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The Effect of EDCs on Health and Human Development

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Potomac Conservancy
And
Georgetown University

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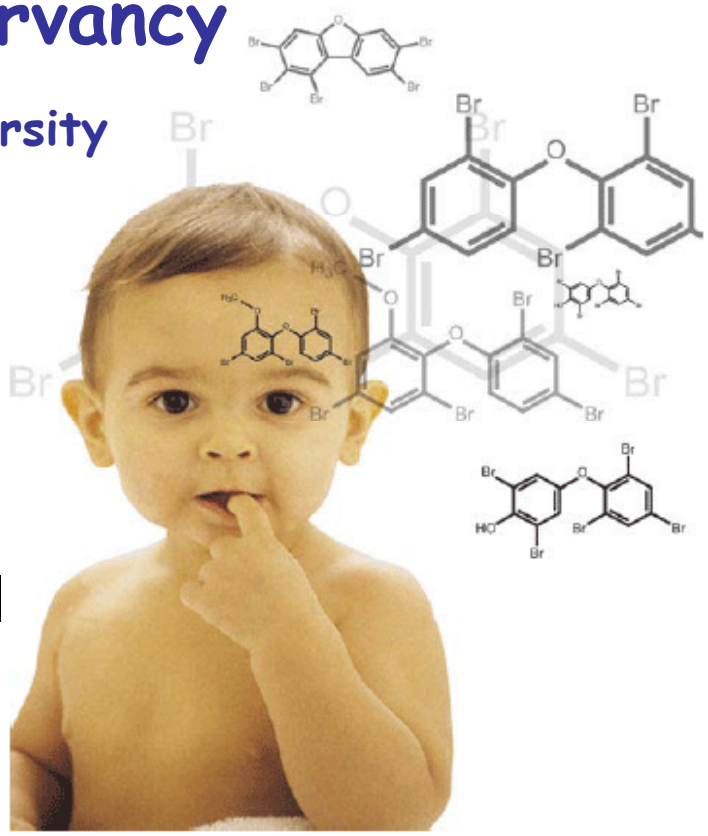
National Institutes of Health

National Institute for Environmental Health Science

With many thanks to Jerry Heindel, Kris Thayer and Thad Schug



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Overview: Main Points

- Pharmaceuticals, fabric treatments, pesticides, fertilizers and other chemicals can disrupt the endocrine systems and can be found in water.
- Exposure to environmental chemicals, including **EDCs** in the environment may play an important role in the etiology of diseases… along with nutrition, infection and stress.
- Development is the most sensitive window for the effect of environmental chemicals, including EDCs in the susceptibility to diseases later in life.

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Overview

- Endocrine Disruptors in the Environment
- Developmental Basis of Disease
- Epigenetics
- Developmental Basis of Disease: Examples
  - Arsenic and Skin Cancer
  - DES and Obesity
- Summary

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Endocrine Disruptors

- 60-80,000 chemicals in commerce
- Subset are toxic
- Subset of those that are toxic are EDCs
- "An endocrine disruptor (ED) is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes ***adverse health effects*** in an intact organism, or its progeny, or (sub) populations (WHO/I 2002)"
- **Adversity** "A change in morphology, physiology, growth, *reproduction, development or lifespan* of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences (WHO/IPCS 2004)"

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Characteristics of EDC Toxicity

- **Low dose effects**
  - High dose effects are different from low dose effects
  - Non-monotonic dose responses
- **Wide range of effects**
  - Endocrine signaling govern all tissues/organs
  - Nuclear and membrane receptors, neurotransmitters, metabolism
- **Persistent and latent effects**
  - Developmental exposure most sensitive window
  - Transgenerational effects (vinclozolin, dioxin, BPA, phthalates)
- **Ubiquitous exposure**
  - Consumer products
  - Pharmaceuticals
  - Industrial products

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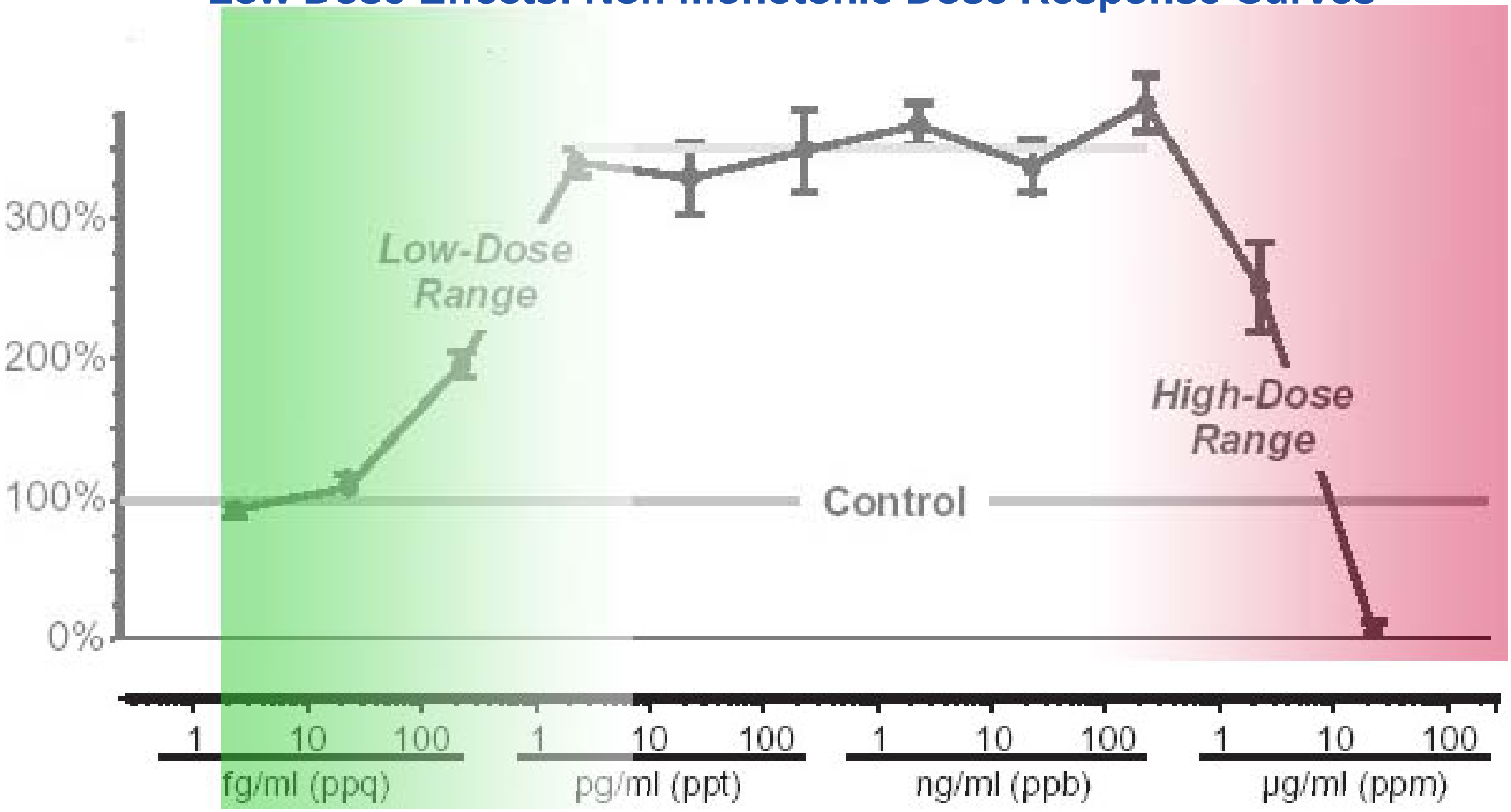
Low Dose Effects: Non monotonic Dose Response Curves



Nonmonotonic curves are common

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Household Products in the Environment

- **Antiinflamatory/Analgesics**
  - Ibuprofin,/Naproxen, Aspirin,
- **Antiepileptics**
- **Psychiatrics/Antidepressants**
  - Diazepam
- **Cancer Therapeutics**
- **Lipid regulators**
  - Artovstatin
- **Steroids**
  - 17alphaethinylestradiol,
  - 17 beta estradiol
  - Estrone
- **Pesticides**
  - Organophosphate
  - Carbamates
  - Pyrethroids
  - Organochlorine
- **Beta Blockers**
  - Metoprolol
  - Atenolol
- **Antibiotics and Antimicrobials**
  - Sulphonamides
  - Tetracyclines
  - Triclosan
- **Stimulants**
  - Caffeine
- **Diuretics**
- **Personal Care Products**
  - Insect repellants
  - Antioxidants
  - Preservatives
  - Soaps
  - Sunscreens
  - Fragrances/cosmetics
  - Toothpaste

Secondary Sources of EDCs

- **Water**
  - Municipal wastewater discharge (drinking water)
  - Agricultural fields
  - Concentrated animal feeding operations
  - Landfill leachates
  - Urban runoffs
- **Contaminated Food (concentration up the food chain)**
  - Animals
  - Plants
- **Inhalation from combustion of pharmaceuticals**
- **Dermal**
  - soil/patches

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Examples of Endocrine Disruptors

HERBICIDES
2,4,-D
Alachlor
Amitrole
Atrazine
Linuron
Metribuzin
Nitrofen
Trifluralin

FUNGICIDES
Benomyl
Ethylene thiourea
Fenarimol
Hexachlorobenzene
Mancozeb
Maneb
Metiram - complex
Tri-butyl-tin
Vinclozolin

INSECTICIDES
Aldicarb
beta-HCH
Carbaryl
Chlordane
Chlordecone
DBCP
Dicofol
Dieldrin
DDT and metabolites
Endosulfan
Heptachlor / H-epoxide
Lindane (gamma-HCH)
Malathion
Methomyl
Methoxychlor
Oxychlordane
Parathion
Synthetic pyrethroids
Transnonachlor

INDUSTRIAL CHEMICALS
Bisphenol - A
Polycarbonates
Butylhydroxyanisole (BHA)
Cadmium
Chloro- & Bromo-diphenyl ether
Dioxin (2,3,7,8-TCDD)
Furans
Lead
Manganese
Methyl mercury
Nonylphenol
Octylphenol
PBDEs
PCBs ★
Pentachlorophenol
Penta- to Nonylphenols
p-tert-Pentylphenol
Phthalates
Styrene

RED= Found in water

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Developmental Origin of Adult Disease Barker Hypothesis

- 1989 David Barker found an inverse relationship between birthweight and death from heart disease in England and Wales.
- Studies confirmed by "Dutch Hunger Winter" when food supplies to occupied Netherlands were cut off by Nazis. Individuals born during this time had high incidence as adults of insulin-resistance.

Fetal Origin of Adult Disease (FEBAD) confirmed for

Coronary heart disease

Hypertension

Type II diabetes

Table 1. Hazard ratios for coronary heart disease according to body size at birth[a]

	Hazard ratio (95% CI)	No. of cases/No. of men
Birthweight (g)		
<2500	3.63 (2.02–6.51)	24/160
–3000	1.83 (1.09–3.07)	45/599
–3500	1.99 (1.26–3.15)	144/1775
–4000	2.08 (1.31–3.31)	123/1558
>4000	1.00	21/538
P for trend	0.006	
Ponderal index (kg m^{-3})		
<25	1.66 (1.11–2.48)	104/1093
–27	1.44 (0.97–2.13)	135/1643
–29	1.18 (0.78–1.78)	84/1260
>29	1.00	31/578
P for trend	0.0006	

Cheryl Walker

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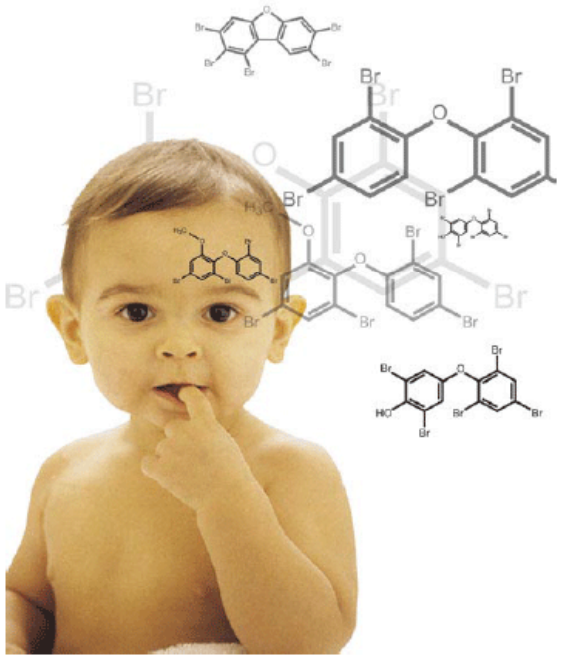
Why are kids more sensitive?

- **Development (in utero and first few years of life) is a sensitive time for exposure to environmental chemicals**.
  - Tissues/organs forming,
  - Lack of DNA repair,
  - Poor liver metabolism,
  - Developing immune system,
  - Lack of blood/brain barrier**,**
  - ↑ metabolic rate
  - Programming (epigenetic marks set)



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Developmental Basis of Disease II

- **Functional changes**: changes at gene and cell level….looks normal but at molecular level
  - altered gene expression (altered proteins),
  - altered protein activity
  - and/or altered number of cells

  that persist…after exposure gone…. and lead in increased sensitivity to disease later in life.
- Functional changes are not detected in typical teratology studies.
  - Need developmental exposures and lifetime examination for multiple disease outcomes.
  - Need ‘omics endpoints.

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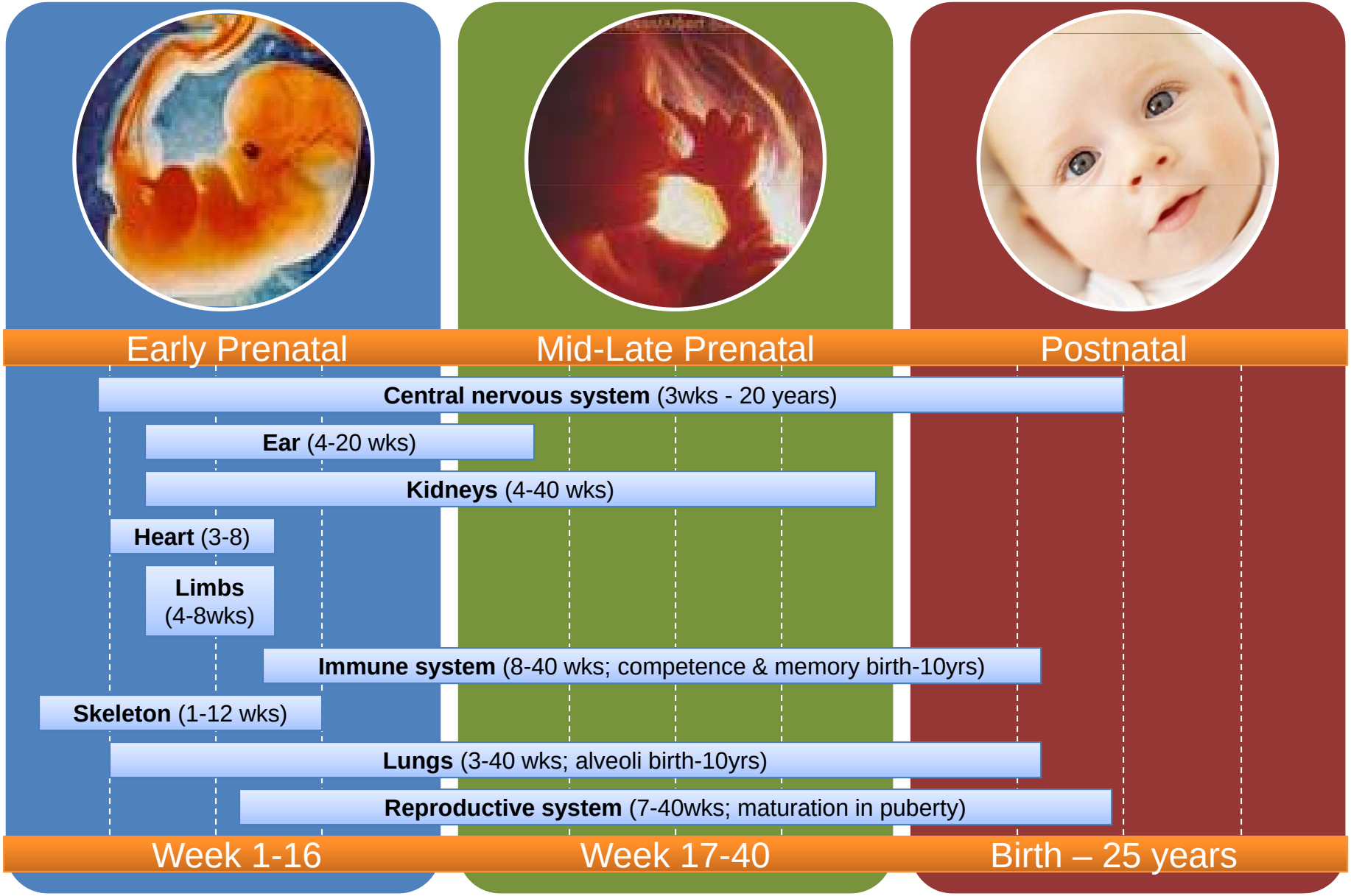
Stages of Prenatal and Postnatal Organ Development



Source: Altshuler, K; Berg, M et al. *Critical Periods in Development,* OCHP Paper Series on Children's Health and the Environment, February 2003.

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Developmental Basis of Disease: Environmental Focus

- Environmental exposure during development (single/multiple.)
- Effect depends on chemical, timing, dose, genetic background, nutrition, stress, and infections.
- Functional change…altered programming (gene expression) or altered protein regulation that last lifetime.
- The pathophysiology can manifest as:
  - the occurrence of a disease that otherwise would not have happened;
  - an increase in risk or earlier occurrence of a disease that would normally be of lower prevalence;
- Variable latent period.
- No measurable biomarker like birth weight. Thus, new more sensitive biomarkers of exposure and effect are needed.

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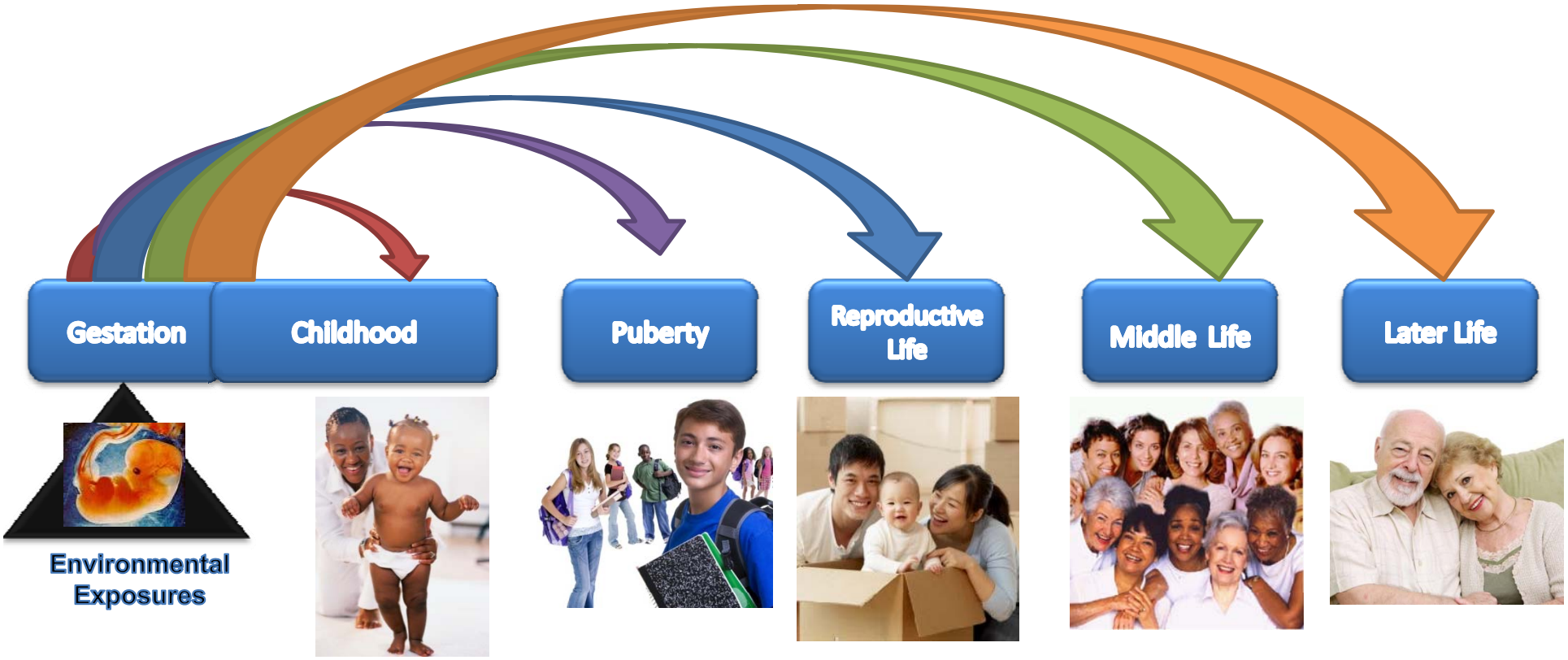
Developmental Origins of Disease:

Developmental Exposures Lead to Disease Throughout Life



How can it be that developmental exposures can cause effects that persist long after the exposure?

A Paradigm Shift in Toxicology

Epigenetics… "chemical" modifications of **DNA** and **chromatin** which are heritable and affect genome function (transcription, replication, recombination), but don't affect DNA backbone.

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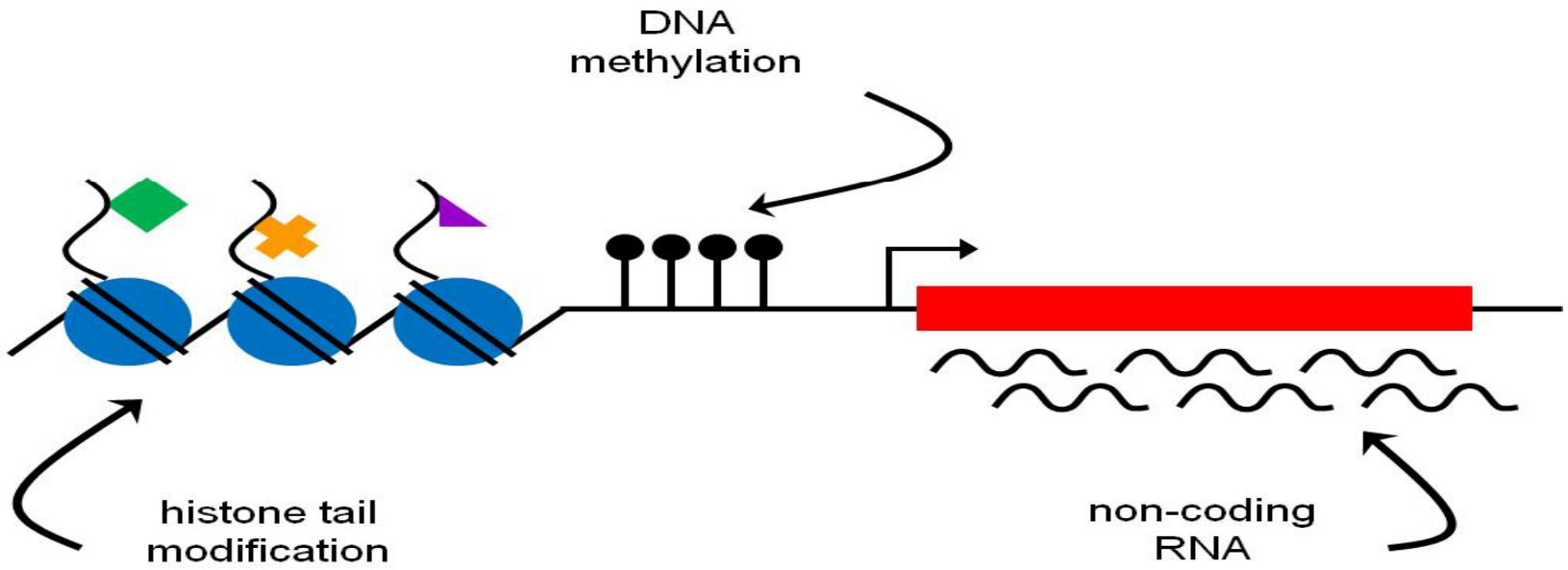
Developmental Programming: Epigenetics

•During development genes are turned on and off in a precisely controlled temporal pattern to form both the tissues and the regulatory pathways controlling them.

•The control of gene expression is via alterations in the epigenetic system (Chromatin remodeling and DNA methylation) which is most active during development.



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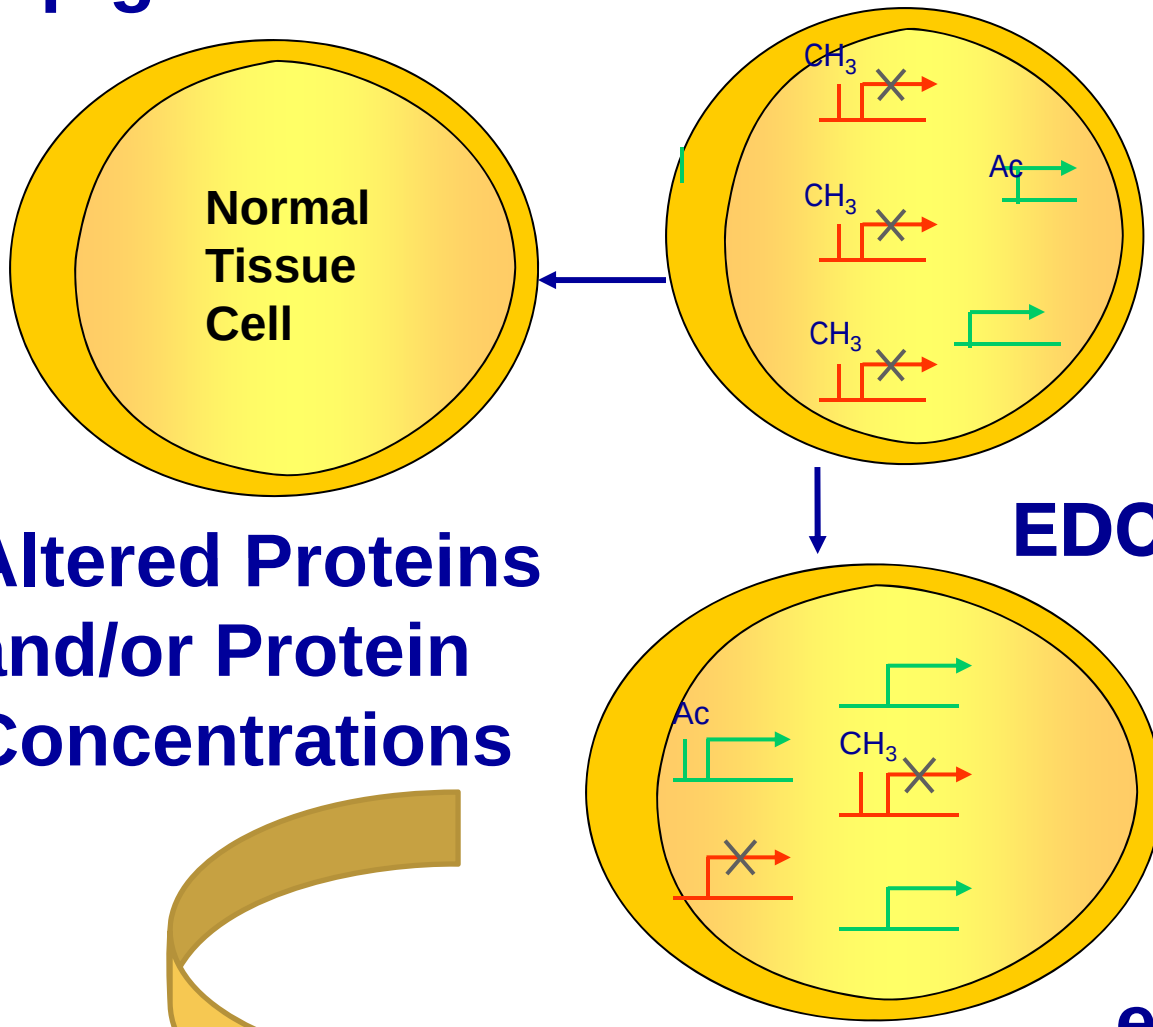
Epigenetic/Environmental Basis of Disease!



Normal Differentiating Cell (Development)

EDCs

Altered Proteins and/or Protein Concentrations

Altered Gene Expression (persists due to epigenetic marks)

Disease / Dysfunction

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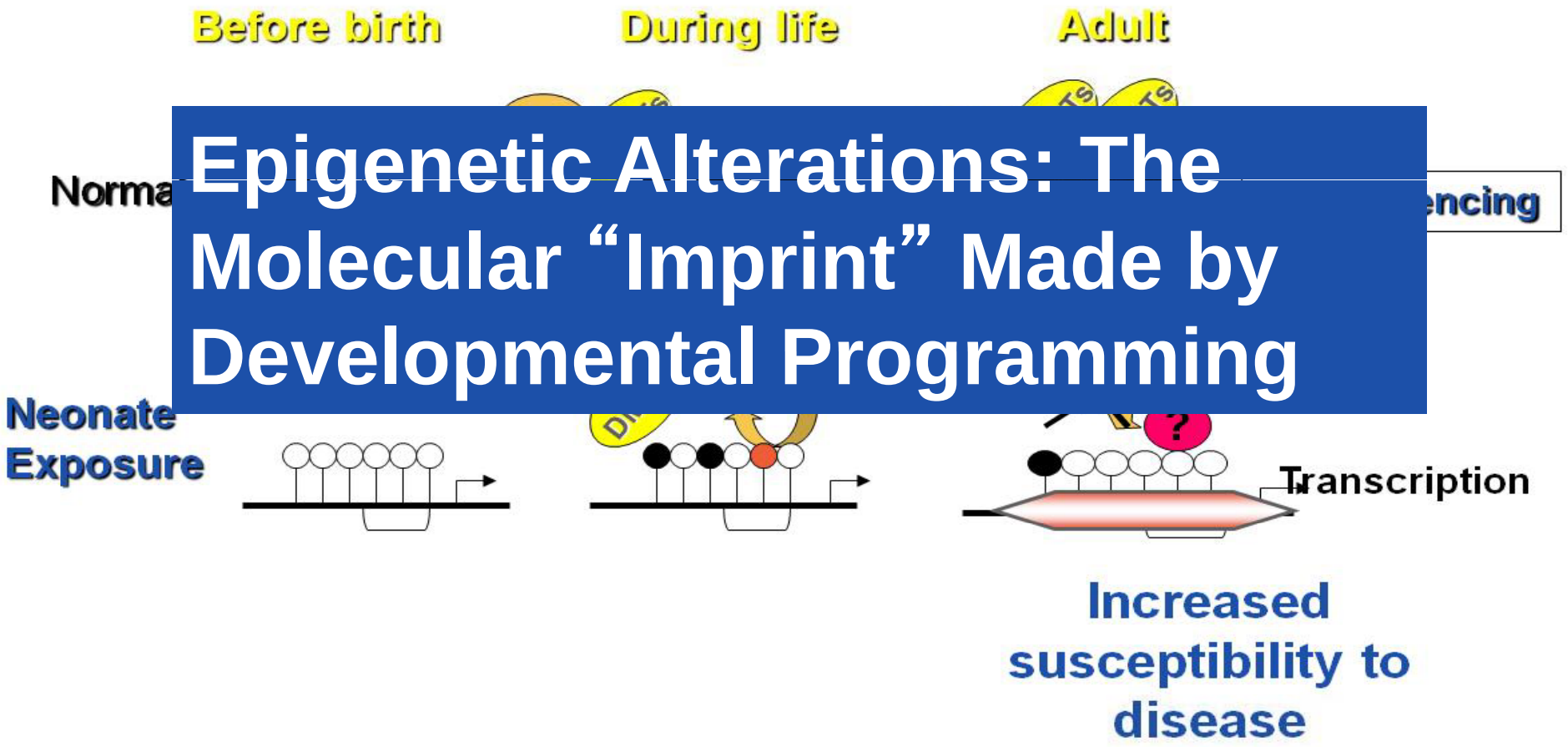
Developmental Basis of Disease: Environmental Exposure and Epigenetics



Epigenetic Alterations: The Molecular "Imprint" Made by Developmental Programming

UNIVERSITY OF Cincinnati
Shuk Mei Ho
Cancer Research Group

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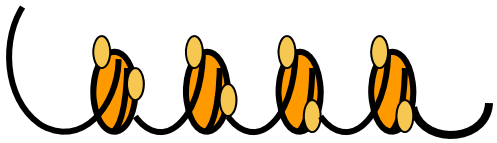
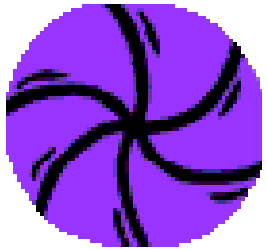
Environmental factors (development)

(Chemicals, diet, drugs, stress, behavior)



Epigenetics
(stable but plastic)

Genetic polymorphisms
(born with)



Inter-individual variability

Susceptibility to Disease, Toxicants, Drugs, Altered behavior

Shuk mei Ho

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Developmental Diseases

- **Reproductive/Endocrine**
  - Breast/prostate cancer
  - Endometriosis
  - Polycystic ovary syndrome
  - Fertility
  - Diabetes/metabolic syndrome
  - Puberty
  - Obesity
- **Brain/Nervous System**
  - Alzheimer's disease
  - Parkinson's disease
  - ADHD/learning disabilities
- **Pulmonocardiovascular**
  - Atherosclerosis
  - Asthma
  - Chronic obstructive pulmonary disease
  - Heart disease/hypertension
- **Immune/Autoimmune**
  - Systemic/tissue specific autoimmune disease
  - Immunosuppression

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Chemicals Known to be Important in Developmental Basis of Disease

- Environmental Estrogens
  - Diethylstilbestrol
  - Genistein
  - Bisphenol A
- Tributyl Tin
- Phthalates
- Dioxin/PCBs
- Arsenic
- Atrazine
- Smoking/ETS/ Air Pollution
- Methylmercury/Lead/arsenic
- LPS
- Lead
- Vinclozolin
- Polybrominated diphenyl ethers (PBDE)

NON Mutagenic Effects

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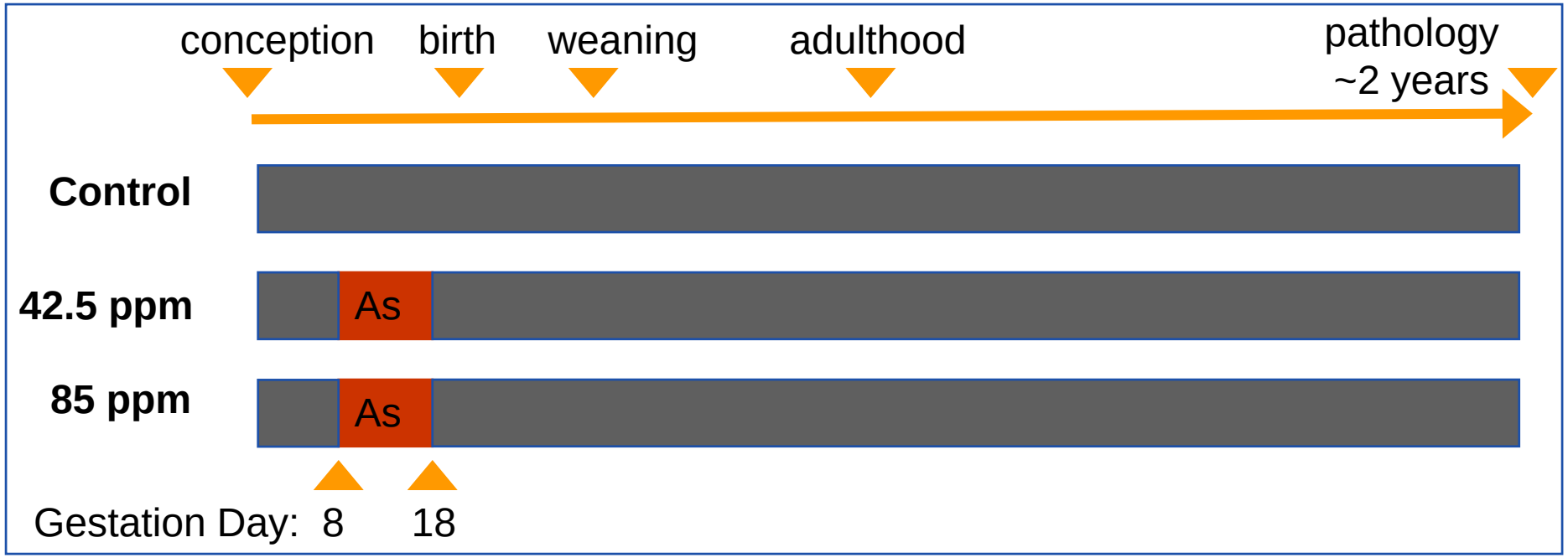
Developmental Exposure to Inorganic Arsenic and Carcinogenesis in Mice



Notes:

- Known human carcinogen, GR, AR, PR, ER, TR, RXR
- Arsenic (as $NaAsO_2$) given in maternal drinking water (well tolerated)
- No other treatment after gestation day (GD) 18
- Now done in several strains (C3H, CD1)

M. Waalkes NIEHS

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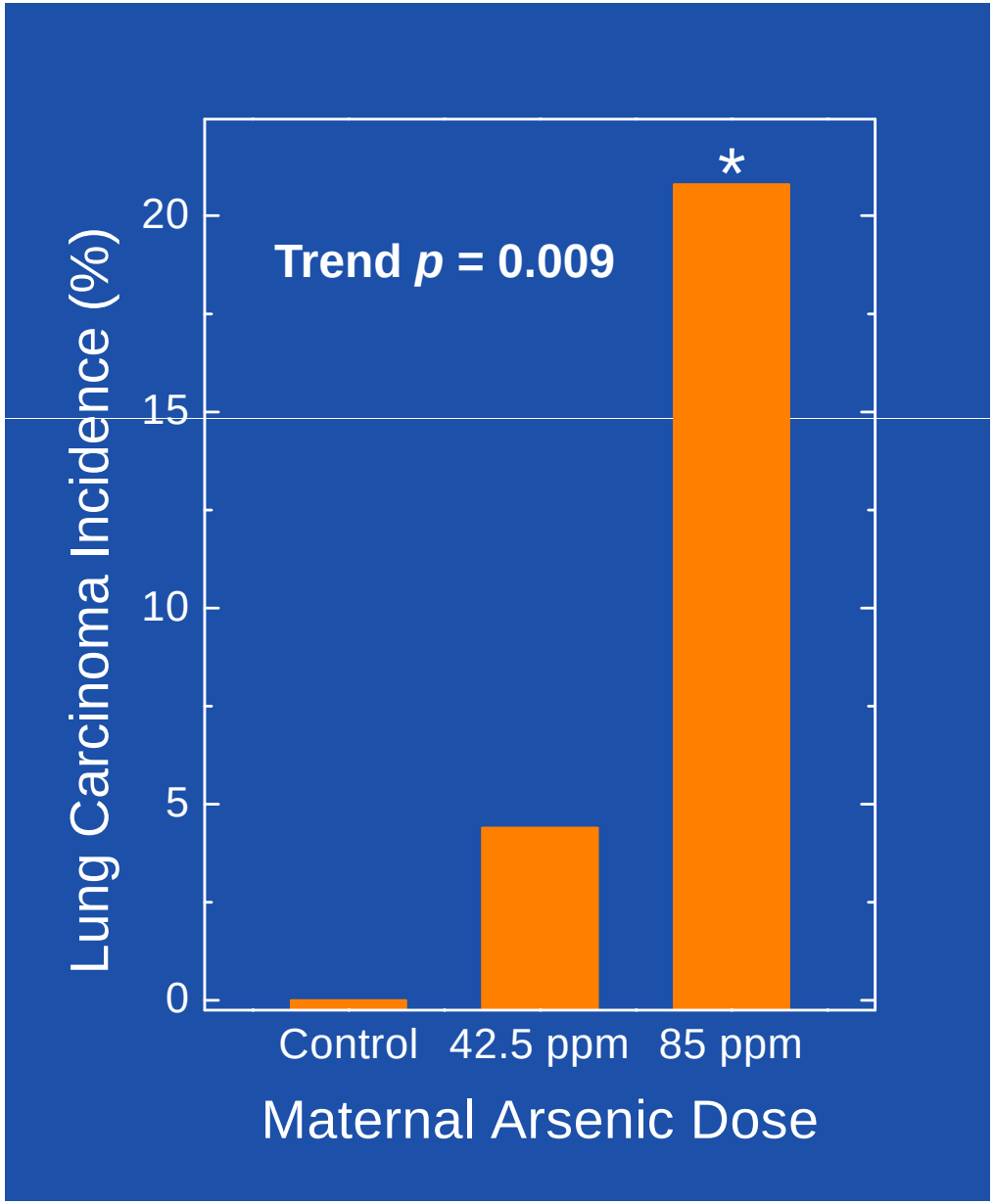
Arsenic Carcinogenesis: Female C3H Offspring

- **Arsenic is a Lung carcinogen**
  - ❑ Example: Lung carcinoma in adult female C3H offspring
  - ❑ Also tumors or preneoplasia in liver, adrenal, UB, ovary, uterus, oviduct, vagina, cervix, etc.
    - In adulthood
    - In other strains too
    - Long after arsenic exposure ends



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Mouse Model Issues

People are exposed during all periods of their lives.
We only tested the fetal life stage in mice.
Testing at any one stage is not “environmental”

In Utero

Tested here: sensitivity high in mice

Childhood

Sensitivity unknown

Adolescence

Sensitivity unknown

Adulthood

Usually negative in mice: but not “environmental”

M. Waalkes

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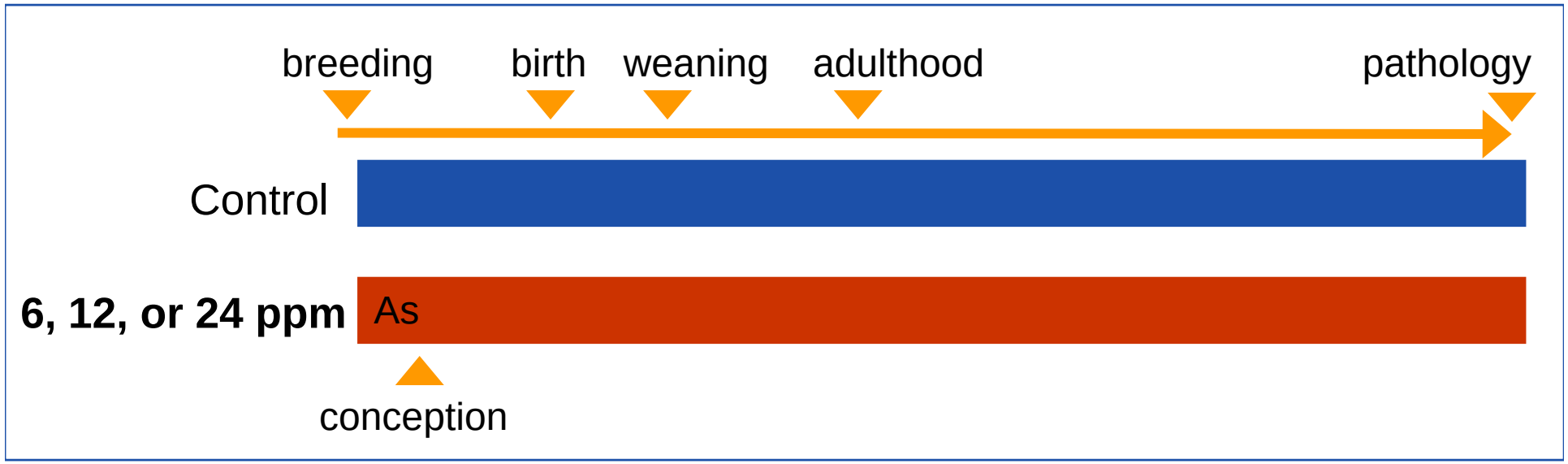
Whole Life Arsenic Exposure and Carcinogenesis in CD1 Mice



Notes:
- Arsenic (as NaAsO2) given in drinking water (well tolerated)
- Mice observed for up to 2 years
- Arsenic washes out rapidly

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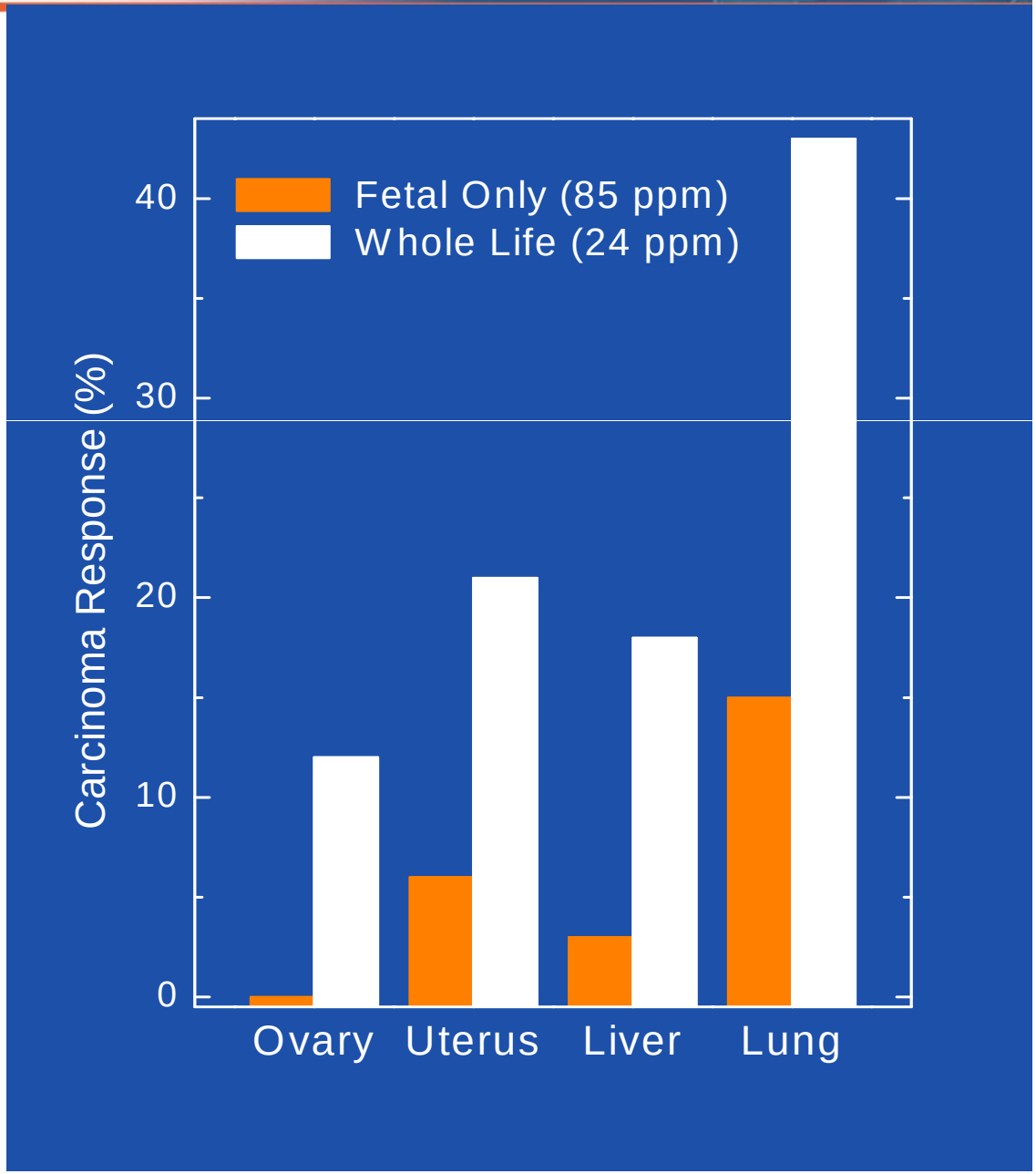
Whole life Exposure Compared to Developmental Exposure

- **Doses approaching human exposure**
  - Increase response in many target tissues
    - Example: female offspring
  - Target tissues the same
    - **Response higher in whole life… with lower doses**



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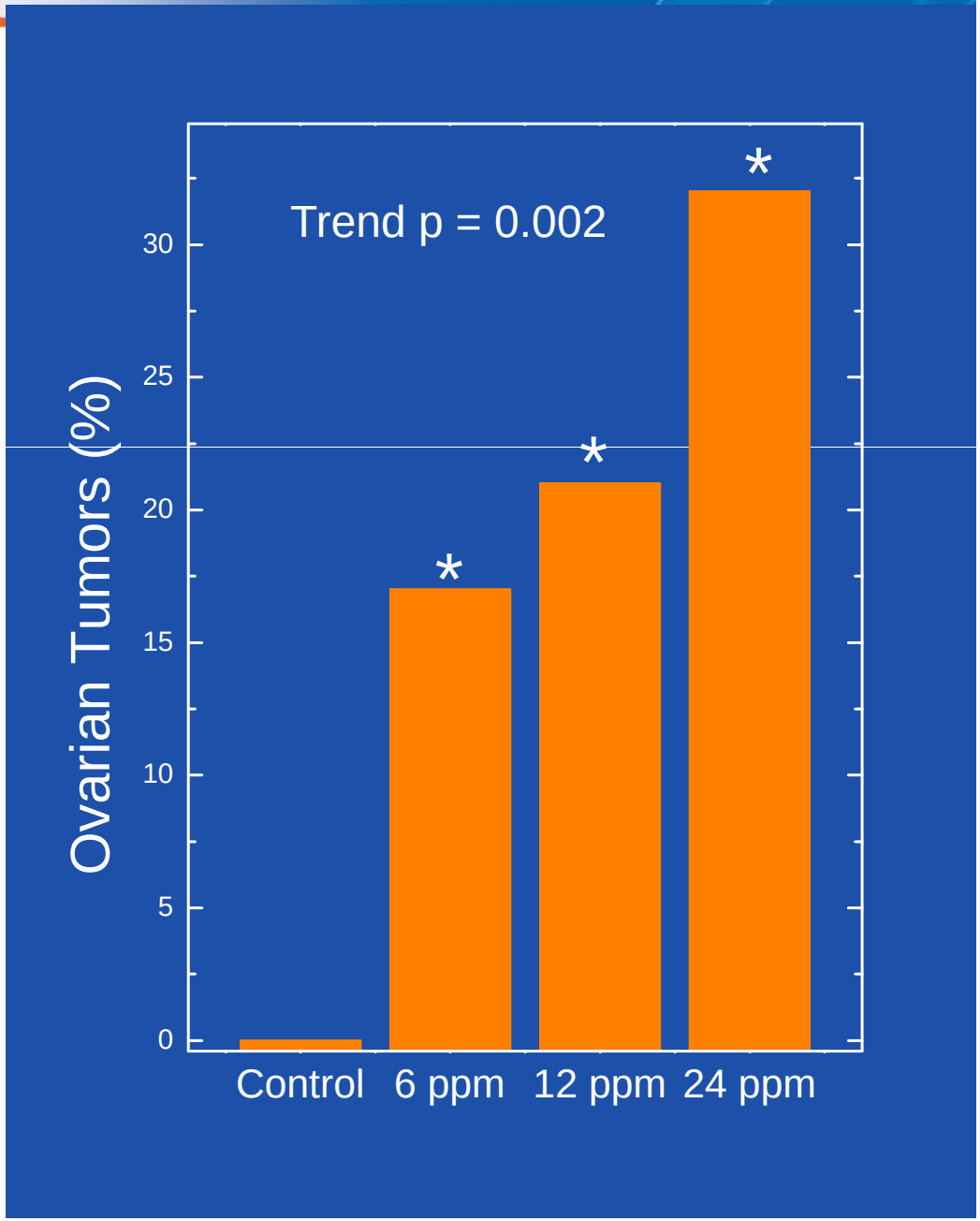
Other Results with Whole life Exposure

- **Get tumors even at 6 ppm**
  - Example:
    - Female ovarian tumors
    - Also get adrenal tumors
  - Clear dose-response
  - While doses are higher than human exposures, they produce blood levels in the range of humans.



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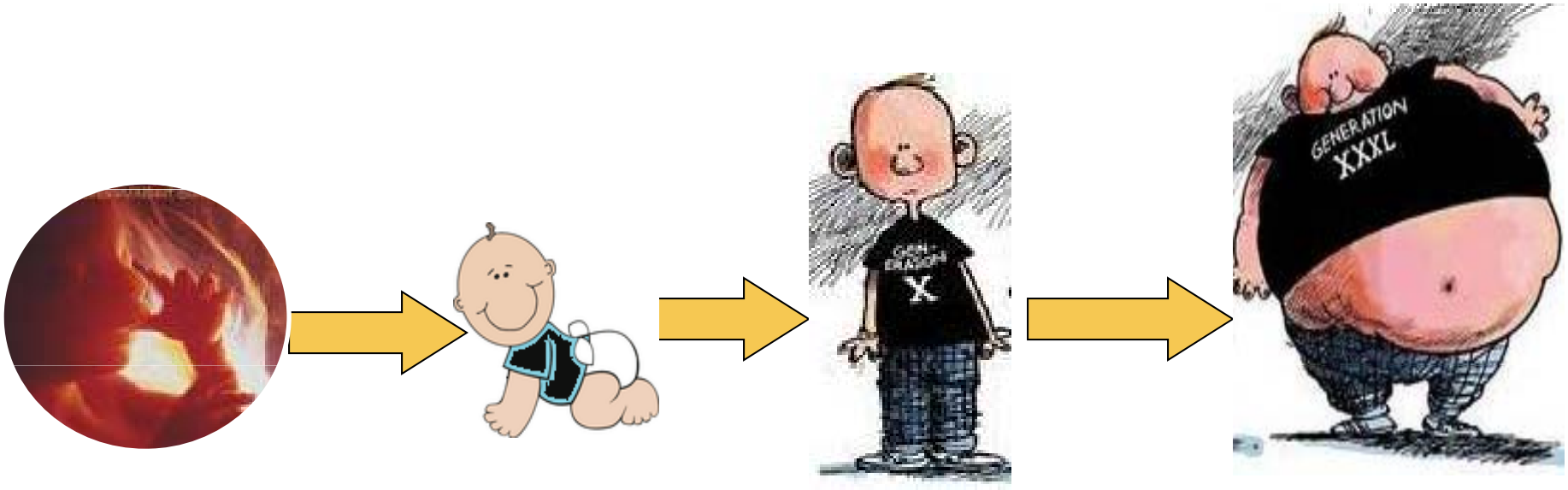
Developmental Basis of Disease: Obesity



Does sensitivity to develop obesity have its origins during development…and do environmental chemicals exposures play a role?

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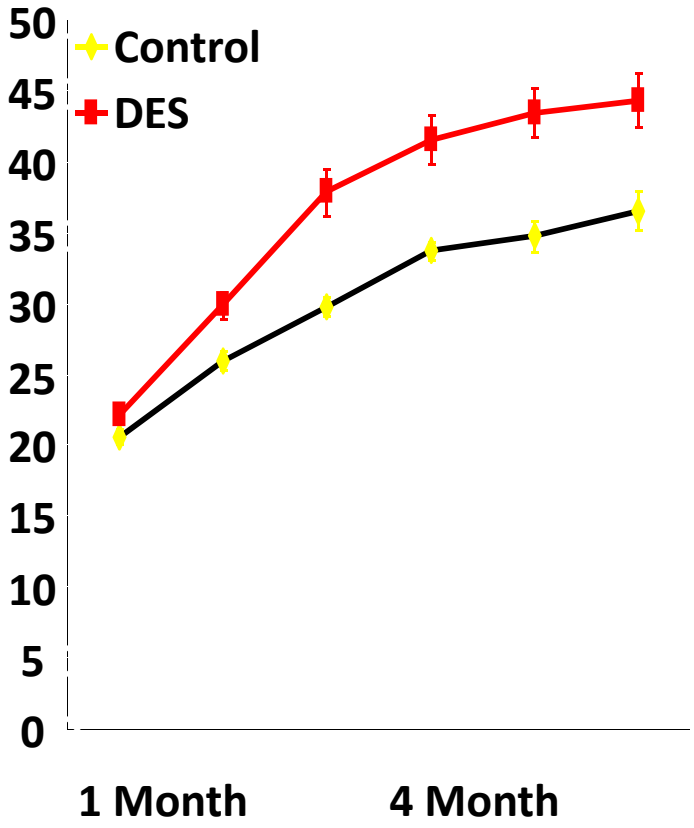
Developmental Exposure to DES and Weight Gain
Proof of Principle



Exposure of CD-1 mice to DES for 5 days at birth results in increased weight gain starting at puberty in female mice. No change in food intake or exercise. Newbold et al.

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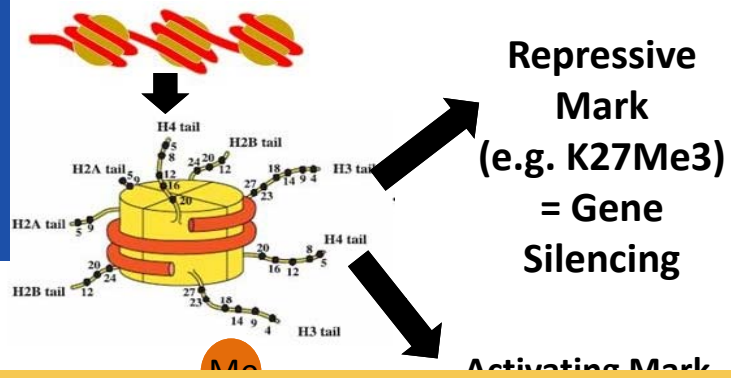
Xenoestrogens Activate Nongenomic Signaling to Regulate Histone Methyltransferase Activity



ER + DES

Identification of an "imprint" left by developmental programming such as altered histone methyl marks may be useful for identification of exposed individuals and as a biomarker for disease susceptibility in adult life.

AKT → HMT → Inactive

Chromatin

Ligand Activation

Non-genomic signaling

Phosphorylation of Histone Methlytransferases

Cheryl Walker

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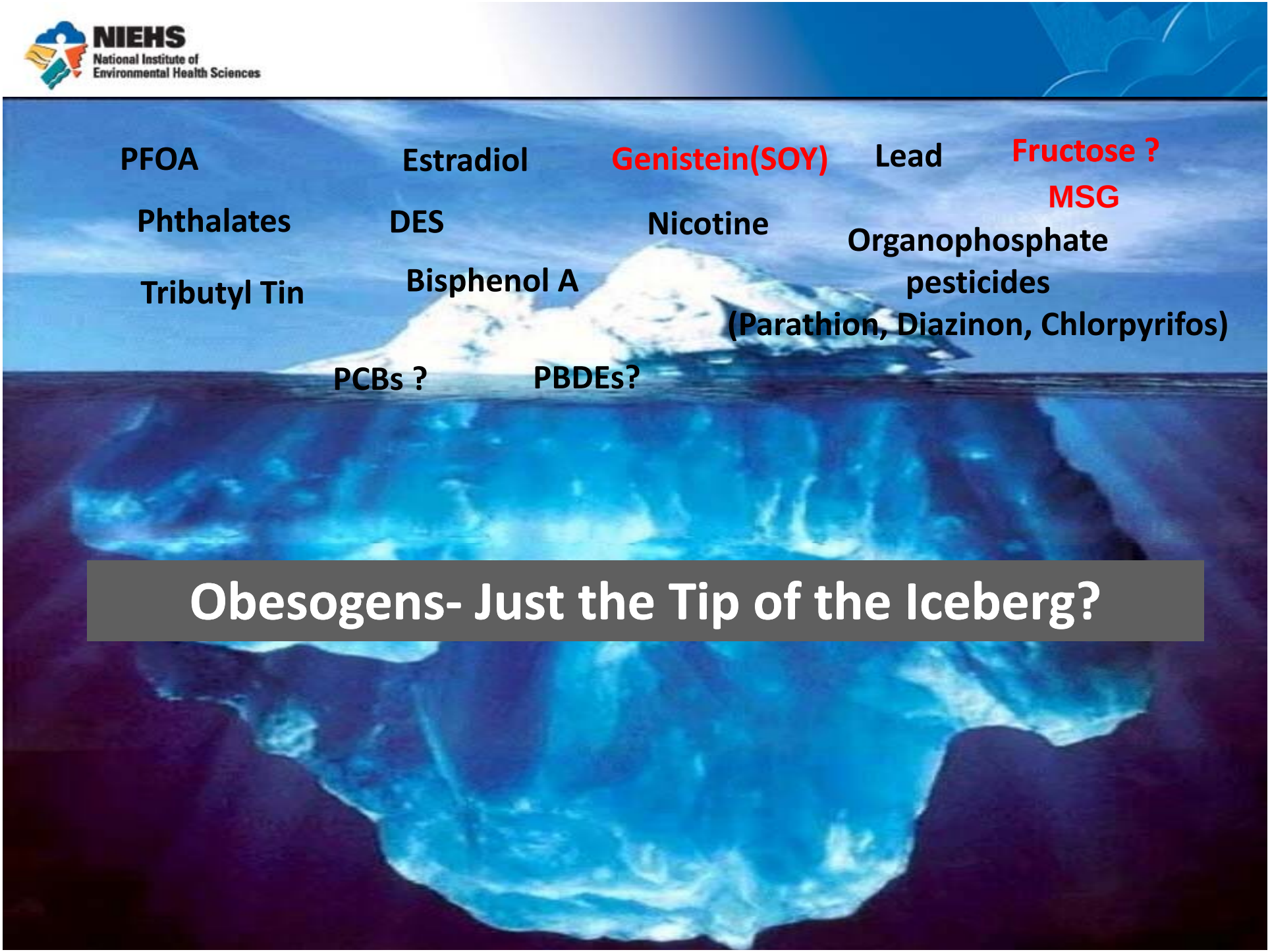

Obesogens- Just the Tip of the Iceberg?

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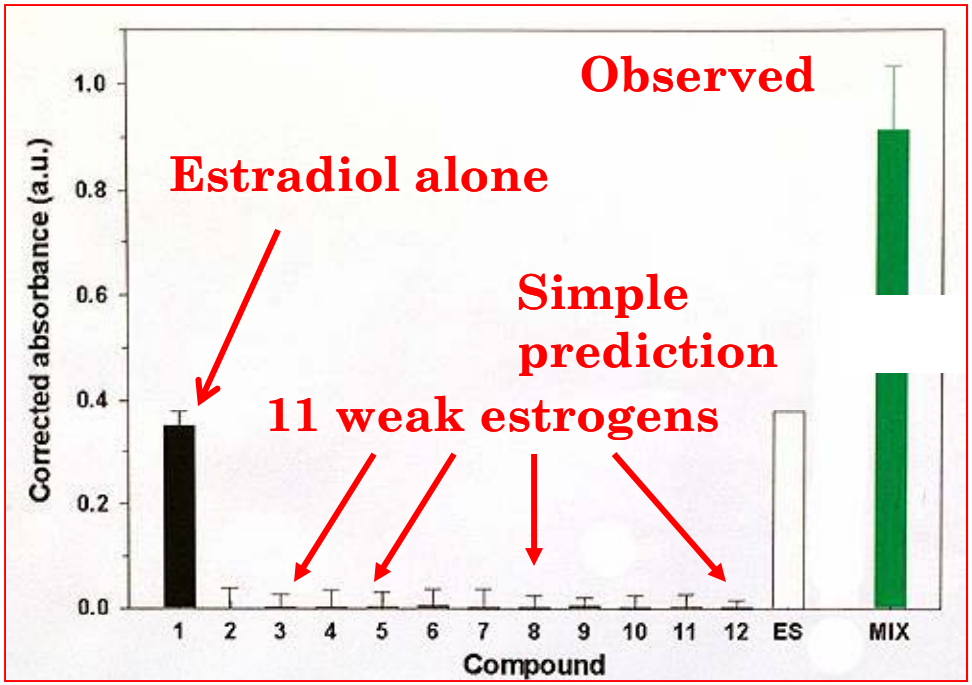
Can multiple chemicals at low doses with no individual effects be important in disease etiology?

Something From Nothing!



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It is possible and even probable...

that developmental exposure to low ppb levels of a mixture of environmental chemicals…(found in majority of Americans, all of which have been shown in animal studies to result in disease later in life)

- **Nicotine (smoking)**
- **BPA (polycarbonate)**
- **Genistein (soy)**
- **TBT (polyvinyl plastic)**
- **Pfoas (teflon/scotchgard), PBDEs (flame retardants)**
  Phthalates (plastic)
- **P0Ps (pesticides)**
- **Atrazine (herbicide)**
- **Arsenic**
- **ETC…….**

Plus now add Pharmaceuticals !

could jointly contribute (due to additively of effects) to disease later in life….in combination with nutrition and the genetic background.

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The Developmental Basis of Disease Changes Everything

- It changes focus from adults to development for the cause of disease.

GOOD NEWS:

- This paradigm changes the focus from curing a disease to prevention and intervention strategies to reduce disease incidence.
- The perinatal period is a window of opportunity for disease prevention.
- Epigenetics along with genomics and proteomics may provide sensitive biomarkers of developmental exposures which could aid in development of prevention and intervention strategies.

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Summary

- Are EDCs found in the environment…YES
- Are EDCs found in the environment at levels that could cause human disease/dyfunction?
- Unclear! More research needed.
- If it is possible that the low levels could be toxic what would be the most sensitive window of sensitivity?
- Development!
- What sensitive endpoints would be needed to pick up activity of low levels of environmental pharmaceuticals?
- ‘Omics (genomics, proteomics, epigenetics)
- What is the best approach?
- Precautionary Principle while working to reduce exposure.