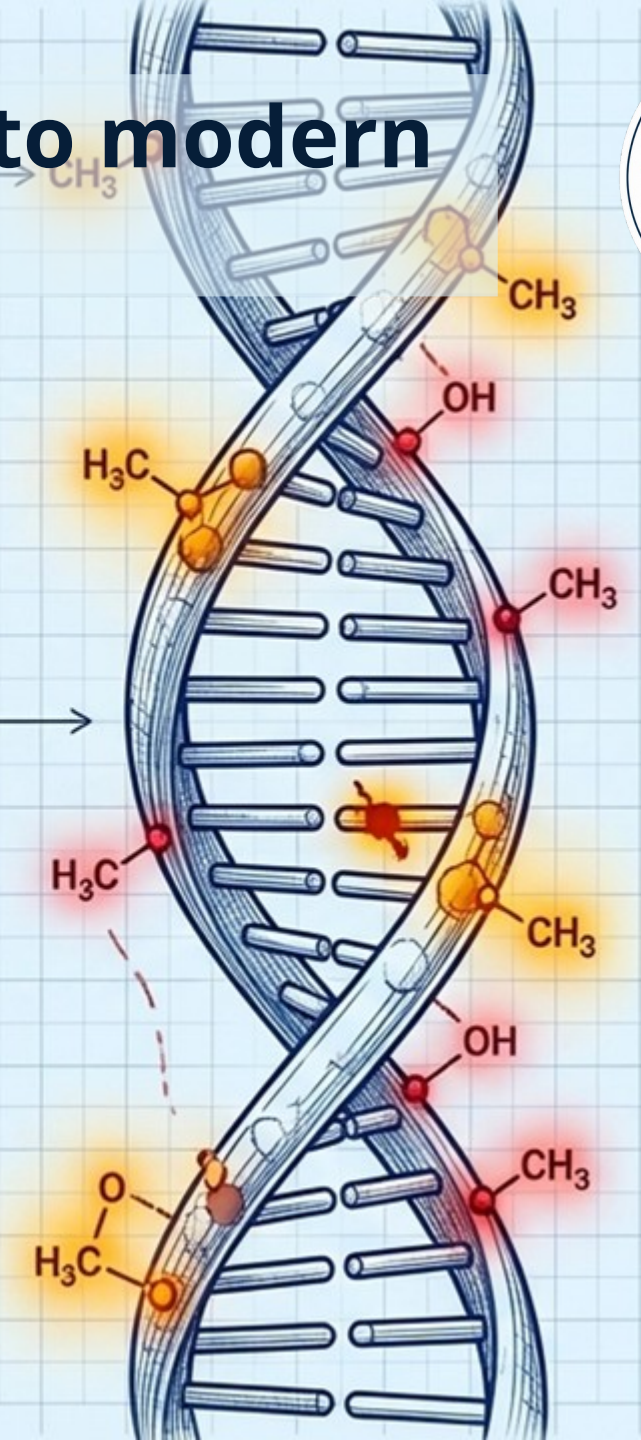
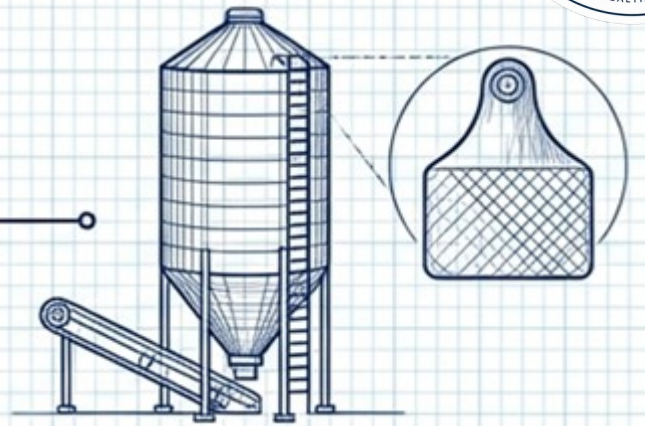
The One Health approach to modern toxicology

Diethylstilbestrol (DES): Epigenetic Legacy, Sentinel Animals and One Health

From “miracle drug” to foundational model for transgenerational epigenetics and the fetal origins of adult disease



The Illusion of the Miracle Drug (1940s-1970s)



Between 2 and 8 million women may have been exposed during pregnancy in the USA alone; DES was also used in livestock production.

Human use

Massively prescribed to pregnant women under the mistaken belief that it could prevent miscarriage and obstetric complications. Also used for lactation suppression and hormonal therapies.

Veterinary use

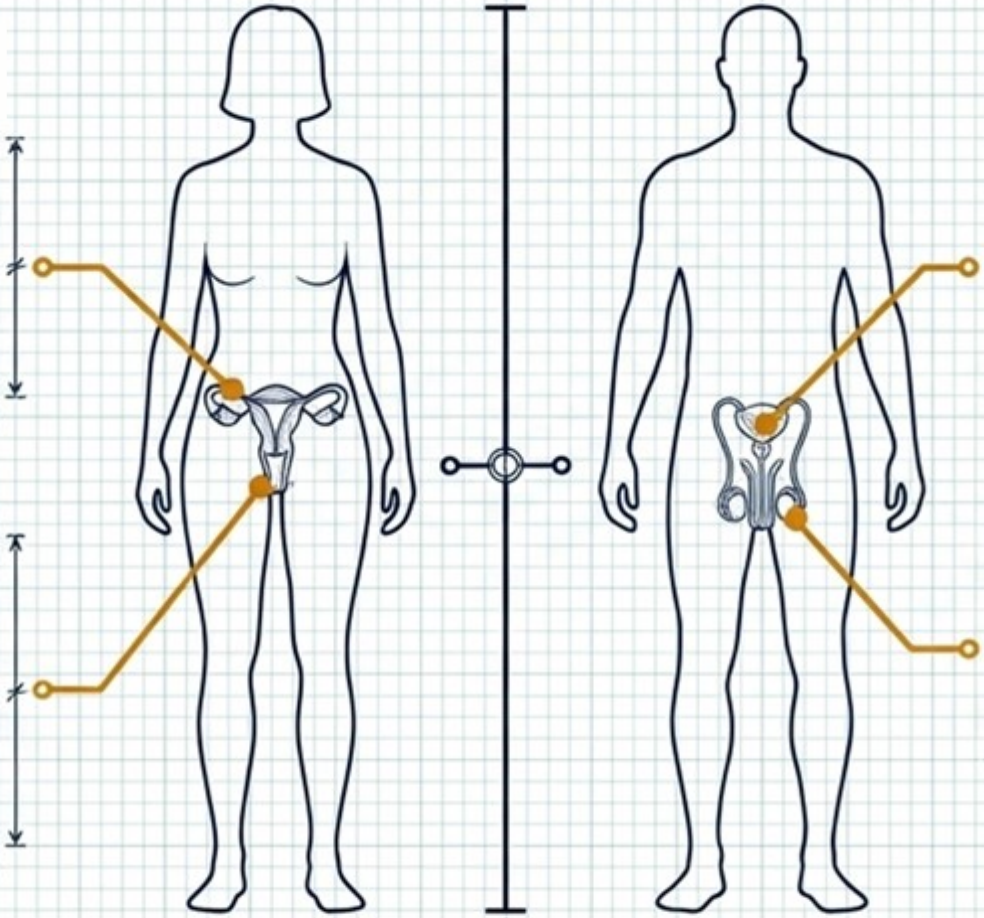
Widely used as a potent growth promoter in beef cattle; approved by the FDA in 1954 as Stilbosol.

1971: USE DURING PREGNANCY DISCONTINUED. THE DISCOVERY OF DEVASTATING SIDE EFFECTS ON DEVELOPING FETUSES DESTROYS THE MYTH OF THE PLACENTA AS AN IMPENETRABLE BARRIER.

DES daughters (F1)

Primary outcome: Early post-pubertal clear-cell vaginal adenocarcinoma (A very rare tumor form).

Structural damage: reproductive tract malformations; infertility, miscarriage and preterm birth.



The sons of DES (F1)

Genital anomalies: such as hypospadias (urethra malformation) and epididymal cysts.

Functional damage: testicular hypotrophy and reduced sperm quality and quantity.

The Transgenerational Legacy (F2 and Beyond)

Beyond direct exposure: DES toxicity can project its effects beyond the generation exposed directly in utero.

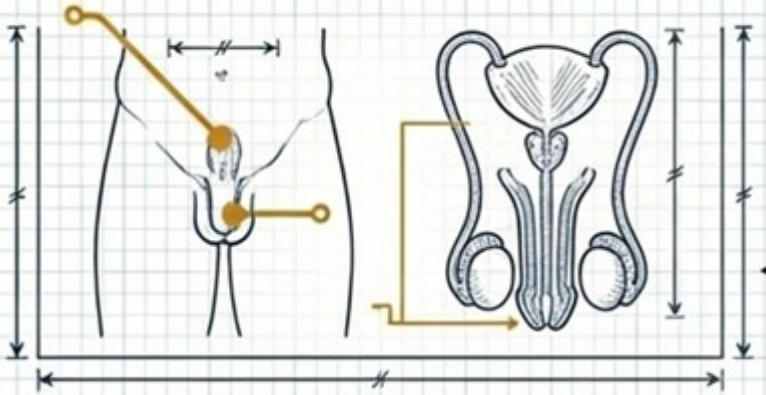
F0
(Mother)

F1
(Offspring)

F2
(Grandchildren)

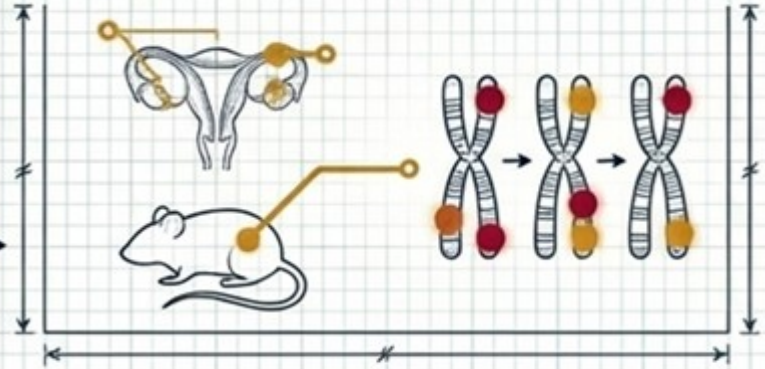
Human evidence: F2 male

Studies have reported increased genital anomalies in male grandchildren of DES-exposed women; evidence for female grandchildren remains under investigation.



Validation: F2 Animal Models

Mouse studies show that adverse effects of perinatal DES exposure may be transmitted to subsequent generations through the germ line.

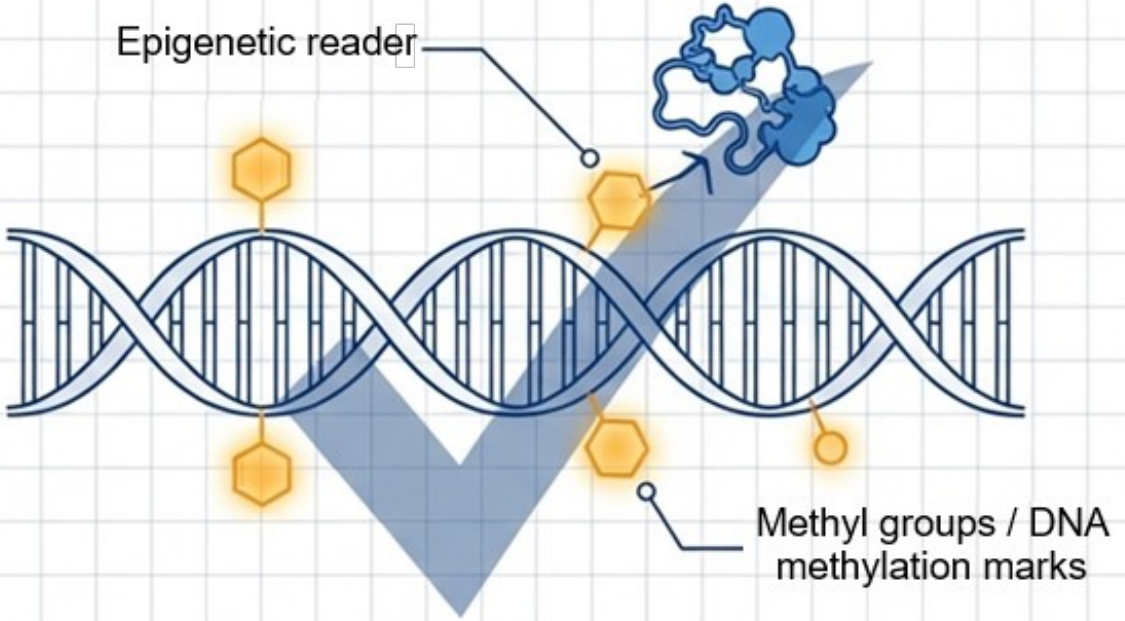


The Mechanism: Not Mutation, but Epigenetics

False myth: genotoxicity

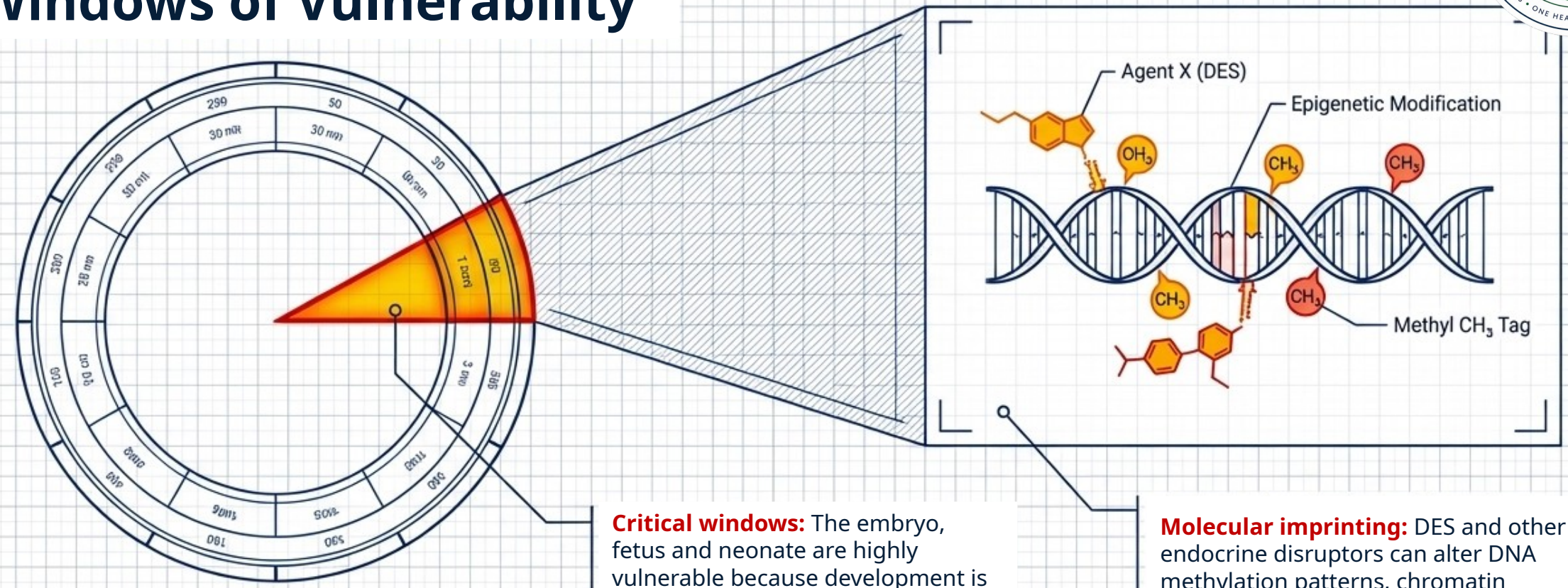
The observed malformations are not primarily explained by classical DNA-sequence mutations: the genetic “hardware” may remain unchanged.

Reality – Epigenetic alteration



DES acts as a model endocrine disruptor, leaving molecular marks on chromatin and altering gene expression and phenotype: the biological “software”. **Persistence:** Epigenetic marks may persist over time and help explain delayed and heritable health effects.

Molecular Action and Windows of Vulnerability



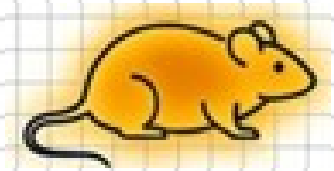
Critical windows: The embryo, fetus and neonate are highly vulnerable because development is hormone-driven and detoxification systems are still immature

Molecular imprinting: DES and other endocrine disruptors can alter DNA methylation patterns, chromatin regulation and estrogen-responsive

Timing matters: The same dose may have very different effects depending on the developmental window of exposure.

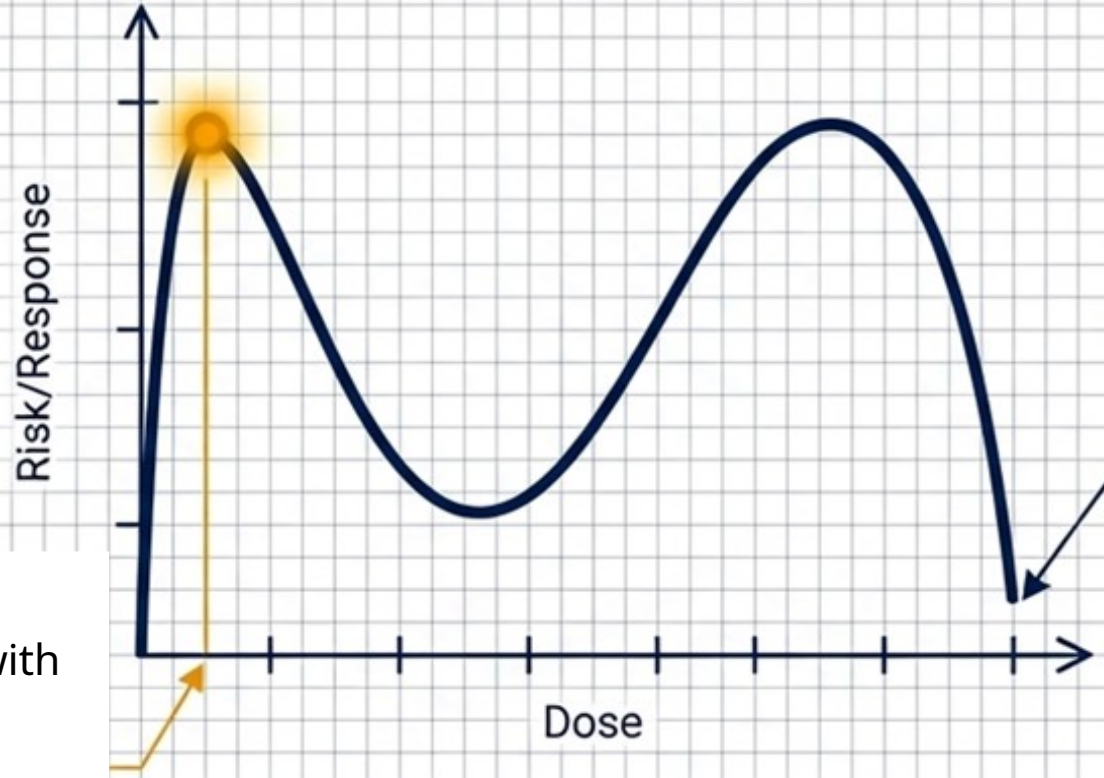
Beyond Reproduction: DES as an Obesogen

L'esposizione perinatale a basse dosi riprogramma i meccanismi di differenziazione degli adipociti (cellule adipose).



Low-dose effect (1ppb)

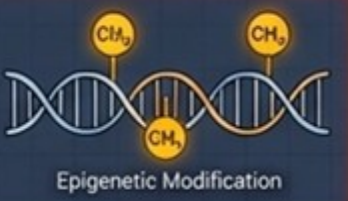
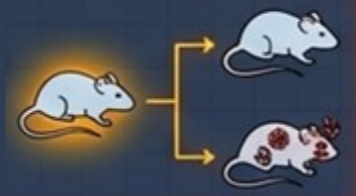
In experimental models, low doses have been associated with adult obesity and metabolic alterations and can produce different and more subtle long-term outcomes.



High doses (1000 ppb)
May cause weight loss

The Paradoxical Effect: The toxicology of DES is characterized by a non-monotonic dose-response curve.

Synthesis: The Birth of the **DOHaD** Paradigm (Developmental Origins of Health and Disease)

Classical toxicology (ante 1971)	Post DES paradigm
The dose makes the poison (linear relationship)	Very low doses can produce systemic effects when exposure occurs during vulnerable developmental windows.
Permanent damage is expected to require overt genetic mutation (genotoxic damage)	The damage is epigenetic: invisible at birth, it alters gene expression in the years that follow. 
For the fetus, the placenta is treated as a protective wall.	Transgenerational Inheritance: Today's environmental pollutants (endocrine disruptors) can reprogram the health of future generations. 

The Epigenetic Legacy of DES

The role of animals: from production tools to environmental sentinels.

Core message: Farm animals, laboratory mice and companion animals have revealed how endocrine disruption can move from managed exposure to long-term biological warning.
One Health relevance: The animal body becomes an early detector of shared chemical environments.

Three Acts of Endocrine Disruption

Act I – Exploitation (the 1950s–1970s)

Intentional use of DES as a livestock growth promoter to increase production efficiency and profit.

Act II – Experimentation (2000 - present)

The CD-1 mouse as a model revealing the obesogen hypothesis and developmental reprogramming (DOHaD).

Act III – Exposure (Present)

Companion animals and wildlife as collateral victims and sentinels of indoor and outdoor chemical contamination.

The Sabotage Mechanism: Xenoestrogens and Receptors

Normal signaling: The receptor binds endogenous estrogen and triggers a physiological response.

Mimicking disruptor: An EDC such as DES can bind as if it were the natural hormone, producing abnormal or amplified responses.

Blocking disruptor: An EDC can occupy the receptor without activating it, blocking the biological signal.

Gli xenoestrogeni non si limitano ai recettori nucleari; alterano i circuiti di feedback nell'ipotalamo, nell'ipofisi e nelle gonadi.

Act I: The Zootechnical Boom and the Growth Illusion

1947 - Purdue University: First demonstration that subcutaneous DES implants (42-48 mg) could stimulate growth in heifers.

1954 - Iowa State College / FDA: Oral DES administration approved (10 mg/day); declared benefits included weight gain (15%) and improved feed conversion (10%).

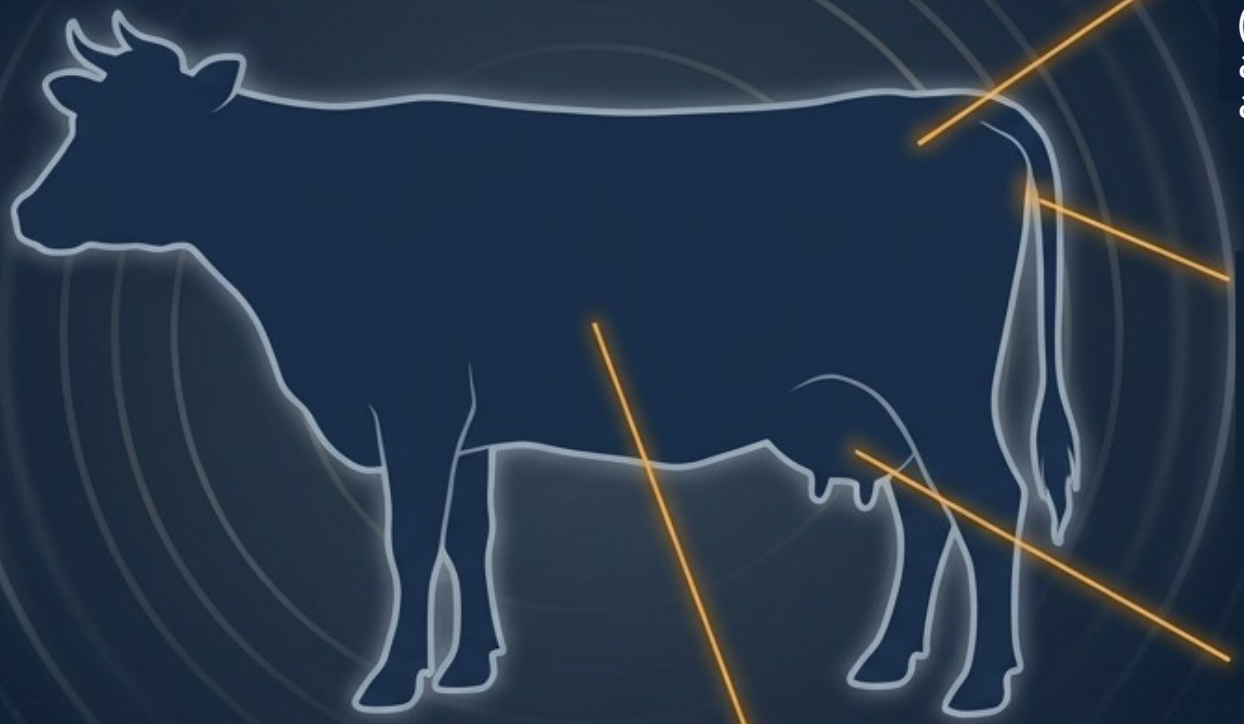
1960s:
A large proportion of feedlot cattle received DES.

1971
Human tragedy (clear cell adenocarcinoma in daughters exposed in utero).

1979
Definitive FDA prohibition of DES use in beef production.

The price of artificial mass

Commercial success: The production benefit masked a profound endocrine disturbance.



Pelvic Area: “Hooky” conformation (prematurely mature appearance and skeletal alteration).

Reproductive area: Marked vulvar swelling, prolonged estrus and abnormal sexual behavior.

Mammary area
Abnormal and premature mammary development

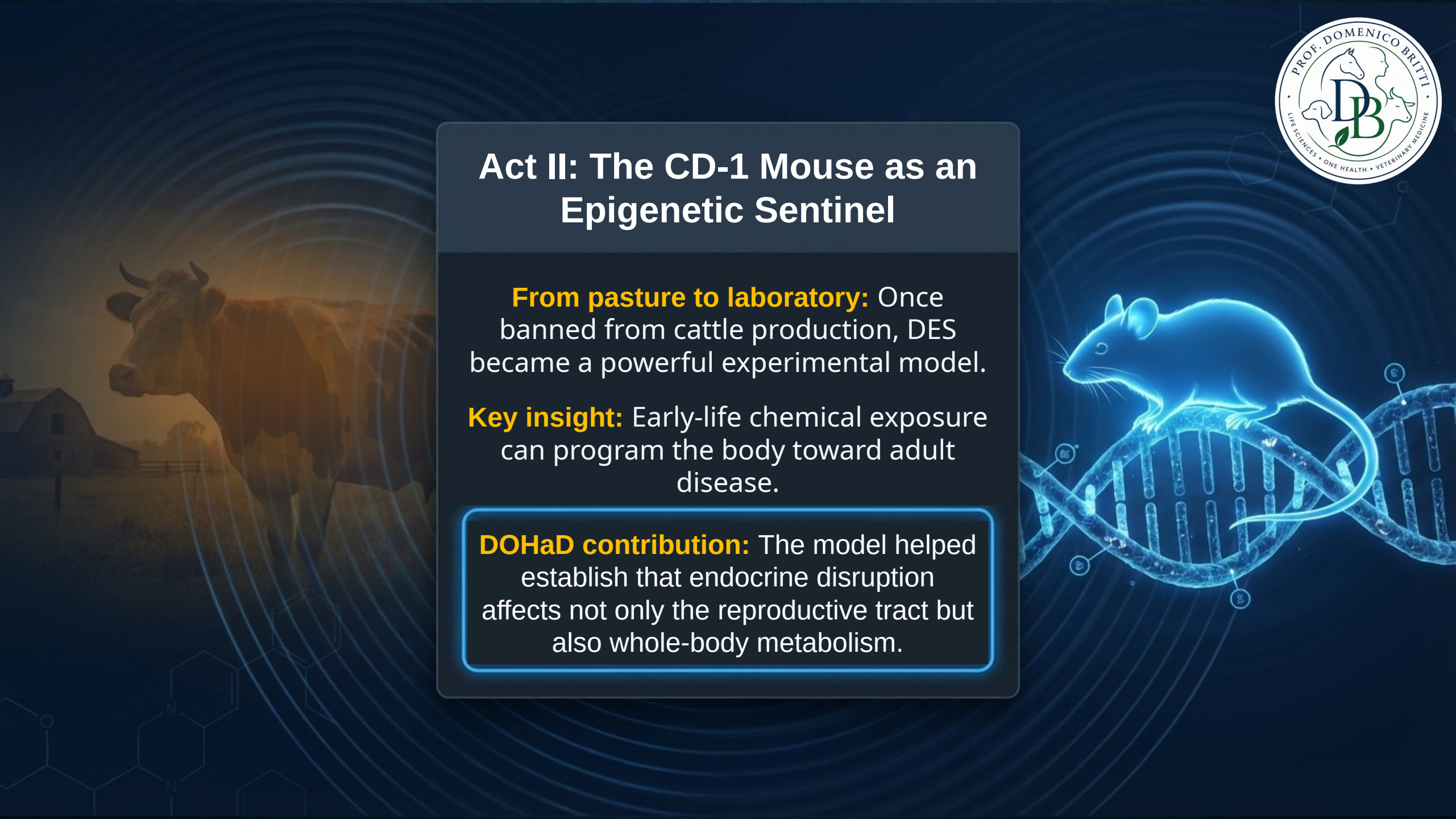
Body composition: reduced carcass grade and unnatural increase in lean mass at the expense of physiological fat.

Act II: The CD-1 Mouse as an Epigenetic Sentinel

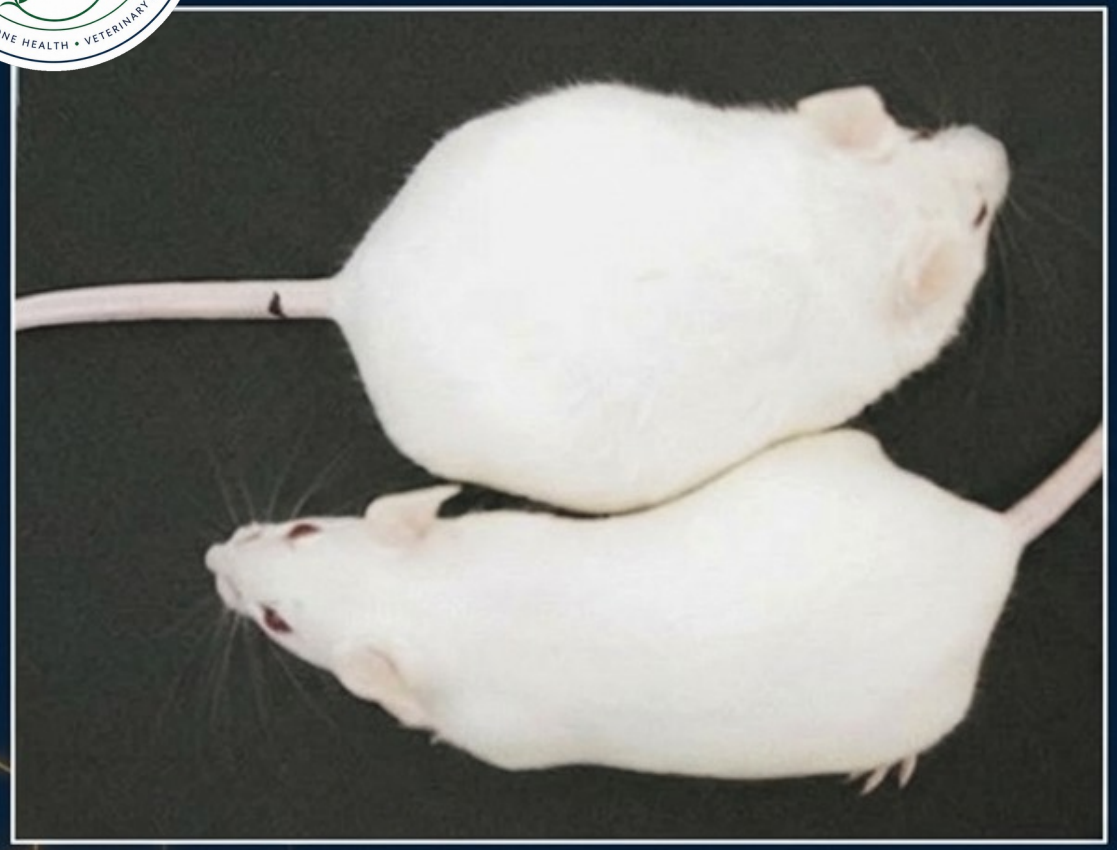
From pasture to laboratory: Once banned from cattle production, DES became a powerful experimental model.

Key insight: Early-life chemical exposure can program the body toward adult disease.

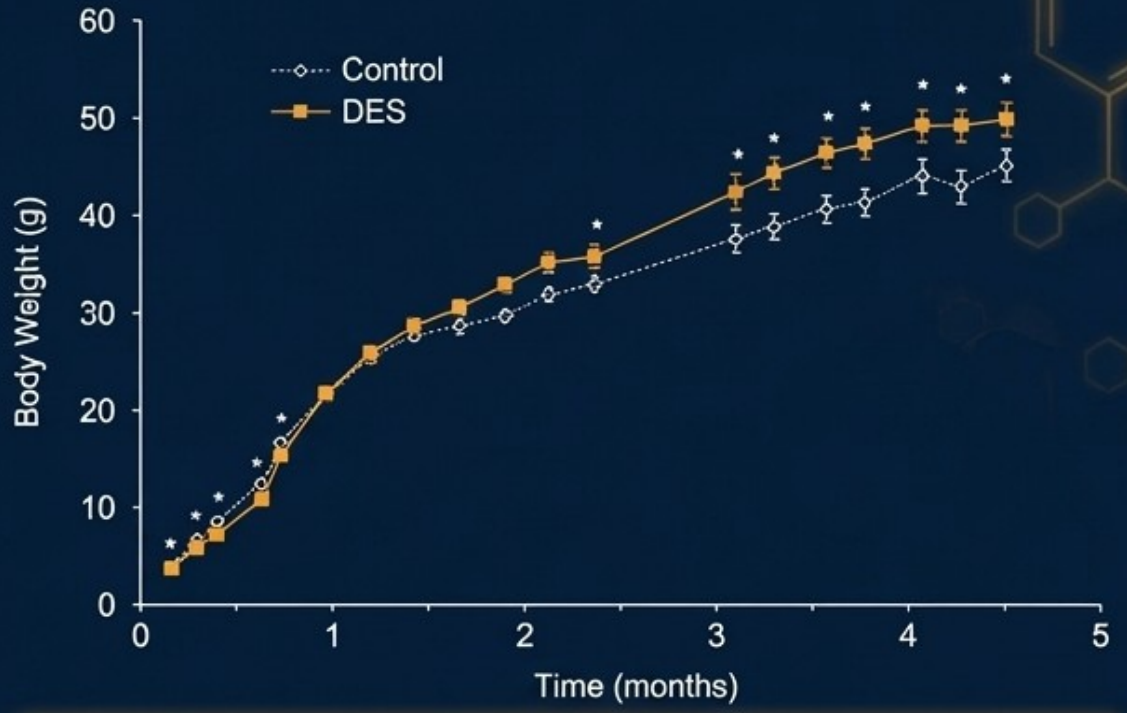
DOHaD contribution: The model helped establish that endocrine disruption affects not only the reproductive tract but also whole-body metabolism.



The Obesogen Hypothesis: A Time Bomb



Visual comparison at 4 months of age.
Obesity is not only a matter of overfeeding; it may reflect early-life chemical reprogramming.



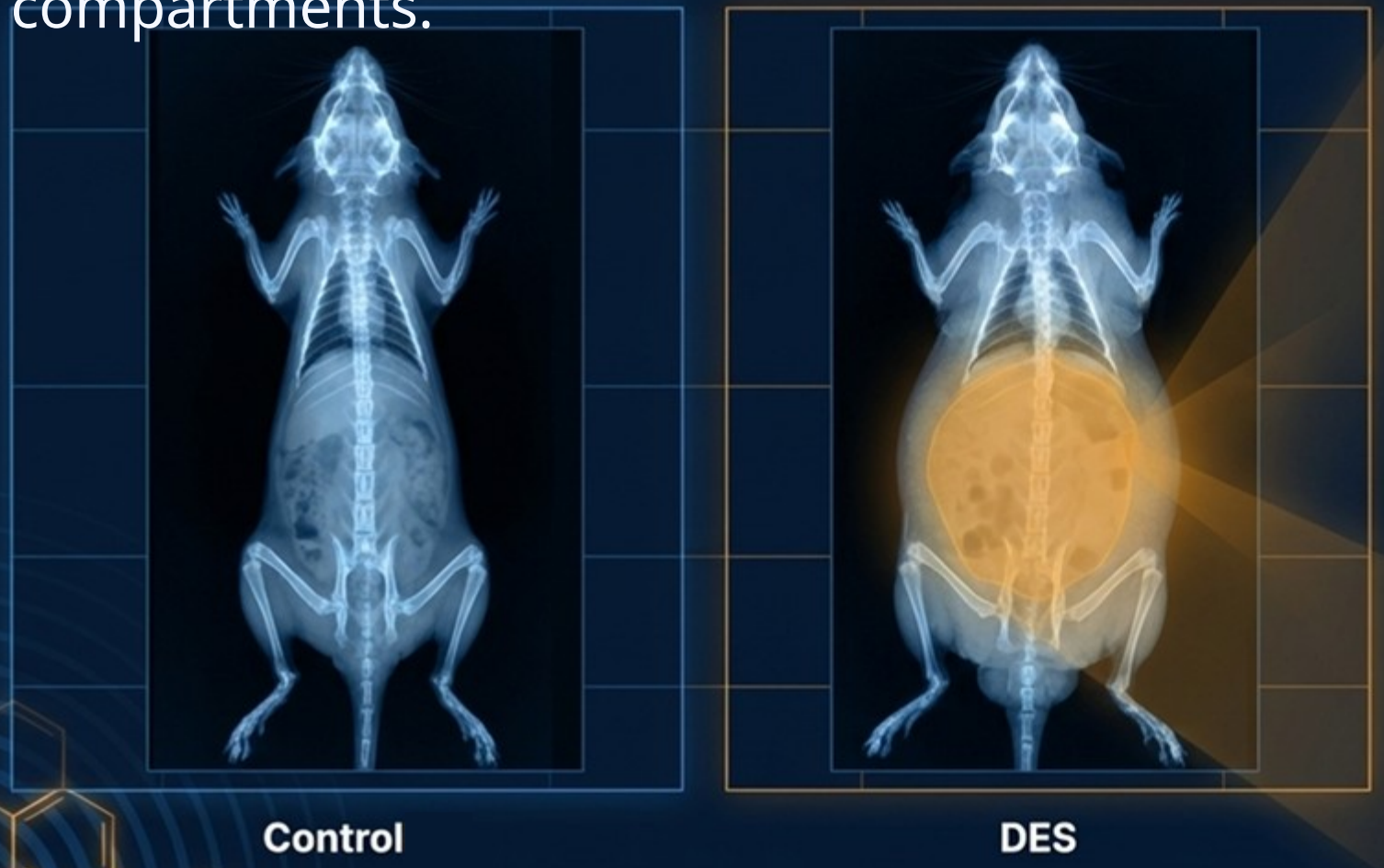
The Protocol: Neonatal exposure (Days 1–5) to DES doses (1 mg/kg).

The Paradoxical Effect (Growth Curve):

- Phase 1 (Treatment): Drastic reduction in initial body weight.
- Phase 2 (Puberty): Rapid catch-up growth period.
- Phase 3 (2+ Months): Surpassing control subjects and development of permanent obesity in adulthood.

Sotto la Pelle: L'Invasione Adiposa

Fat distribution: DES exposure does not only increase mass; it can shift fat deposition toward pathological compartments.



Data Points (Age 6–8 Months):

- **Dramatic increase in estimated fat mass** (40.8% in the DES group vs. 29.9% in controls).
- **Specific and excessive accumulation in the inguinal fat pads** (0.207 g vs. 0.108 g) **and retroperitoneal fat depots** (0.555 g vs. 0.291 g).

Implicazione Clinica:

Questo schema di grasso addominale in eccesso è il fattore predisponente a disfunzioni metaboliche e cardiovascolari, in analogia con alcune dinamiche patologiche umane.

Metabolic Chaos and Genetic Reprogramming

Gli estrogeni ambientali alterano l'espressione dei geni coinvolti nello sviluppo degli adipociti e nella distribuzione del grasso.

The Delayed Echo

Days 1-5
Neonatal Exposure Window

Month 2+
Development of obesity in adulthood

The Genetic Imprint (Mouse Uterus at 19 Days):

Thbd Gene — Downregulated (OFF)

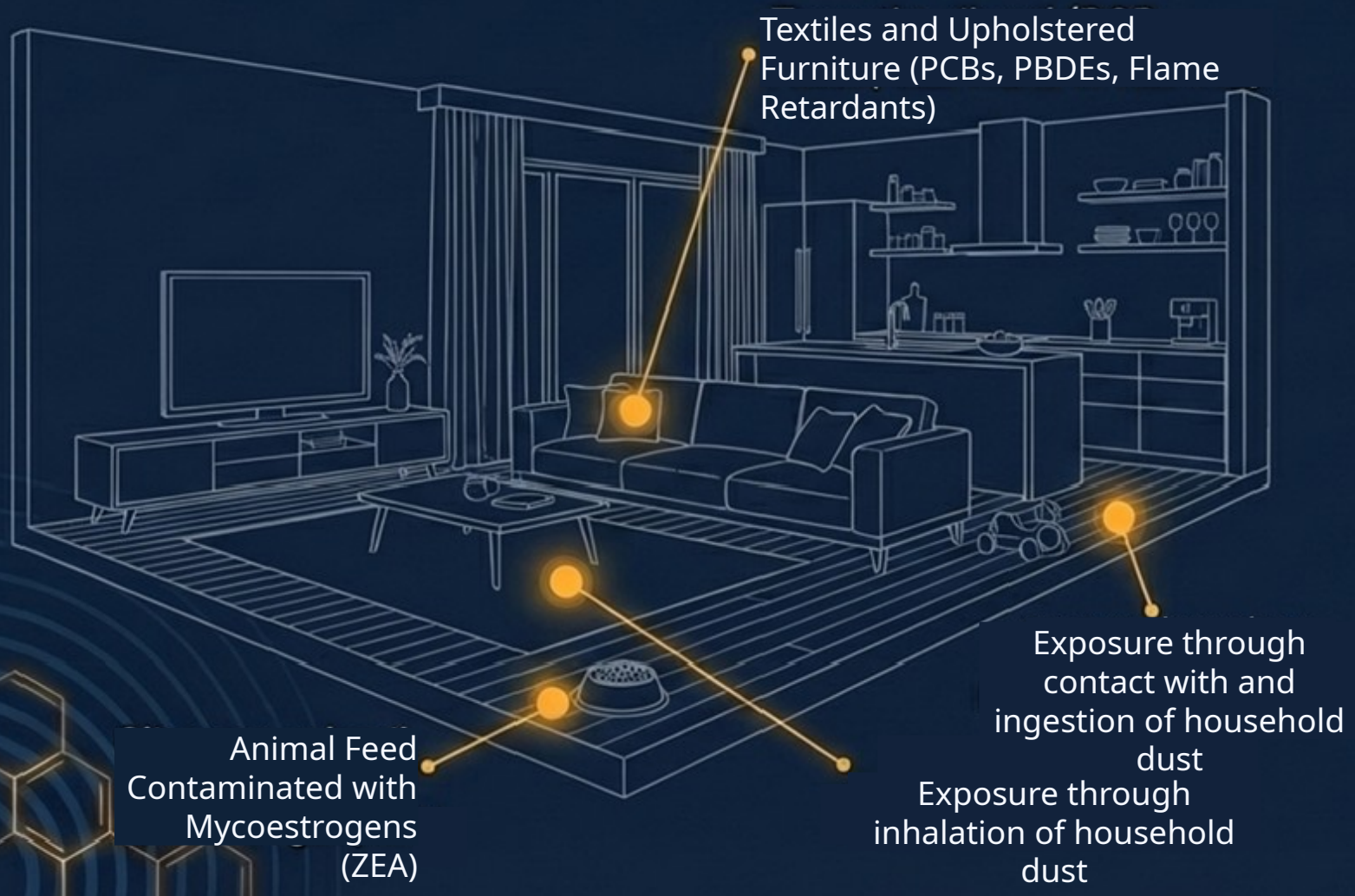
Nr2f1 Gene — Downregulated (OFF)

Sfrp2 Gene — **Upregulated (ON)**

Glycemic Warning: Altered glucose metabolism has been detected as early as 2 months of age, before the actual development of excess body weight. Obesity may reflect early-life biological programming, in addition to diet, lifestyle and environmental factors.

Act III: Companion Animals and the Modern

Shared spaces: Dogs and cats share our indoor and outdoor environments, making them contemporary sentinels of chemical exposure.



New culprits

After DES, a wide range of xenoestrogens remains relevant: BPA, phthalates, PCBs, PBDE flame retardants and mycoestrogens such as ZEA.

Primary pathways:

Contaminated pet food, inhalation or ingestion of domestic dust, and transplacental or lactational transfer.

Testicular Dysgenesis Syndrome (TDS) in Dogs

Mechanistic analogy: In analogy with human observations, in utero exposure to EDCs such as phthalates, DEHP and PCB 153 can interfere with fetal Leydig-cell testosterone secretion.

In utero exposure

Clinical manifestations of Testicular Dysgenesis Syndrome (TDS)

At birth

Increased incidence of cryptorchidism and hypospadias has been associated with contaminant exposure.

Young/adult animals

Poor sperm quality and altered germ-cell proliferation.

Oncological risk

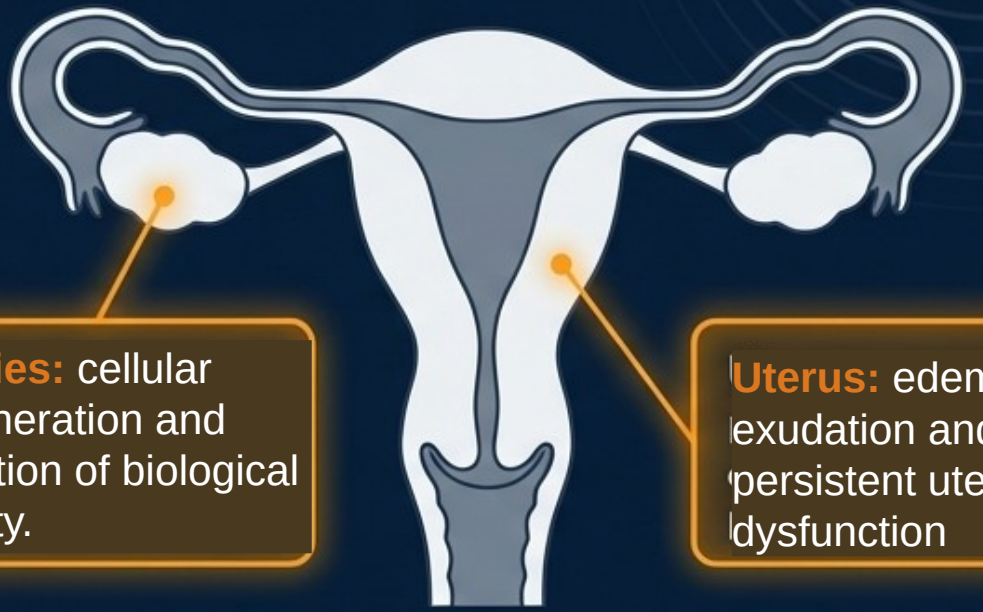
Pre-cancerous lesions and seminomas may represent later-life outcomes.

Female Reproductive Toxicity: The Zearalenone Case

Unique vulnerability:
Female dogs are particularly sensitive because of long progesterone- and prolactin-dominated cycles.

Role of ZEA: Zearalenone is a fungal mycoestrogen (Fungal estrone-like endocrine disruptor)
frequently found in commercial pet food.

TARGET ORGANS



Ovaries: cellular degeneration and inhibition of biological activity.

Uterus: edema, exudation and persistent uterine dysfunction

Clinical outcome: Possible predisposition to the endometritis-pyometra complex under continuous low-dose exposure.

Maternal Legacy: Transmission and Bioaccumulation

Ecological example: In Arctic sledge dogs, litters from mothers fed contaminated seal blubber showed altered sex ratios with a strong female bias.

Reference: Sonne et al. (2008). *Greenland sledge dogs (Canis familiaris) develop liver lesions when exposed to a chronic dietary low dose of an environmental organohalogen cocktail.* **Environmental Research, 106(1), 72–80.** DOI: 10.1016/j.envres.2007.08.010

Synthesis Matrix: The Interspecific Impact of EDCs

	 Cattle - production	 CD-1 mice - model	 Dogs and cats - domestic sentinels
Source of Exposure	Intentional exposure through feed or implants for profit	Controlled neonatal exposure	Accidental exposure from home environment and diet
Main Compounds	**DES as the main pharmaceutical estrogen**	DES, BPA or genistein	PCBs, PBDEs, phthalates and ZEA
Phenotypic Inheritance	Accelerated growth, pelvic changes and abnormal estrus.	Epigenetic reprogramming, DOHaD and adult obesity	Sindrome da Disgenesia Testicolare (TDS), piometra, alterazione del rapporto tra sessi

The Chemical Mirror

Beyond models: Animals are not only experimental models for human disease.
Sentinel value: From the agricultural boom to our homes, animals are often the first to live, accumulate and reveal invisible exposures.

One Health conclusion: Understanding epigenetic inheritance of endocrine disruption in animals helps us understand the unseen biological cost of our shared chemical environment.
Closing message: What happens to them echoes in us.

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