2010)

Anti-androgenic activity




(Miyagawa, *Endocrinol*, 2015)

Inhibition of prostaglandin synthesis

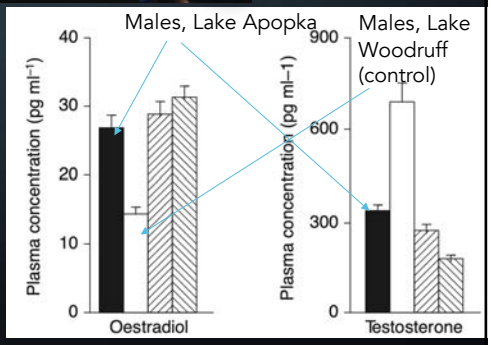



(Liu, *PLoS One*, 2012)

53

# Organochlorine exposure and reproductive health of male alligators from lake Apopka

- Lake Apopka (Florida) was contaminated by a mixture of organochlorine compounds.
- p,p'- DDE, trans-nonachlor, mirex and endrin are present at p.p.b. (µg/kg) concentrations in the serum of juvenile alligators
- Male alligators had higher oestradiol and lower testosterone levels than those from a control lake not contaminated; they also had a smaller phallus size
- Experimental studies confirmed the ability of DDT metabolites to induce sex reversal



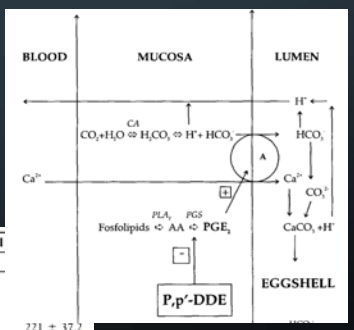

(Guillette Jr, *Reproduction*, 2001)

54

# DDT and reproductive potential of birds

- Decreases in the size of bird species around the US Great lakes (fear of a "silent spring", R. Carson)
- These lakes have been contaminated by various organochlorine compounds
- Various biological mechanisms may have been implied, including a thinning of the eggshell (and hence a reduced litter size)

- DDT has been shown experimentally to induce a thinning of eggshells
- At a finer scale, this may be explained by the inhibition of prostaglandins (implied in the shell synthesis) by DDT metabolites




| Time | EI Control | EI DDE | EI Diff. % | p,p′-DDE level ug/g w.w. |
|---|---|---|---|---|
| Pretreatment | 2.25 | 2.22 | −1.3 | N.D. |
| 0–10 Days | 2.37 | 2.19 | −7.6* | 7.8 ± 0.95 |
| 10–20 Days | 2.40 | 2.07 | −13.8* | 17.3 ± 1.14 |
| 20–30 Days | 2.41 | 2.00 | −17.0* | 25.3 ± 3.4 |
| 35–45 Days | 2.38 | 1.95 | −18.0* | 51.4 ± 6.1 |
| | | | | 221 ± 37.2 |
| | | | | 342 ± 38.1 |

N.D. = not detectable, w.w. = wet weight; f.w. = fat weight (hexane/ether extraction). The statistical significance (t-test) of the difference between control and p,p′-DDE treated groups are denoted *$p < 0.001$. Eggshell Index was calculated as: eggshell weight (mg)/length (mm) × breadth (mm). Data from (79).

(Lundholm, *Comp Biol Phys*, 1997)

55

# DDT effects in humans? Breast cancer

DDT assay (1959-67)

Child Health and Development Studies (CHDS)

**Follow-up of exposed (F0) women until age 50**

**(1960-1998)**

No overall association with breast cancer risk overall.

Possible increased risk in relation to DDT for women aged <14 yrs in 1945 (when DDT use became widespread)

DDT has anti-androgenic and **oestrogenic properties**.
Oestrogens exposure is implied in breast cancer risk.
**Could DDT exposure increase breast cancer risk?**

(Cohn, *EHP*, 2007)

Delivery

**Follow-up of offspring (F1 cohort)**

**(1960-2010)**

**Association between DDT pregnancy level and breast cancer incidence**

Early-life exposure to DDT is a possible cause of breast cancer

(Cohn, *JCEM*, 2015)

56

# Breast cancer: implication of estrogenic compounds

- Breast cancer is the most frequent cancer in the EU (405,000 cases/year)
- First connection between breast cancer and the ovary made in 1896 (quoted by Khan, *J Biol Chem*, 2010)
- Reproductive life factors are associated with breast cancer risk. They are also associated to the level of endogenous reproductive hormones
- The protein levels of ERα and of the progesterone receptor (PR) are elevated in premalignant and malignant breast lesions.
- Estrogens stimulate cancer cells proliferation
- The first anti-cancer drug efficient against breast cancer is Tamoxifen, a compound with anti-estrogenic properties. Other efficient therapies such as ICI 182780 bind to ER.
- Breast cancer carcinogenesis has been hypothesized to be influenced by endogenous hormones or exogenous hormonally active substances that alter estrogen metabolism (Davis DL, *EHP*, 1997)

57

# Breast cancer: some certain or likely risk factors

## Genetic polymorphisms

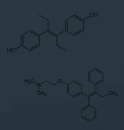
*BRCA1, BRCA2, TP53…*

## Reproductive life factors linked to estrogen exposure

Low number of pregnancies
Early menarche, late menopause
Low duration of breastfeeding

## Estrogenic drugs

Diethylstilbestrol (DES)
Hormonal treatments of menopause (Fabre, *Br J Canc*, 2007)
Estroprogestative contraception (CGHFBC, *Lancet*, 1996)



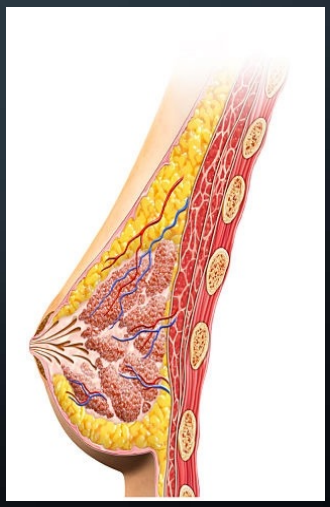
## Chemicals with estrogen-like properties

DDT (early-life) (Cohn, *J Nat Canc Inst*, 2019)
Bisphenol A (Jenkins, *J Steroid Biochem Mol Biol*, 2012)
PCB (Deygas, *Env Res*, 2021)
Xeno-estrogenic burden (Pastor-Barriuso, *Env Health Perspect*, 2016)

## Other environmental factors

Night work (IARC, *Lancet Oncol*, 2019)
Atmospheric pollution (Gabet, *EHP*, 2021)
Dioxin
Alcohol, tobacco smoke
Ionising radiation

**Breast cancer**



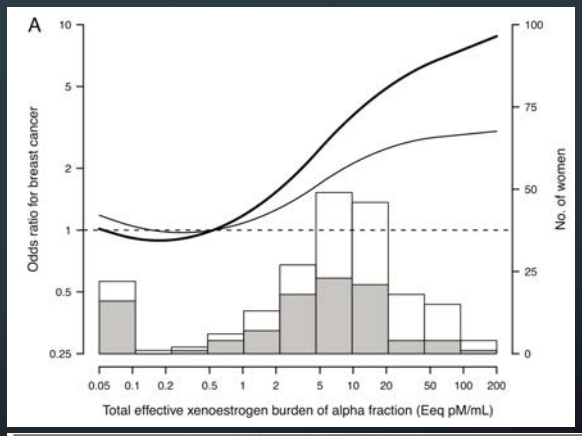
Adapted from (Demeneix & Slama, EDs: Scientific evidence and human health protection, report to the EU Parliament, 2019). Thanks to P Guénel.

58

# Xenoestrogens and breast cancer incidence

**Case-controls study** of 186 incident pre-treatment breast cancer cases and 196 controls. **Assessment of TEXB in cases and controls.**

**TEXB** (total effective xenoestrogen burden)
1. Liquid chromatography used to separate environmental estrogens (a-fraction) from sex-steroids (b-fraction).
2. The estrogenic effect of the extracts is then determined from its proliferative effect on MCF-7 human breast cancer cells




| TEXB | Tertile 1 | Tertile 2 | Tertile 3 | *p* for trend[a] | Undetermined estrogenicity[b] |
|---|---|---|---|---|---|
| Serum TEXB-α[c] (Eeq pM/mL) | ≤ 2.62 | 2.63–8.75 | ≥ 8.76 | | |
| Number of controls/breast cancer cases | 35/18 | 35/32 | 35/57 | | 90/79 |
| Model 1[d] | 1.00 (Reference) | 1.64 (0.74, 3.62) | 3.04 (1.38, 6.70) | 0.005 | 0.83 (0.52, 1.32) |
| Model 2[e] | 1.00 (Reference) | 1.77 (0.76, 4.10) | 3.45 (1.50, 7.97) | 0.003 | 0.73 (0.45, 1.20) |
| Model 3[f] | 1.00 (Reference) | 1.50 (0.55, 4.08) | 1.80 (0.63, 5.09) | 0.32 | |
| Serum TEXB-β[c] (Eeq pM/mL) | ≤ 4.56 | 4.57–11.27 | ≥ 11.28 | | |
| Number of controls/breast cancer cases | 42/21 | 42/43 | 42/58 | | 70/64 |
| Model 1[d] | 1.00 (Reference) | 2.14 (1.06, 4.35) | 3.27 (1.62, 6.61) | 0.002 | 0.86 (0.52, 1.41) |
| Model 2[e] | 1.00 (Reference) | 2.35 (1.10, 5.03) | 4.01 (1.88, 8.56) | 0.001 | 0.97 (0.58, 1.65) |
| Model 3[f] | 1.00 (Reference) | 1.75 (0.65, 4.71) | 3.53 (1.24, 10.0) | 0.02 | |

Abbreviations: Eeq pM/mL, estradiol equivalent in picomolar per milliliter of serum; TEXB-α, total effective xenoestrogen burden of α fraction; TEXB-β, total effective xenoestrogen burden of beta fraction.

(Pastor-Barriuso, *Env Health Perspect*, 2016)

59

# Other long-term possible effects of DDT in humans

(F0 generation)

DDT assay (1959-67)

Increased risk of early age (until 11 years) at menarche in F2 women in relation with the grandmother's (F0) DDT pregnancy levels

| Model Level of Adjustment | Estimated *o,p'*-DDT Association Odds Ratio[2] | (95% CI[3]) | p-value |
|---|---|---|---|
| DDT congeners and F0 Age at Menarche Adjusted | 2.23 | (1.24, 4.01) | 0.0076 |
| + F1 Age at Menarche Adjusted | 2.25 | (1.24, 4.08) | 0.0077 |
| + Race Adjusted | 2.06 | (1.10, 3.87) | 0.0235 |
| + F1 BMI Adjusted | 2.08 | (1.11, 3.90) | 0.0222 |

(Cirillo, *Cancer Epidemiol Biomarkers Prev* , 2021)

(F1 generation)

Increased risk of obesity in middle age in relation to maternal DDT level (La Merrill, *Int J Obes*, 2020)

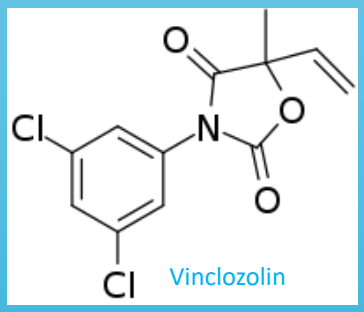
Follow-up in 2010-13, median age, 26

(F2 generation)

60

## Other examples of disruptors of steroid hormones: *disruption of the androgen axis*


(another drug)




Vinclozolin

(another pesticide)

61

## Endocrine (possibly anti-androgenic) properties of currently marketed drugs: the case of mild analgesics

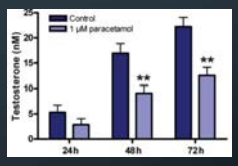
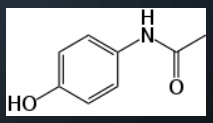
- A few studies in humans suggested that the use of mild analgesics during pregnancy (e.g., acetaminophen/paracetamol, aspirin) can increase the risk of malformations of the male genitalia (hypospadias, cryptorchidism)
- In *in vivo* and *ex vivo* models, analgesics can also alter anogenital distance and testis function at birth, alter testosterone synthesis following early-life or adult exposure.
- At a more fundamental level, analgesics may be able to inhibit androgen synthesis (via an inhibition of the expression of steroidogenic enzymes).
- They also can inhibit prostaglandins.
- Rat studies indicate that androgen deficiency during a critical male programming window (corresponding to 8–14 weeks of gestation in humans) leads to cryptorchidism, hypospadias, compromised fertility and reduction in anogenital distance

(Kristensen, *Nat Rev Endo*, 2016)

Bernard Jégou (1951-2021)

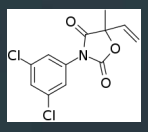
62

# Analgesics and anomalies of male genitalia: from molecular to population evidence

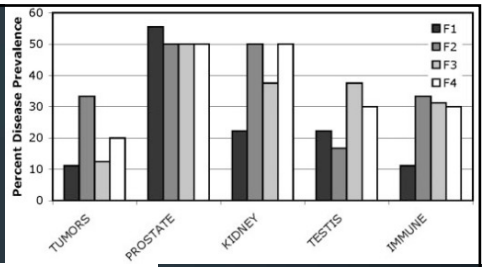
| Individual scale (cohorts) | Individual scale (in vivo toxicology) | Cellular scale (in vitro toxicology) | Molecular scale |
|---|---|---|---|
| Possible increased risk of hypospadias and cryptorchidism (reviewed by Kristensen, *Nat Rev Endo*, 2016) | Lack of convincing association with cryptorchidism in animal models? |  (Kristensen, *Human Reprod*, 2011) |  Inhibition of androgen synthesis |

63

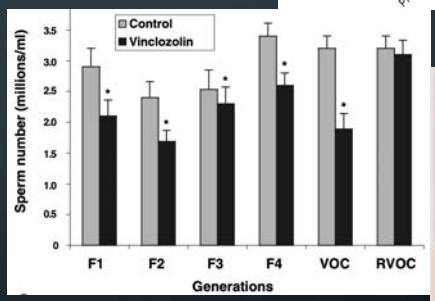
# Vinclozolin transgenerational effects



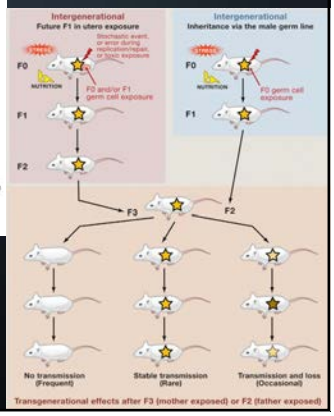
- Vinclozolin, a (now banned) pesticide with anti-androgenic properties has been shown to induce diseases in 4 generations (F1-F4) of male rats following intra-uterine exposure of F1 generation (Anway, *Science*, 2004; *Endocrinology*, 2006)
- Possible suggested mechanism: transgenerational *epi*genetic inheritance (Vaiserman, *Epig Chrom*, 2017; Legoff, *Cell*, 2019).
- Although it exists in plants and nematodes, the existence of this mechanism in mammalians is debated due to in utero genome-wide DNA reprogramming (Heard, *Cell*, 2014). Other mechanisms (including genetic mutations) are possible.




(Anway, *Endocrinology*, 2006)



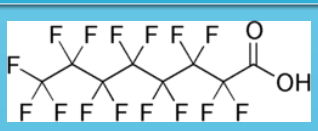

(Anway, *Science*, 2005)




(Heard *Cell*, 2014)

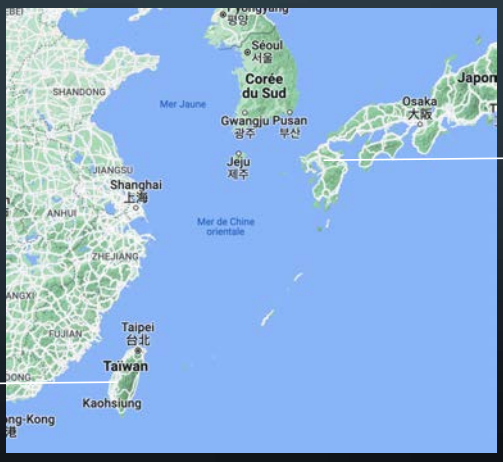
64

## D. Thyroid disruption: PCBs, Perfluorinated compounds (PFCs)


65

## Yu-Cho and Yu-Cheng ("oil disease") PCB contaminations

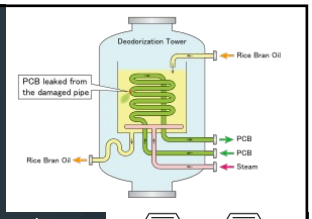


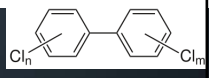

Yu-Cho (1968)
(Fukuoka and Nagasaki areas)

Yu-Cheng (1979)

66

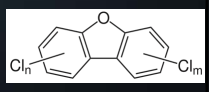
# Yu-Cho and Yu-Cheng dramas




PCBs

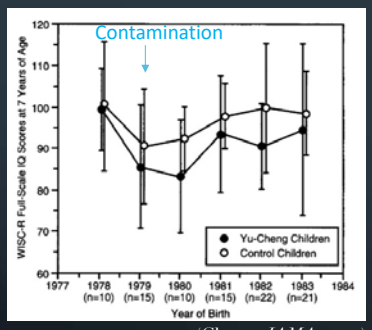
- Contamination of rice bran oil by PCBs used to heat the oil to deodorize it. The contamination also included PCDFs (polychlorinated dibenzo furans, PCBs degradation products)
- The oil was used as a feed supplement for poultry and for cooking
- The first signs in Yu-Cho (1968) were the death of poultry
- Symptoms in humans included dermal and ocular lesions, menstrual cycle disorders, immunosuppression.
- An excess risk of mortality for all cancers, lung and liver cancer (women) were observed (Onozuka, *Environ Health*, 2020).
- A 'Fetal PCB Syndrome' (FPS) was observed in some newborns: dark brown pigmentation of the skin and mucous membrane, gingival hyperplasia, fetal growth suppression, precocious dentition and abnormal calcification of the skul (Yamashita, *EHP*, 1985)




PCDFs

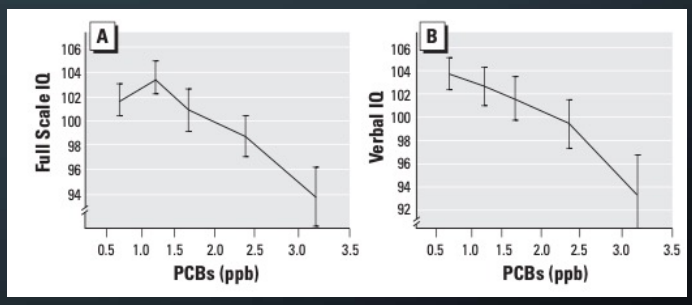
67

# PCB associations with cognition in childhood




(Cheng, *JAMA*, 1992)

Oswego (lake Ontario) cohort, n=156 children 9-11 years

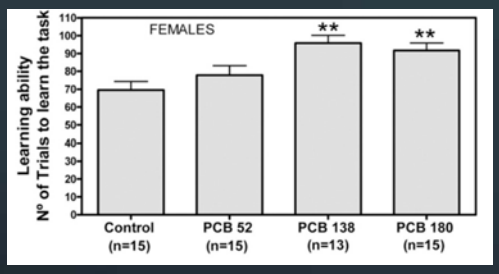


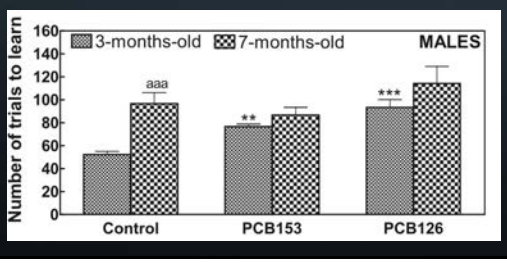

(Stewart, *EHP*, 2008; *Neurotox Toxicol*, 2012)
See also (Ribas-Fito, *JECH*, 2001) for an early review

68

# PCB effects on cognition in animal models

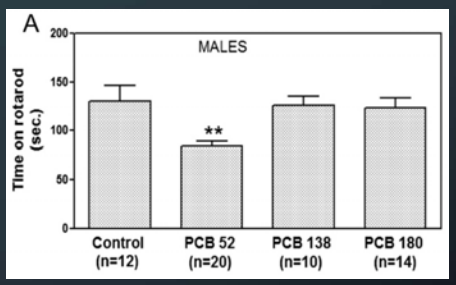
Impairment of learning ability




(Piedrafita, *Eur J Neurosc*, 2007)

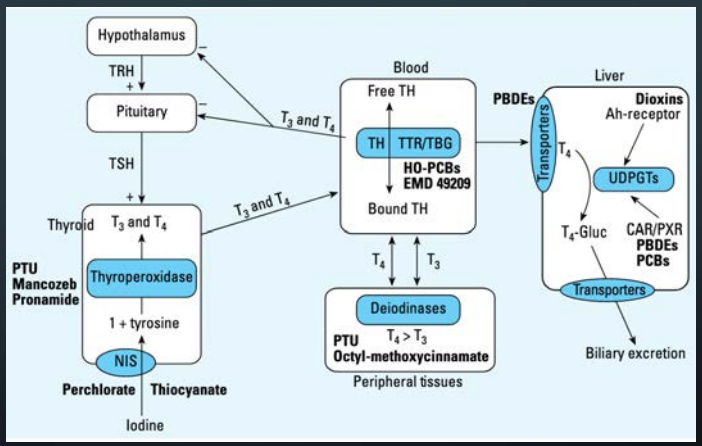
Impairment of motor coordination by PCB 52




(Boix, *Neuroscience*, 2010)

69

# Hypothesized mechanisms of influence various endocrine disruptors on the thyroid axis: *implication of liver metabolism of thyroid enzymes*




(Miller, *Env Health Perspect*, 2009)

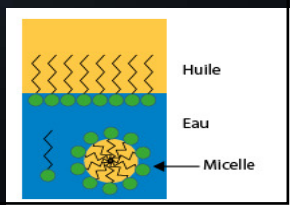
Abbreviations: Gluc, glucose; HO-PCBs, hydroxyl-PCBs; NIS, sodium/iodide symporter; PBDE, polybrominated diphenyl ether; PTU, propylthiouracil; T4-Gluc, T4-glucuronide; TBG, thyroid-binding globulin; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; TTR, transthyretin; UDPGT, uridine diphosphate glucuronyl-transferase. Xenobiotics that block, inhibit, or up-regulate these processes are shown in bold
Modified from Crofton 2008.

70

(PFOA)

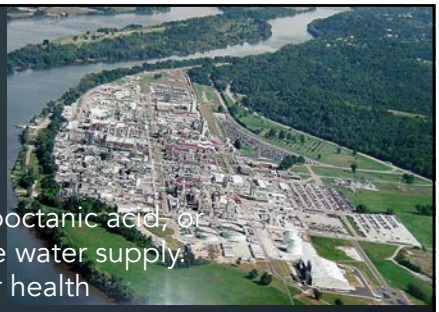
# Perfluoroalkyl substances (PFASs)

- PFASs are organic compounds containing fluorine atoms attached to an alkyl chain: $-C_nF_{2n}-$
- There are over 4,000 different PFASs (https://comptox.epa.gov/dashboard/chemical-lists/PFASSTRUCT)
- They are persistent in the environment
- Some have surfactant properties: both water soluble and lipophilic
- Uses include *fluoropolymers* coating (e.g., in frying pans: Teflon), firefighting foams, ski wax, stain repellents
- Currently, the compounds most studied in terms of health effects are PFOA and PFOS, two 'historical' perfluorinated compounds




71


# PFOA - The C8 trial

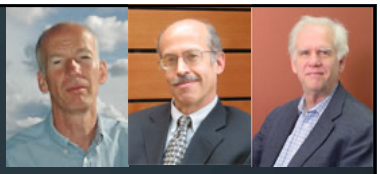


- A DuPont manufacturing facility producing PFOA (perfluorooctanic acid, or C8) in Parkersburg, West Virginia and Ohio contaminated the water supply.
- 2001: Community residents sued DuPont in a class action for health damages given toxicological knowledge about PFOA hazards
- 2004: Settlement of the class action. Both parties accepted to establish a scientific panel (C8 Science Panel) with 3 epidemiologists with the task to determine whether PFOA was indeed linked to health of the community.
- The Science panel was asked to reach a judgment about whether disease was "more probably than not" linked to PFOA.
- Large research funds ($70 million) were provided to the panel
- 12 studies were conducted over 5 years
- If one or more diseases was found linked to PFOA, people with the disease in question would be free to sue DuPont for damages

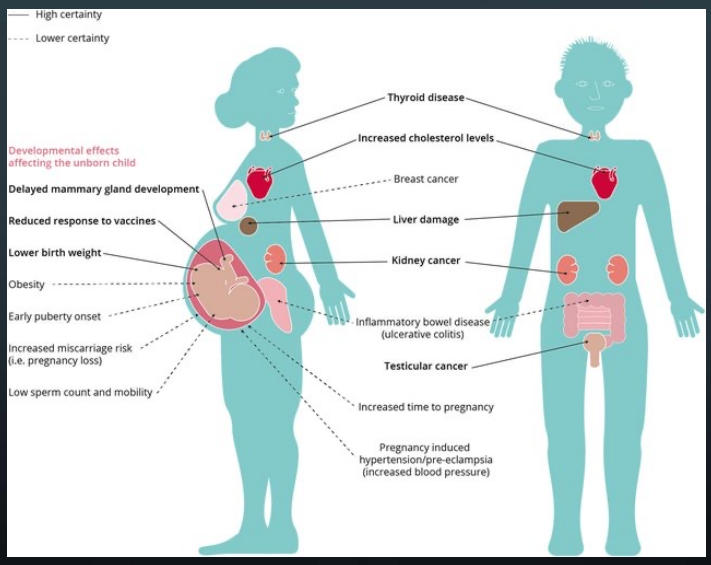
(Steenland, *Epidemiology*, 2014)

72

# Conclusions of the C8 Science Panel
## regarding likely effects of PFOA



T. Fletcher D. Savitz K. Steenland




In 2017, DuPont and its spin-off local company agreed to pay $671 million to settle the lawsuit.
In another settlement in Minnesota, 3M paid $850 million, allowing to restore contaminated water resources.

(Steenland, *Epidemiology*, 2014)
http://www.c8sciencepanel.org/publications.html
Figure from (Fenton, *Env Tox Chem*, 2020)

73

# Lecture overview

Lecture #5

A. Introduction: classifying toxicants
B. Endocrine disruption – Generic considerations
C. Disrupting the estrogenic axis: DES, DDT
D. Disrupting the thyroid axis: PCBs, PFASs

Interactions with nuclear receptors *(seminar of W. Bourguet)*

Lecture #6

E. Characterizing effects of non-persistent compounds in humans
F. Triclosan and bisphenols
G. Social inequalities in exposure
H. Health and societal impact
I. Evaluation of risk management options
J. Risk management

Mixture effects *(seminar of Pr. A. Kortenkamp)*

74