GMO & disease– what do we know?

The Ecology of Ecotoxicology

Today: the tip of the iceberg – things aren't always what they seem…

Warren Porter, Dept. of Zoology, UW, Madison
www.zoology.wisc.edu/faculty/Por/Por.html

Outline

1) Everything is interconnected

2) Basic principles, new definitions

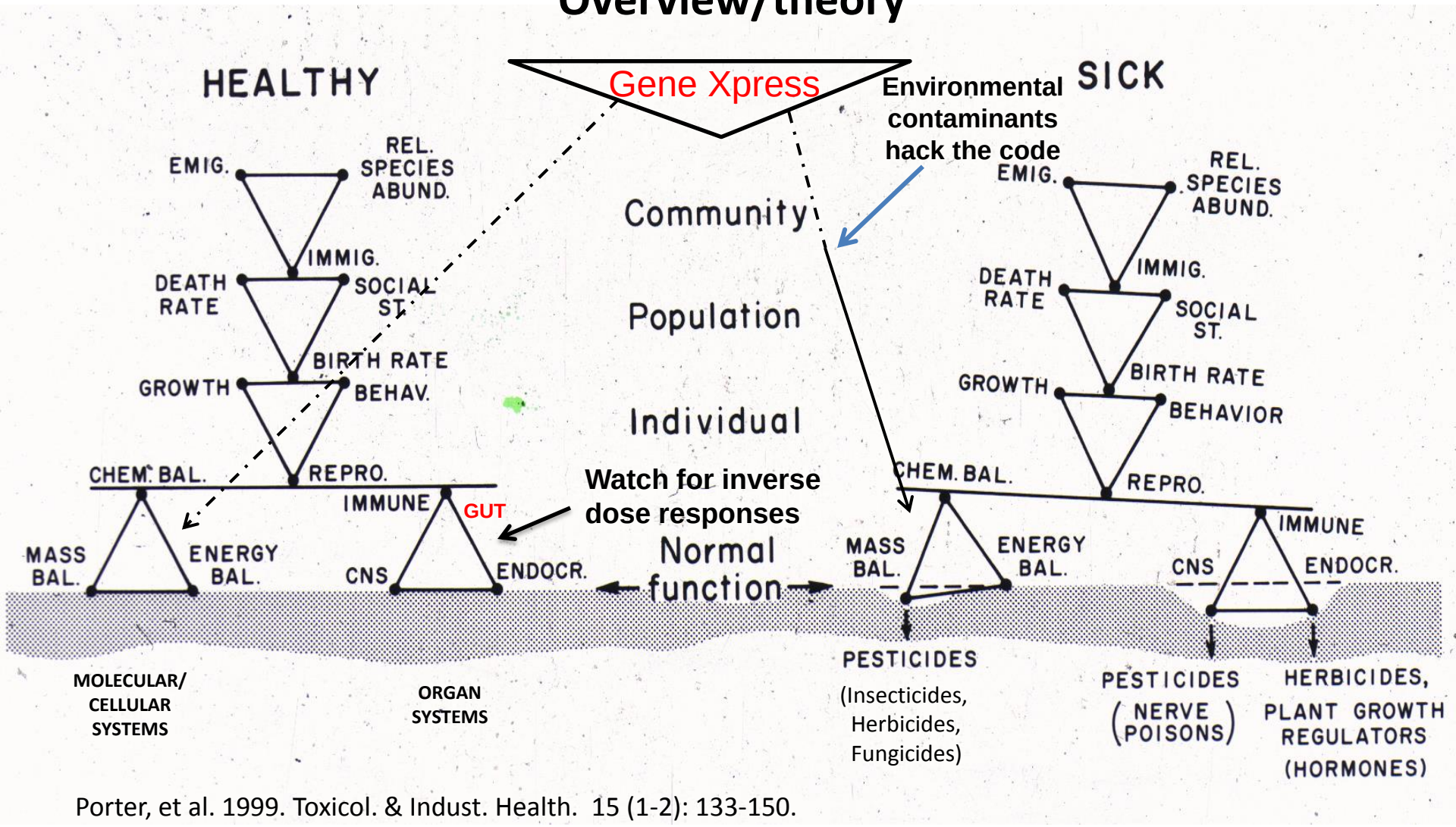
3) How can common pesticide mixtures affect reproduction, sex behavior, learning and immune function?

4) How can common pesticide mixtures induce chronic long term diseases?

5) Safe, effective, inexpensive solutions to these problems

Everything is interconnected

Neurological, endocrine, immune, developmental, genetic effects: Overview/theory



Porter, et al. 1999. Toxicol. & Indust. Health. 15 (1-2): 133-150.

Aldicarb, atrazine, nitrate mixes at environmentally relevant concentrations altered mouse aggression, immune function and thyroid hormone levels.

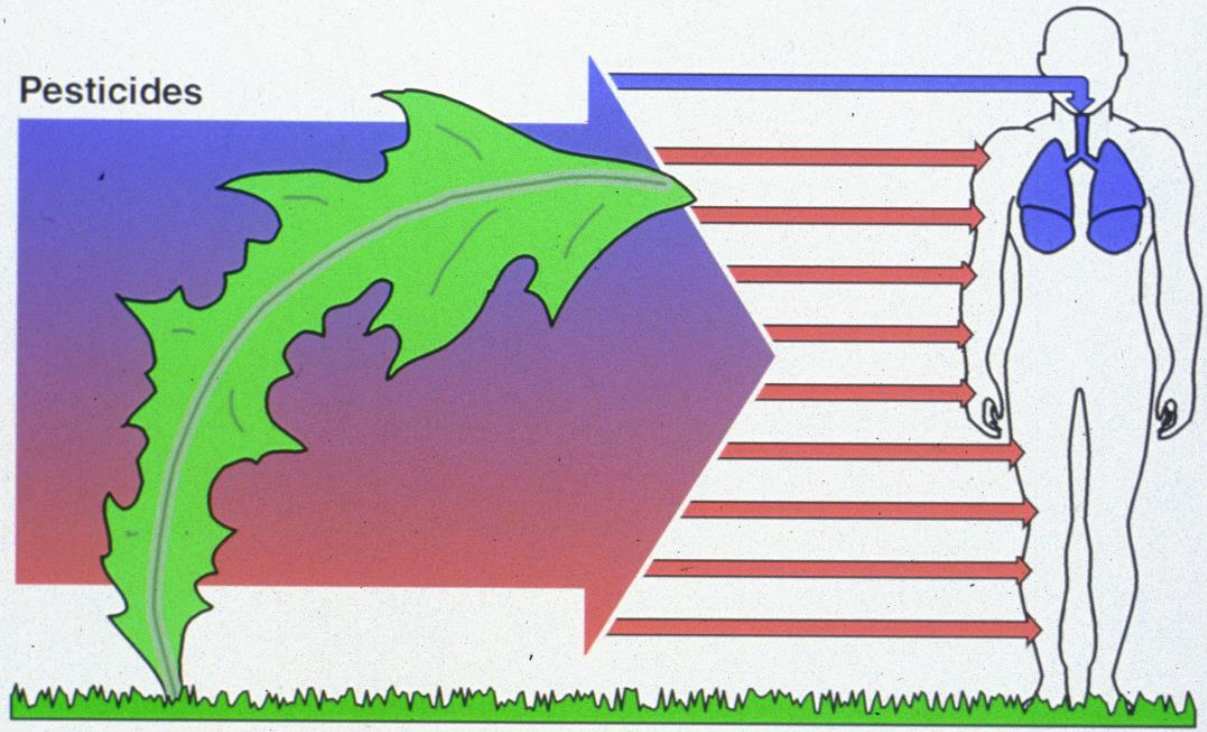
Basic Principles

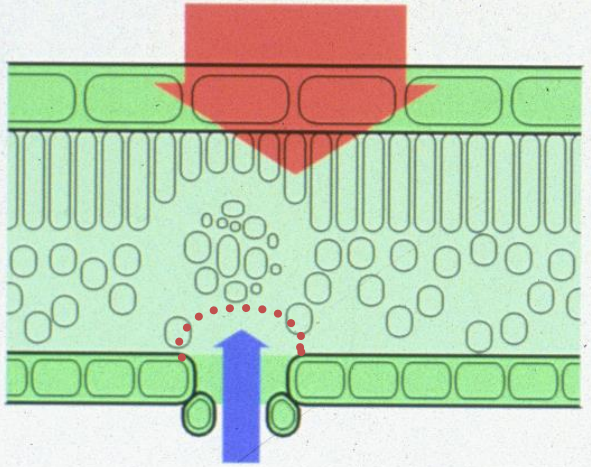
Why entry is easy -> *'other', i.e. 'inert', ingredients*

1) non-ionic (fat soluble) solvents (= no charges) &

2) surfactants

'Inerts' NOT part of EPA registration process ->





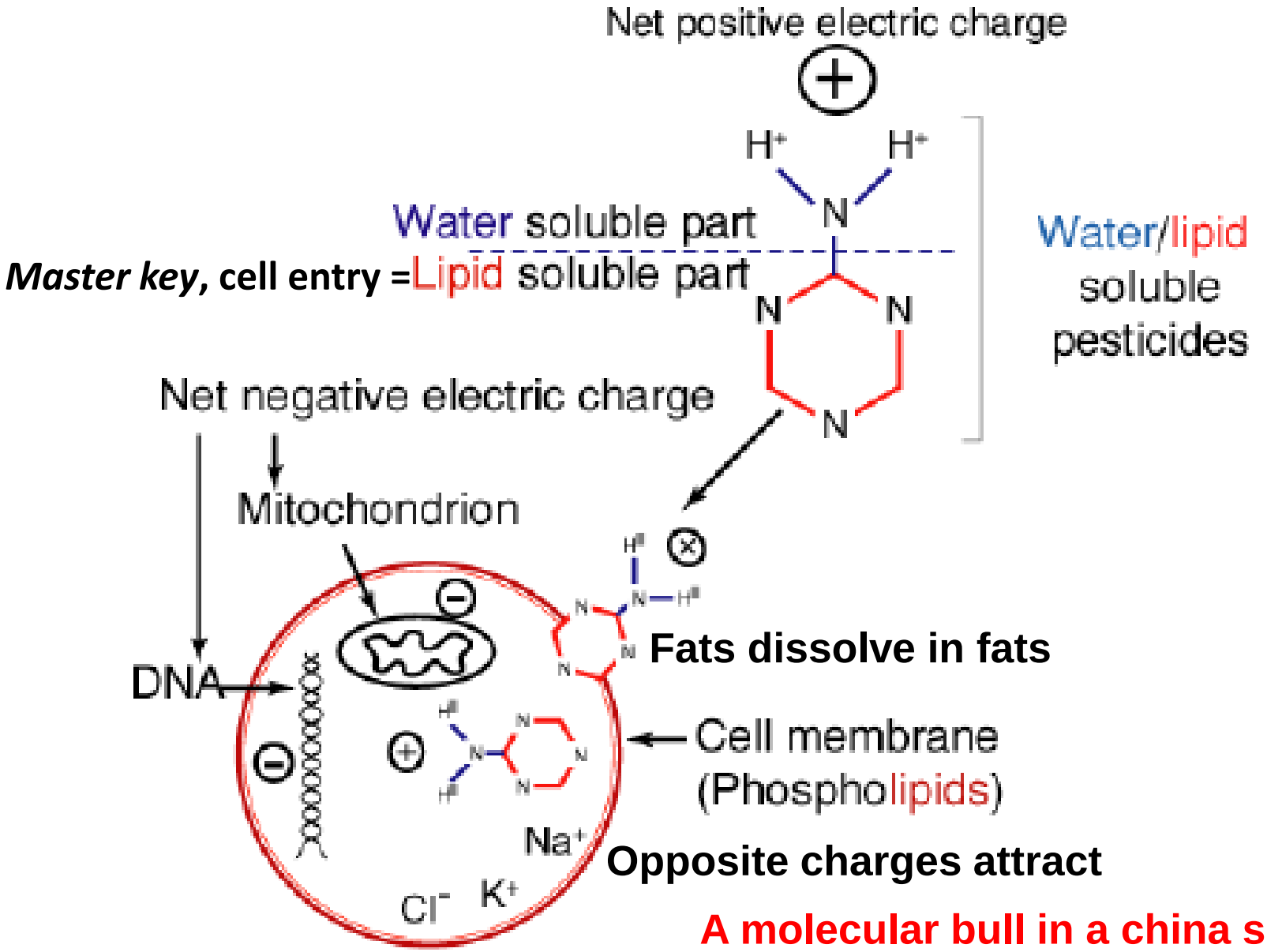
Pesticides + lipid solvents

Pesticides + surfactants

<- Leaf or insect x-section

functionally a 'bait and switch' process

Pesticide structure-function
(Why unintended effects are virtually certain)



***Master key*, cell entry =**

A molecular bull in a china shop

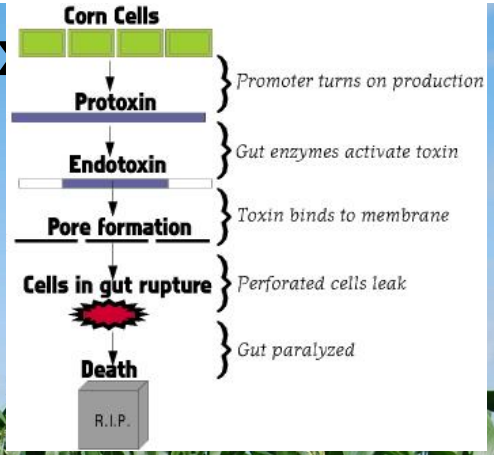
Some new definitions:

PR-GMO crops (Pesticide Resistant)

Roundup (glyphosate) resistant soybeans

PP-GMO crops (Pesticide Producing)

Corn -*Bacillus thuringiensis* (Bt) to
larval control

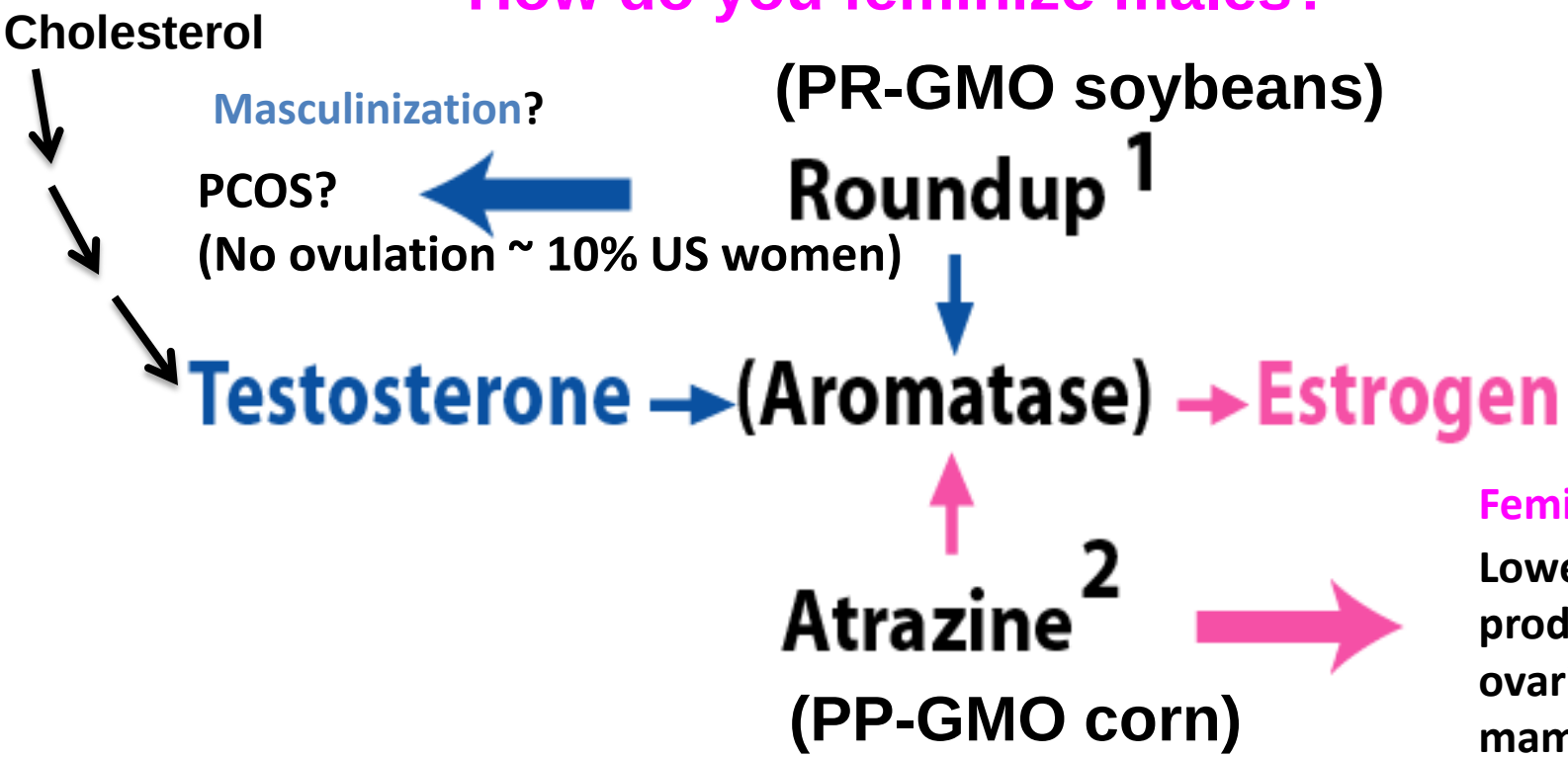


Atrazine - weed control

Reduce mating success

Common herbicides can alter sex hormones at environmentally relevant concentrations

How do you feminize males?



Feminization:

Lower sperm production, ovaries + testes, mammary tissue in male breasts

Changes in sexual orientation

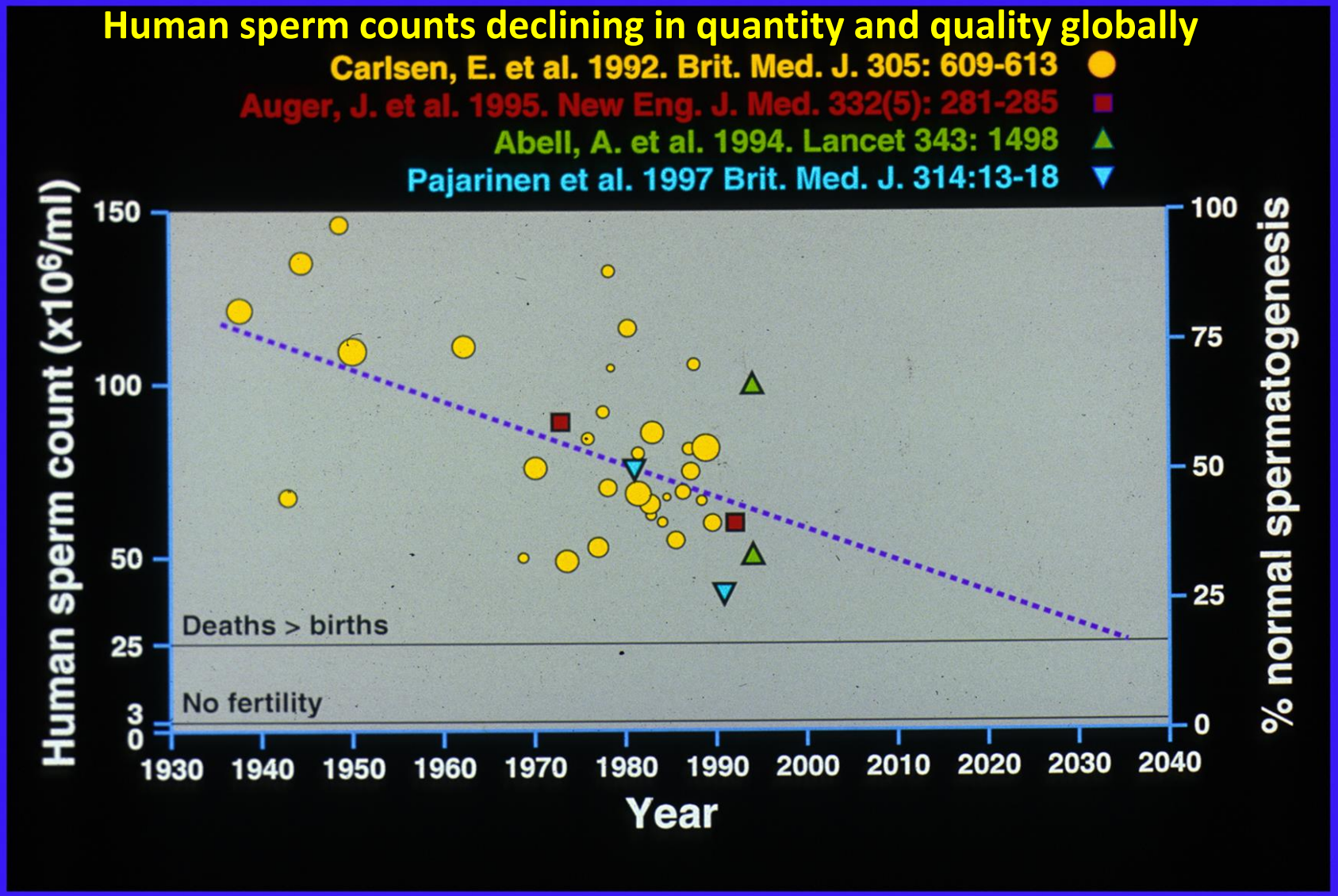
[1] Richard et al. 2005. Env. Health Persp.

[2] Fan et al. 2007. Env. Health Persp.

Evidence for reproductive impairment?

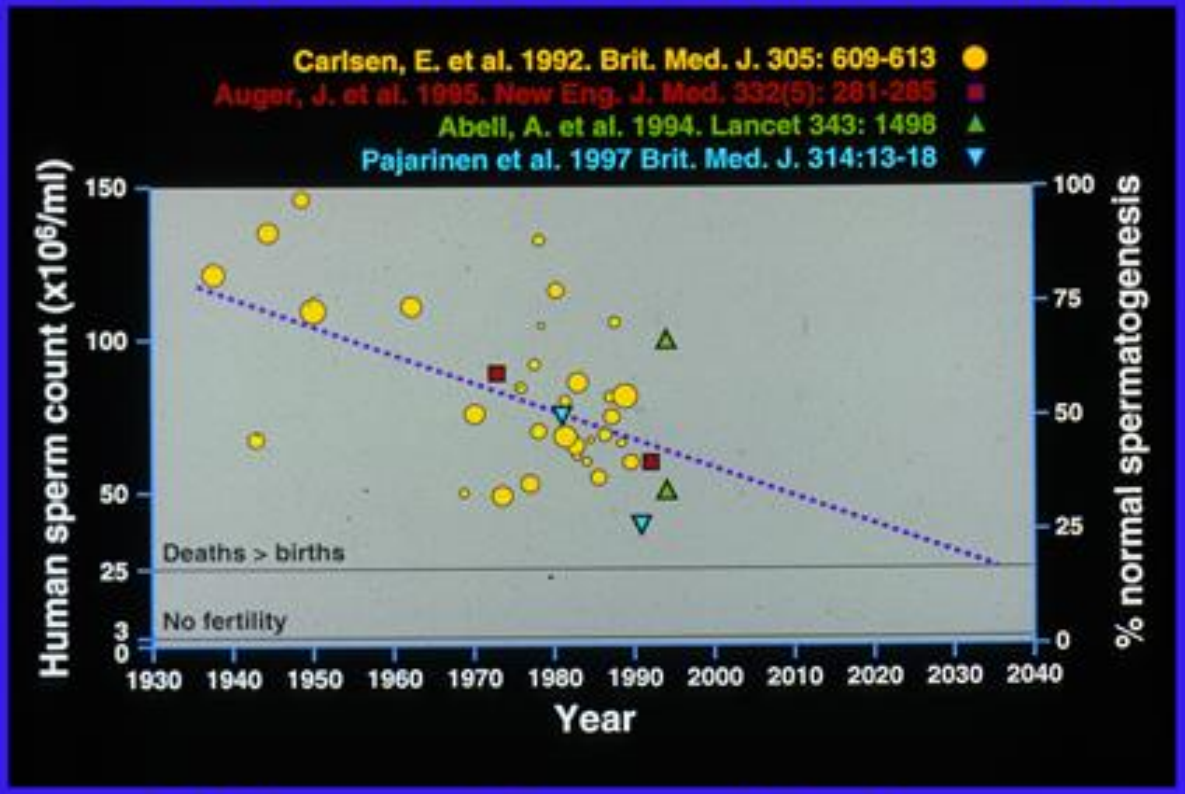
Decrease births: Lower fertility-> reduce mating success

Are we feminizing human males globally?



The human species' current glide path

Human sperm counts declining in quantity and quality globally – xenoestrogen feminization of human males, like the atrazine frogs?



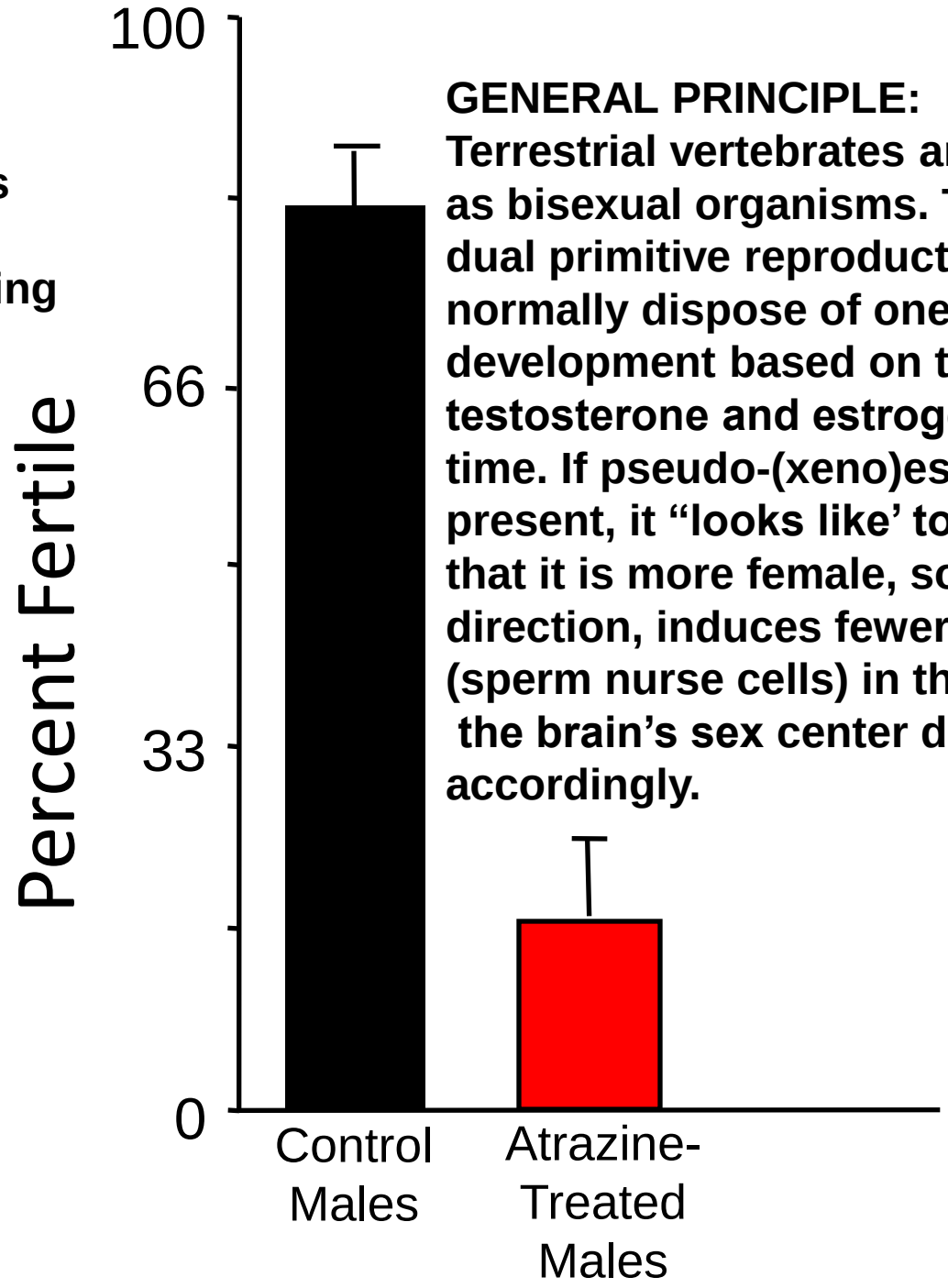
The human species' current glide path

60-70% US males qualified as sperm donors

6-7% US males qualify as sperm donors

1% of Israeli soldiers qualify (2012)
2013 ->1st year ever US whites– more deaths than births

Dr. Tyrone Hayes
UC Berkeley
Atrazine feminizing male frogs at environmentally relevant concentrations



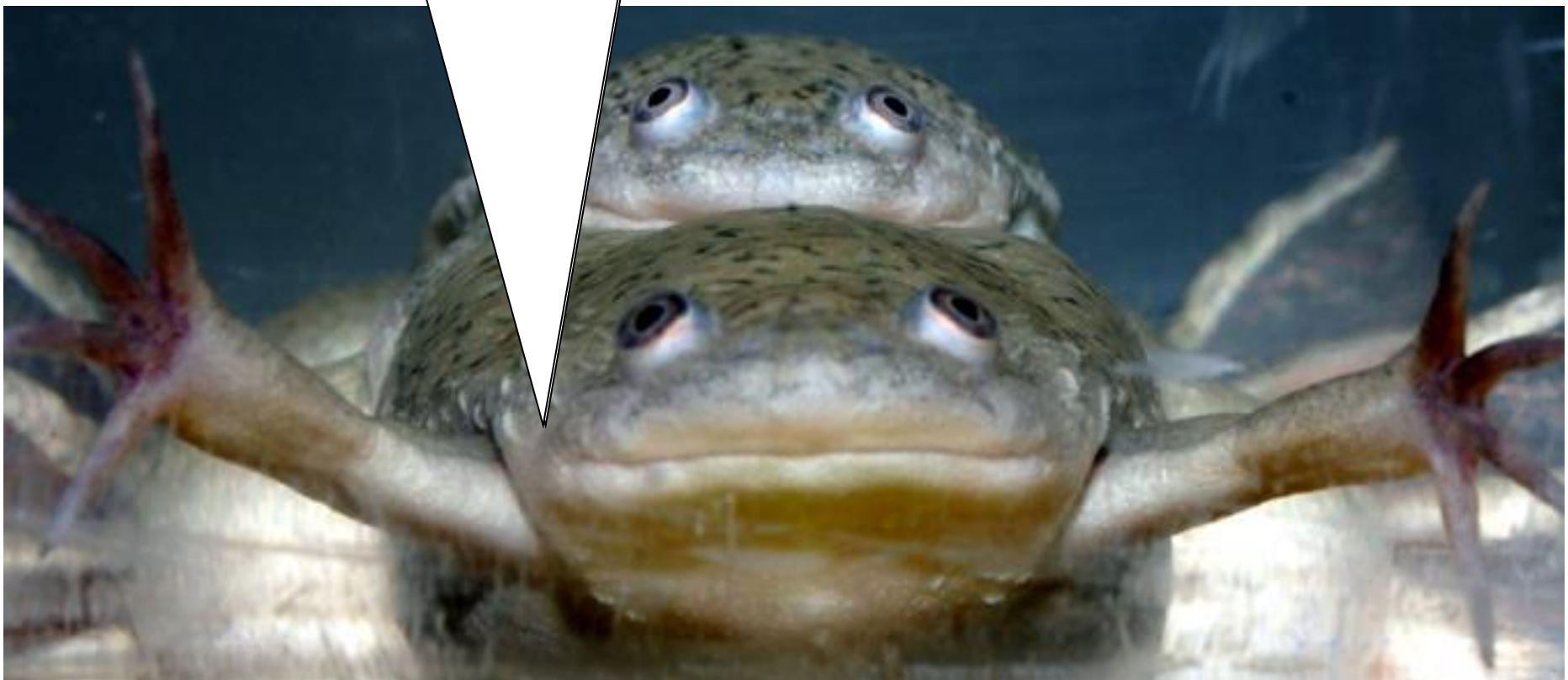
GENERAL PRINCIPLE:
Terrestrial vertebrates are conceived as bisexual organisms. They develop dual primitive reproductive tracts. They normally dispose of one of them during development based on the ratio of testosterone and estrogen at 'decision' time. If pseudo-(xeno)estrogens are present, it "looks like' to a male embryo that it is more female, so it changes direction, induces fewer Sertoli cells (sperm nurse cells) in the testis and alters the brain's sex center development accordingly.

“At this time, EPA believes that no additional testing is warranted to address this issue.”

Tim Pastoor,
Syngenta, makers of Atrazine
Atrazine upregulates aromatase, which elevates estrogen, which elevates breast cancer risk. Syngenta also makes drugs that treat breast cancer.

Reduce mating success

Two male frogs exposed to Atrazine attempting breeding (Prof. Tyrone Hayes, U.C. Berkeley)



Might 'estrogen' changes alter human sexual behavior?

The estrogen receptor is 'promiscuous' - > 12 'partners',

e.g. flame retardants, plasticizers (e.g. nonyl phenol),PCBs, dioxins, pthalates

Steroid Hormones and Brain Development: Some Guidelines for Understanding Actions of Pseudohormones and Other Toxic Agents

by Bruce S. McEwen*

Gonadal, adrenal, and thyroid hormones affect the brain directly, and the sensitivity to hormones begins in embryonic life with the appearance of hormone receptor sites in discrete populations of neurons. Because the secretion of hormones is also under control by its neural and pituitary targets, the brain-endocrine axis during development is in a delicately balanced state that can be upset in various ways, and any agent that disrupts normal hormone secretion can upset normal brain development. Moreover, exogenous substances that mimic the actions of natural hormones can also play havoc with CNS development and differentiation.

This paper addresses these issues in the following order: First, actions of glucocorticoids on the developing nervous system related to cell division dendritic growth and neurotransmitter phenotype will be presented followed by a discussion of the developmental effects of synthetic steroids. Second, actions of estrogens related to brain sexual differentiation will be described, followed by a discussion of the actions of the nonsteroidal estrogen, diethylstilbestrol, as an example of exogenous estrogenic substances. The most important aspect of the potency of exogenous estrogens appears to be the degree to which they either bypass protective mechanisms or are subject to transformations to more active metabolites. Third, agents that influence hormone levels or otherwise modify the neuroendocrine system, such as nicotine, barbiturates, alcohol, opiates, and tetrahydrocannabinol, will be noted briefly to demonstrate the diversity of toxic agents that can influence neural development and affect personality, cognitive ability, and other aspects of behavior.

Env. Health Persp. 1987. Vol. 74: pp. 177-184

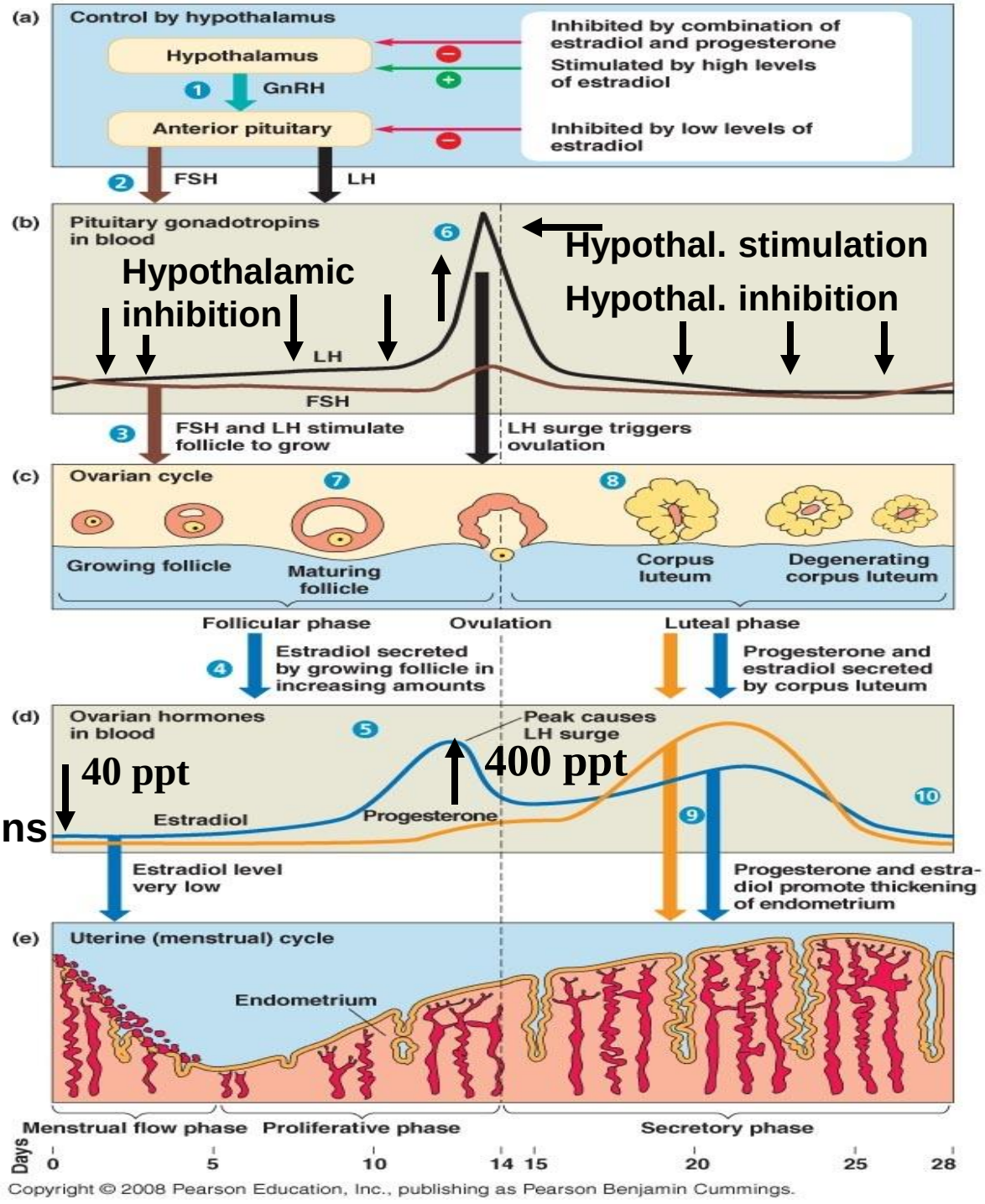
Psychosexual Differentiation and DES

Further insight into actions of estrogens on brain development has come from studies of offspring of mothers exposed to the pseudoestrogen diethylstilbestrol (DES) during pregnancy (*44–46*). In studies thus far completed and published, prenatal DES alters general measures of personality and leads to altered patterns of sexual behavior in adolescence and adulthood that reduce formation of heterosexual relationships (*44*). These differences from carefully matched normal subjects could not be explained by sexual dysfunctions such as vaginismus and dyspareunia, which were low in both groups, but rather appear to be due to psychosocial and neuroendocrine factors related to DES exposure (*44*).

44. Meyer-Bahlburg, H., Ehrhardt, A., Feldman, J., Rosen, L., Veridiano, N., and Zimmerman, I. Sexual activity level and sexual functioning in women prenatally exposed to diethylstilbestrol. Psychosom. Med. 47: 497–511 (1985).
45. Meyer-Bahlburg, H., Ehrhardt, A., Rosen, L., Feldman, J., Veridiano, N., Zimmerman, I., and McEwen, B. Psychosexual milestones in women prenatally exposed to diethylstilbestrol. Horm. Behav. 18: 359–366 (1984).
46. Ehrhardt, A., Meyer-Bahlburg, H., Rosen, L., Feldman, J., Veridiano, N., Zimmerman, I., and McEwen, B. Sexual orientation after prenatal exposure to exogenous estrogen. Arch. Sex. Behav. 14: 57–75 (1985).

Env. Health Persp. 1987. Vol. 74: pp. 177-184

Normal hormone concentrations



Parts per trillion – developing fetal brain – Fred vom Saal

Learning, behavior disorders

Enhanced aggression, shorter memory, alter neuromotor coordination functions

Environmental Health Perspectives Volume 106, Number 6, June 1998

An Anthropological Approach to the Evaluation of Preschool Children Exposed to Pesticides in Mexico

Elizabeth A. Guillette,[1] María Mercedes Meza,[2] Maria Guadalupe Aquilar,[2] Alma Delia Soto,[2] and Idalia Enedina Garcia[2]

[1]Bureau of Applied Research in Anthropology, University of Arizona, Tucson, AZ 85721 USA
[2]Direccion de Investigacion y Estudias de Postgrado, Instituto Tecnológico de Sonora, Obregón, Sonora, México

Yaqui valley, Sonora, Mexico.
US source of winter fruits and vegetables

*The Yaqui Valley in Mexico where our winter fruits & vegetables originate

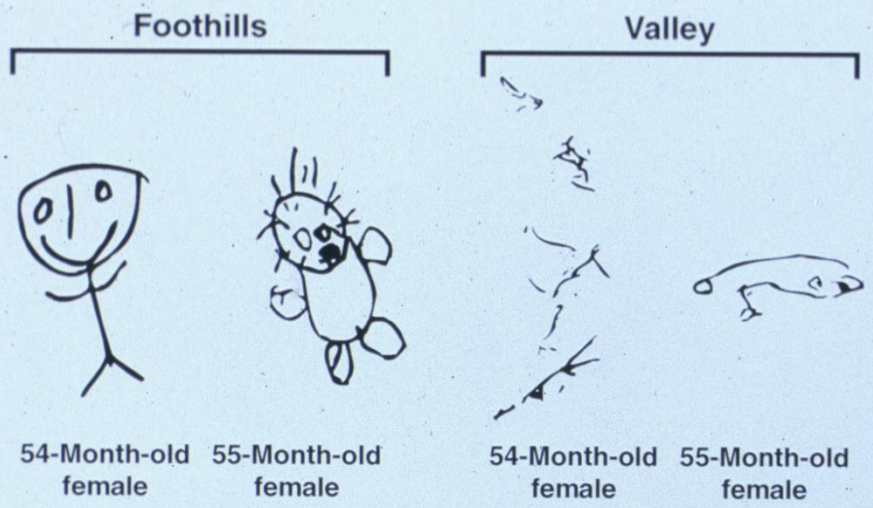


Figure 1. Representitive drawings of a person by 4-year-old Yaqui children from the valley and foothills of Sonora, Mexico.

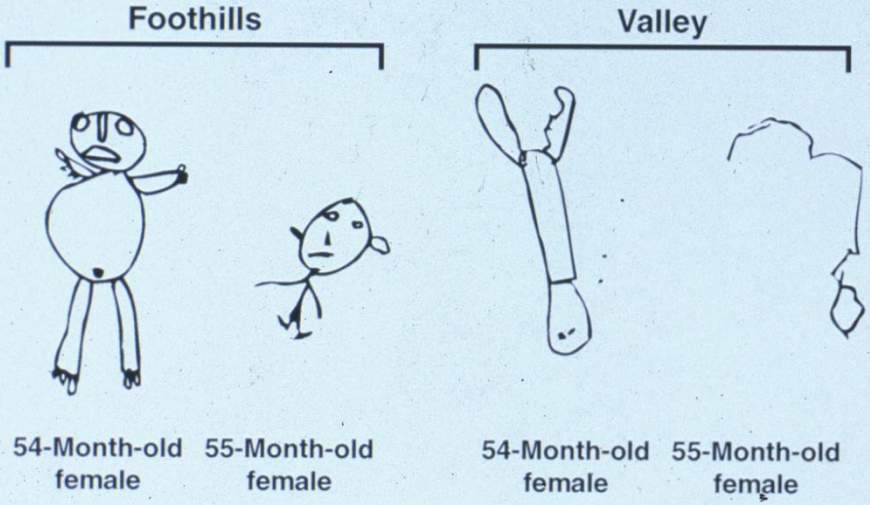


Figure 2. Representitive drawings of a person by 5-year-old Yaqui children from the valley and foothills of Sonora, Mexico.

Unexposed **Exposed**

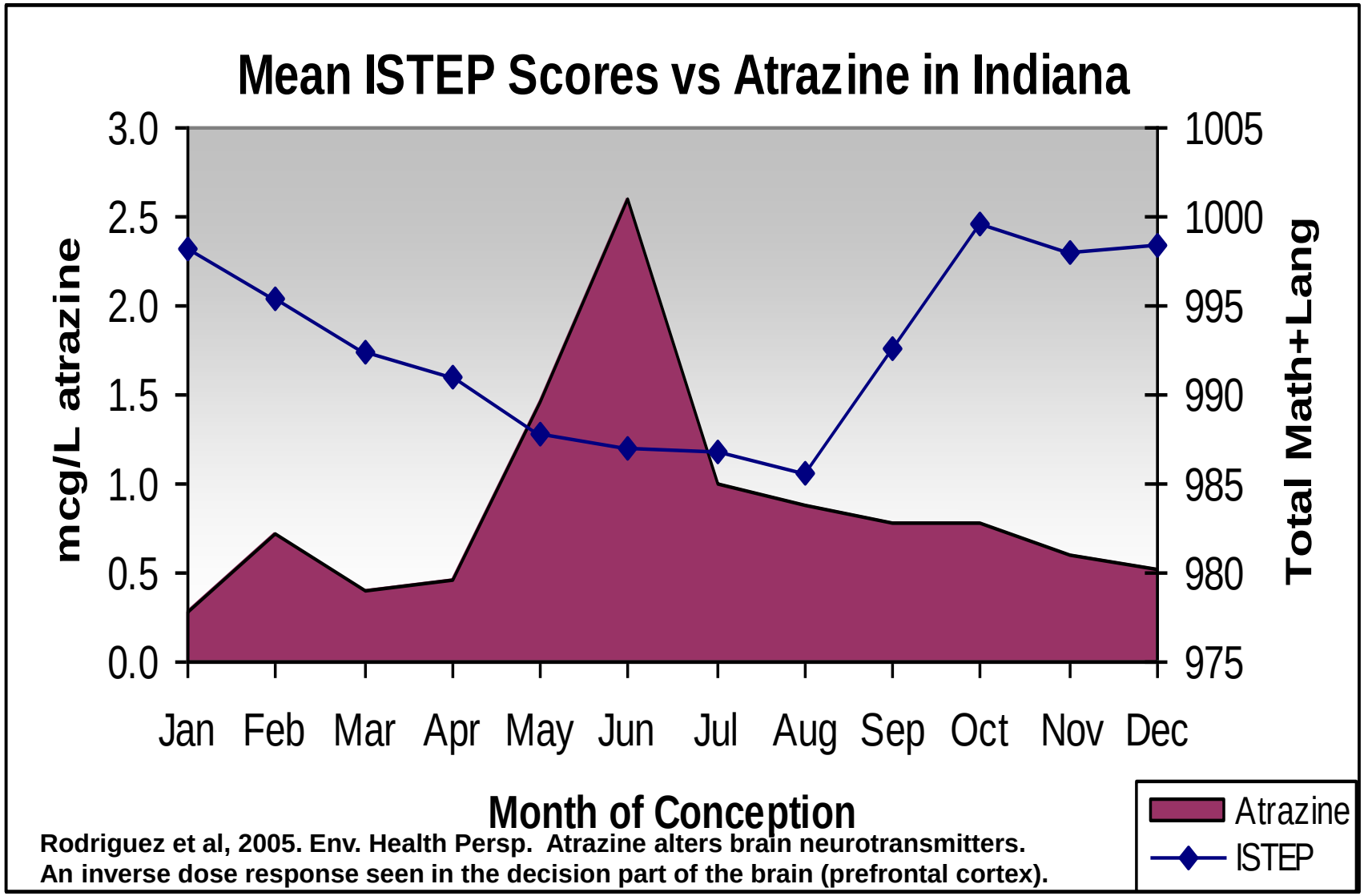
Current Status…

Valley teen boys have mammary tissue in their tender breasts; Valley teen girls have only fat in their breasts.

Children's mothers in the *valley* had very high rates of breast cancer.

What will be our childrens' future? Hint: How much are we spending on remedial education?

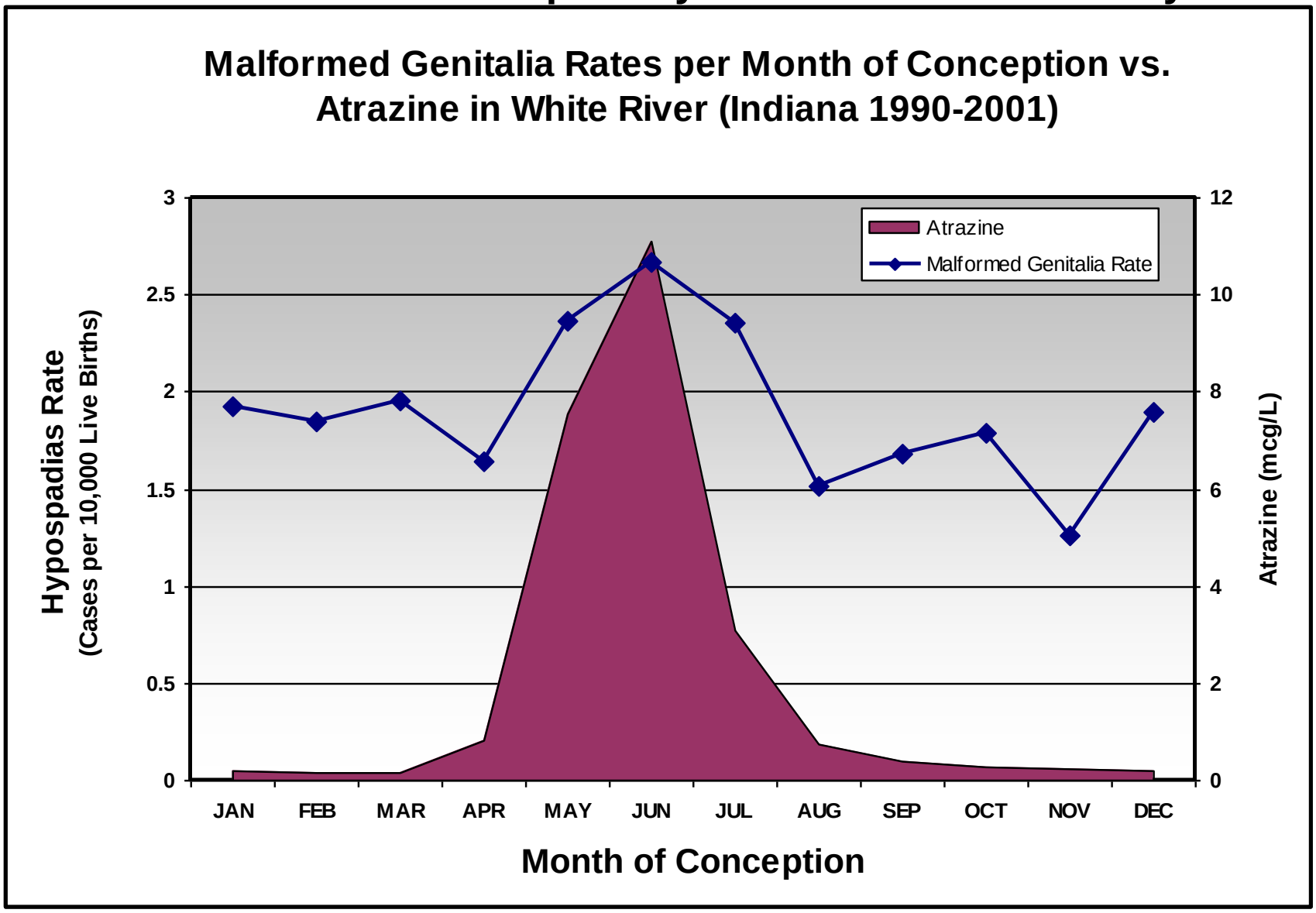
Could learning abilities in humans be altered by an *herbicide*?



Rodriguez et al, 2005. Env. Health Persp. Atrazine alters brain neurotransmitters. An inverse dose response seen in the decision part of the brain (prefrontal cortex).

Dr. Paul Winchester's data: Director of Neonatology, St. Francis Hospital & Health Centers, Indianapolis
Clinical Professor, Indiana University School of Medicine

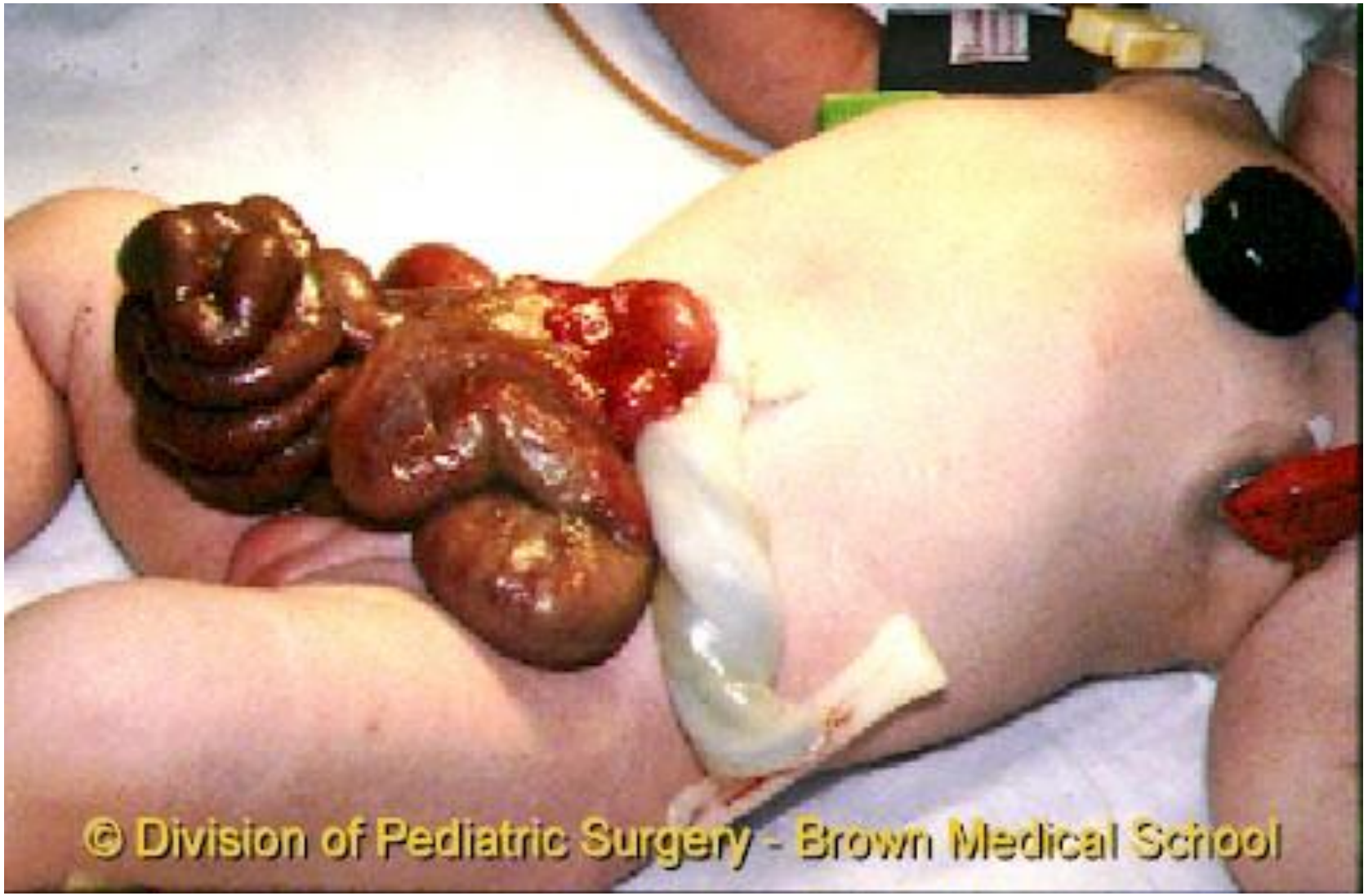
Increased birth defects – primary cause of infant mortality



Dr. Paul Winchester's data: Director of Neonatology,
St. Francis Hospital & Health Centers, Indianapolis
Clinical Professor, Indiana University School of Medicine

Another kind of birth defect

Gastroschisis – a new epidemic from rural areas in Wisconsin and around the country



What does this picture suggest?

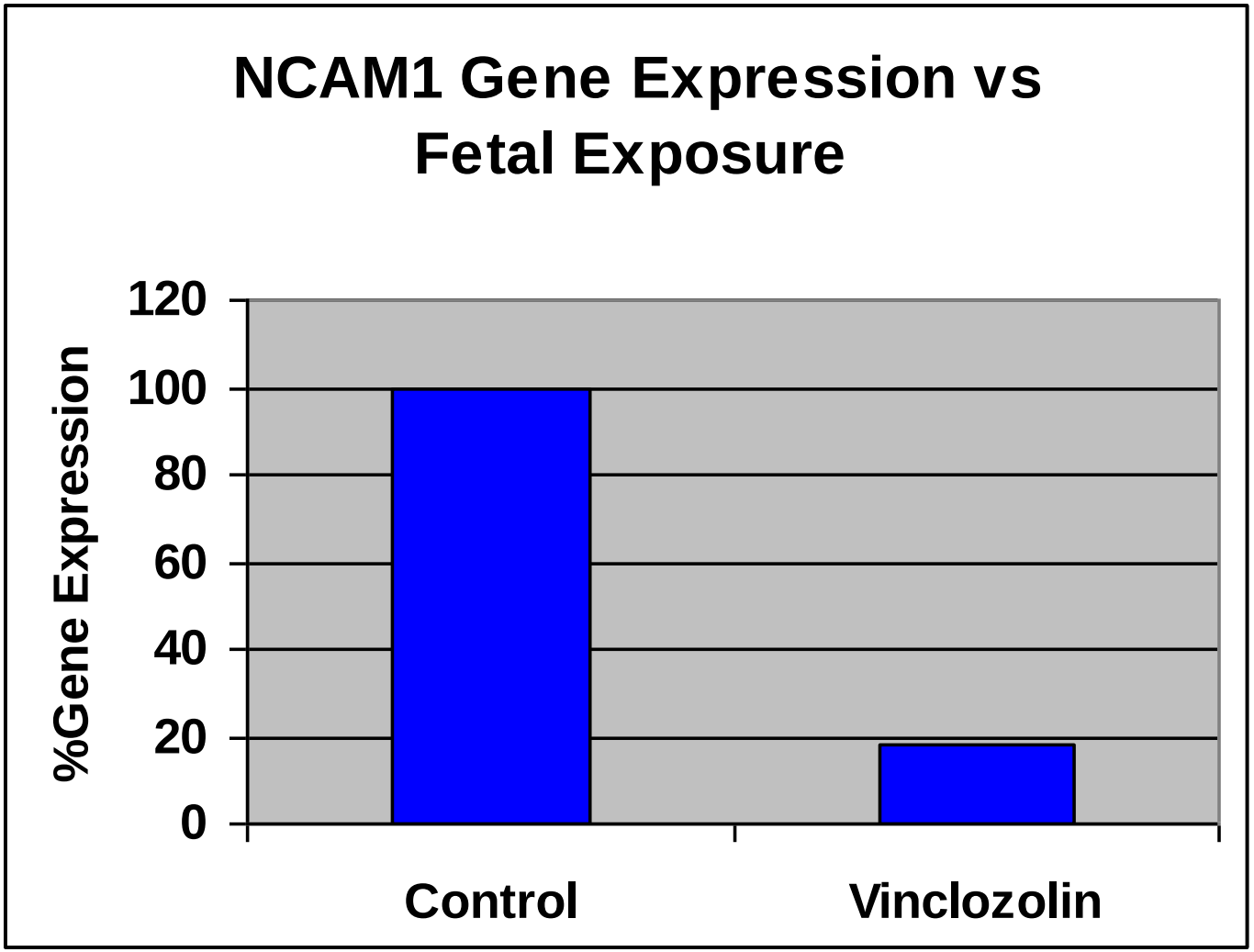
Change in fetal gene expression

1st paper: Epigenetic Transgenerational Actions of Endocrine Disruptors and Male Fertility Matthew D. Anway, Andrea S. Cupp, Mehmet Uzumcu and *Michael K. Skinner*. 2005. Science 308: 1466-1469

Later: Transgenerational (4 generations)Epigenetic Imprinting of the Male Germline by Endocrine Disruptor Exposure during Gonadal Sex Determination

Endocrinology 147(12):5524–5541, 2006

Relative *brain* expression levels of NCAM1



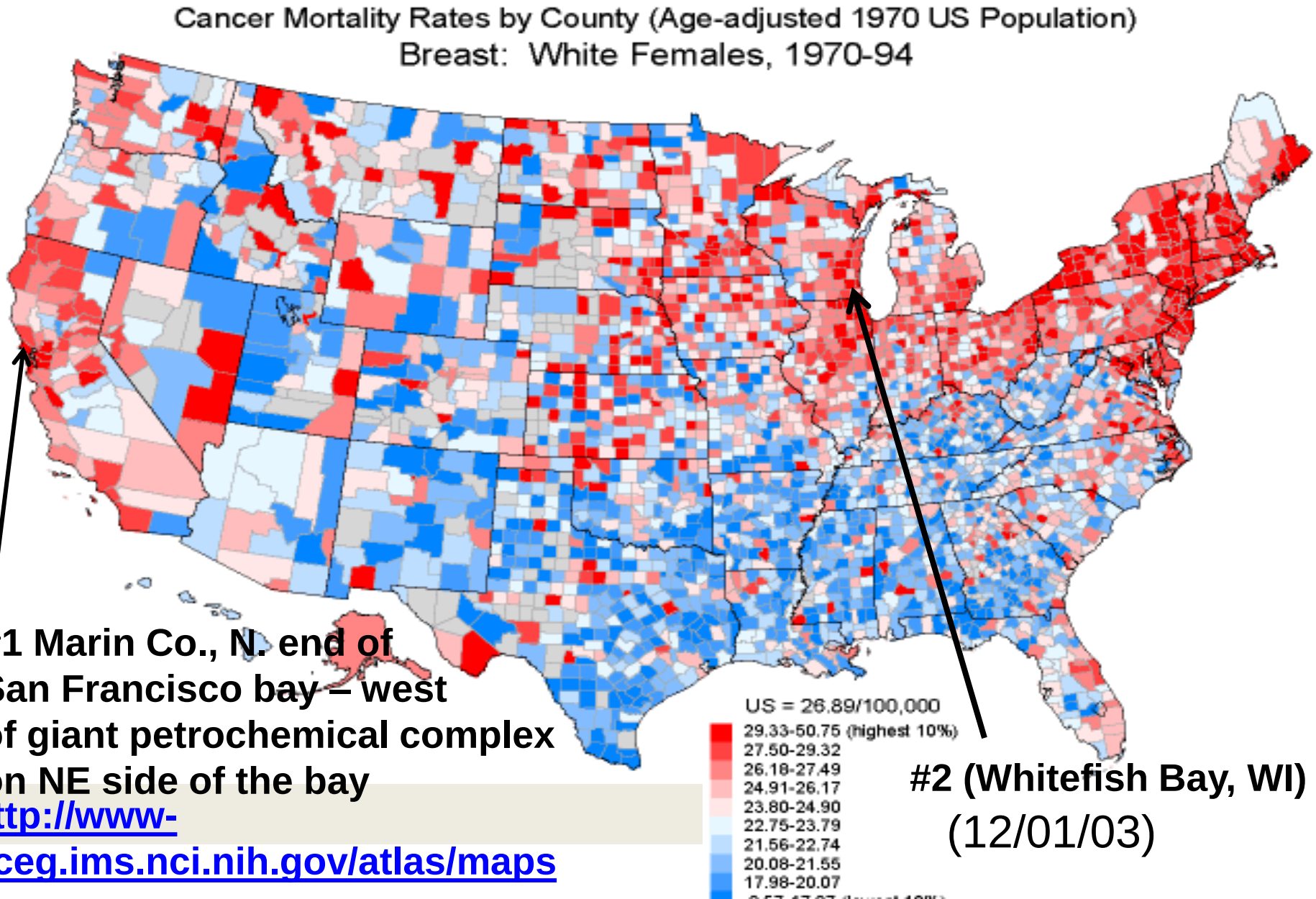
NCAM1 Gene Related Diseases

1) Alzheimers
2) Synovial sarcoma
3) Schizophrenia
4) Mutant-allele-specific amplification (MASA) syndrome
5) Neural tube defects
6) Various tumors

1) Decrease birth rates
2) Increase death rates ->

Increase deaths -> 1) contaminate air

http://dceg.cancer.gov/atlas/maps/brecwf70.gif



#1 Marin Co., N. end of San Francisco bay – west of giant petrochemical complex on NE side of the bay

#2 (Whitefish Bay, WI) (12/01/03)

http://www-dceg.ims.nci.nih.gov/atlas/maps

Increase deaths -> 2) contaminate food

ehp Environmental Health
ehponline.org
PERSPECTIVES
Published by the National Institute of Environmental Health Sciences

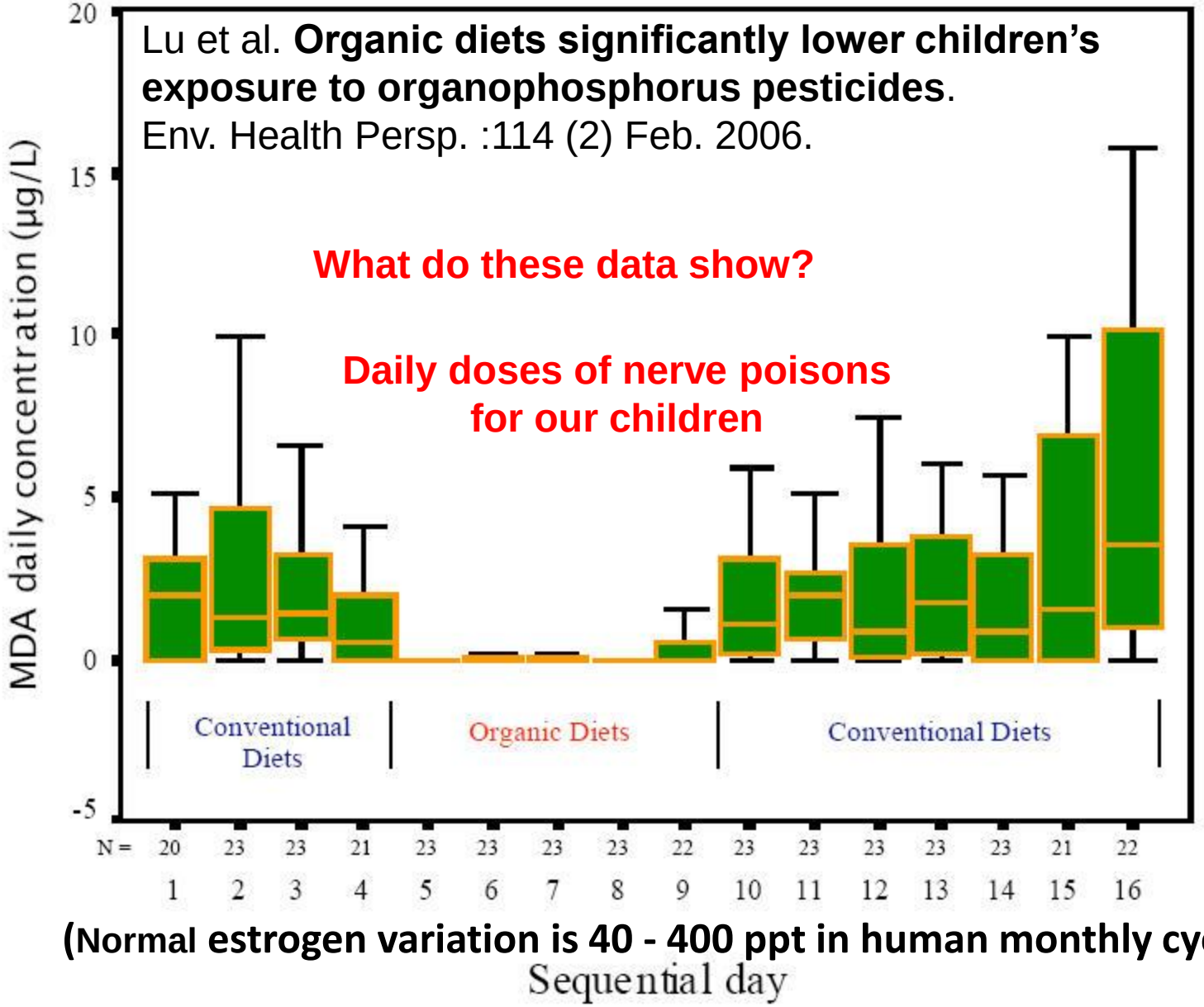
Organic Diets Significantly Lower Children's Dietary Exposure to Organophosphorus Pesticides

Chensheng Lu, Kathryn Toepel, Rene Irish,
Richard A. Fenske, Dana B. Barr, and Roberto Bravo

doi:10.1289/ehp.8418 (available at http://dx.doi.org/)
Online 1 September 2005

MDA = malathion metabolite

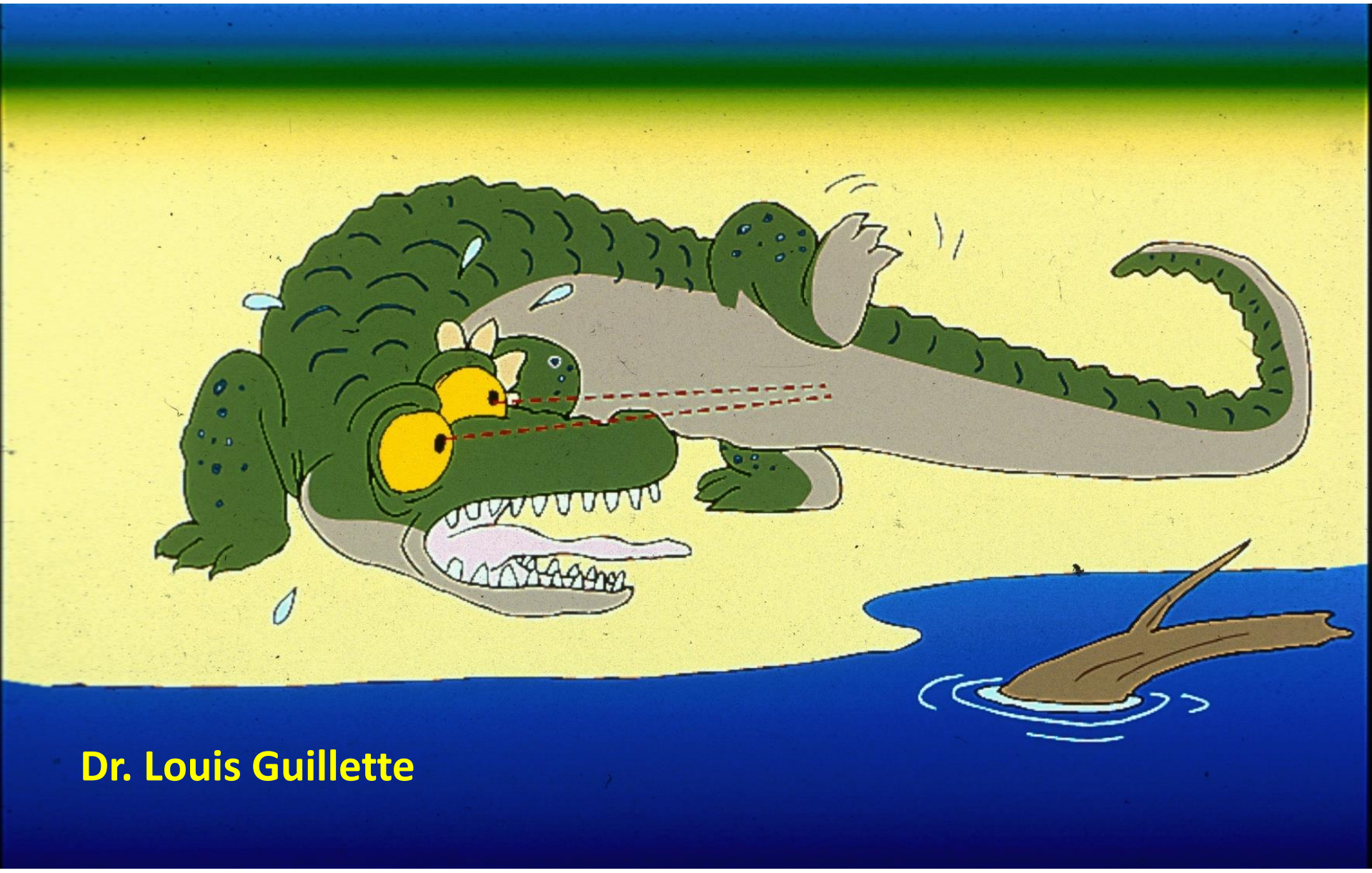
* organophosphate = neurotoxic by design



(Normal estrogen variation is 40 - 400 ppt in human monthly cycle.)

Increase deaths -> 3) contaminate water

A DDT-like compound did this to alligators in Lake Apopka, FL

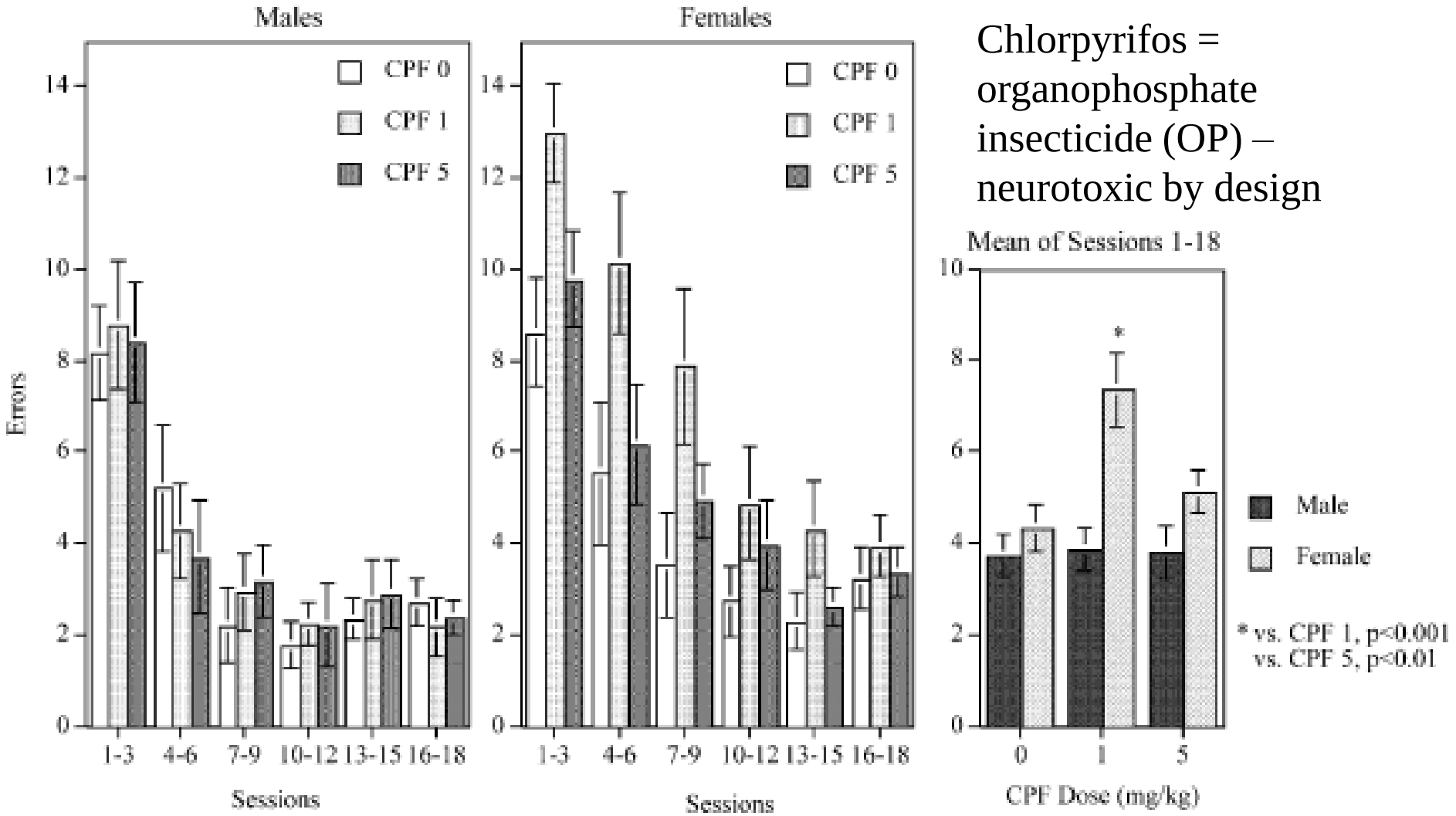


Dr. Louis Guillette

Neuro-endocrine-immune inverse dose effects

Neurological inverse dose – ***female*** learning affected at ***lowest*** dose

Chlorpyrifos = organophosphate insecticide (OP) – neurotoxic by design



Levin *et al*. 2002. Neurotox. and Teratology

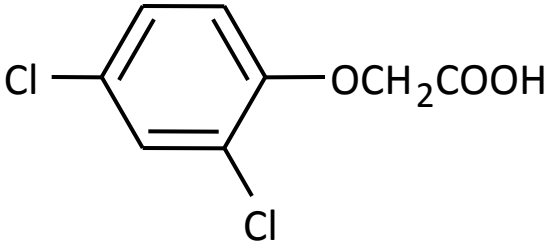
Males impacted at higher doses
Reproductive impairment

Compromised hormone (reproductive) function at low levels

Herbicide induction of abortions and resorptions of embryos?

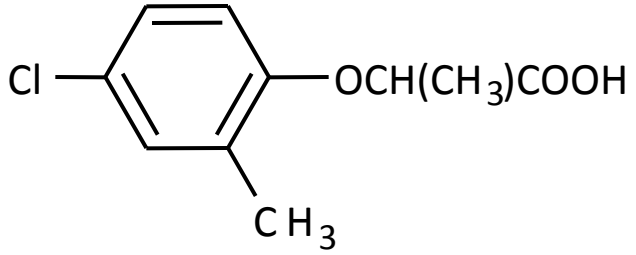
A common lawn chemical mix…

Chlorophenoxyacetic acid derivatives



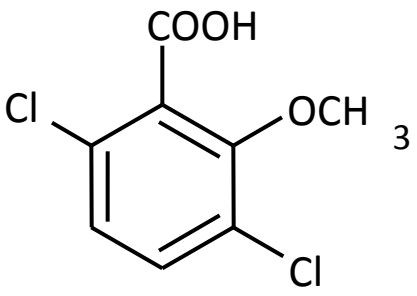
2,4-D (+ 2 small dioxins)

(PR-GMO 2,4-D resistant alfalfa)



Mecoprop

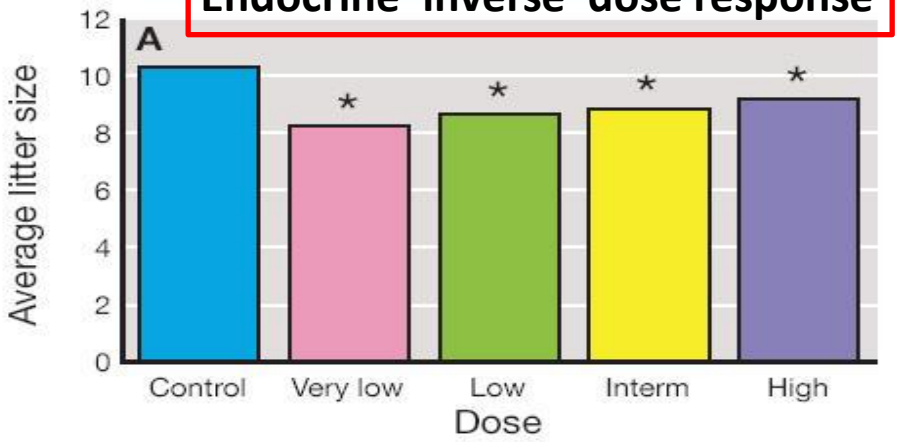
Benzoic acid derivative

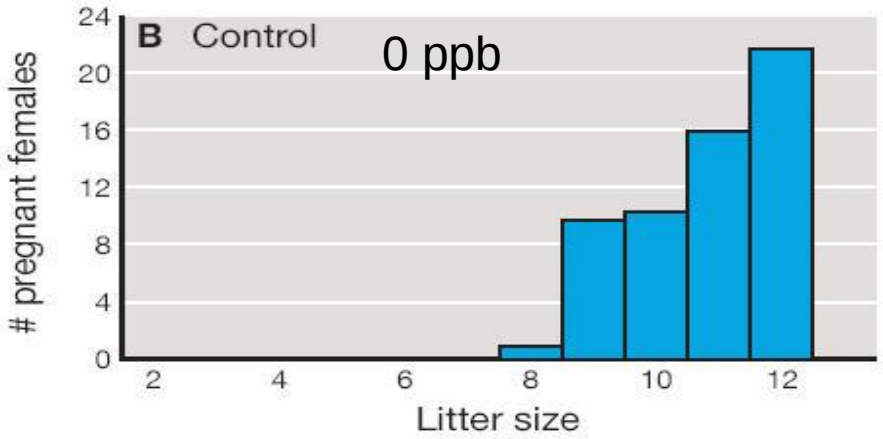


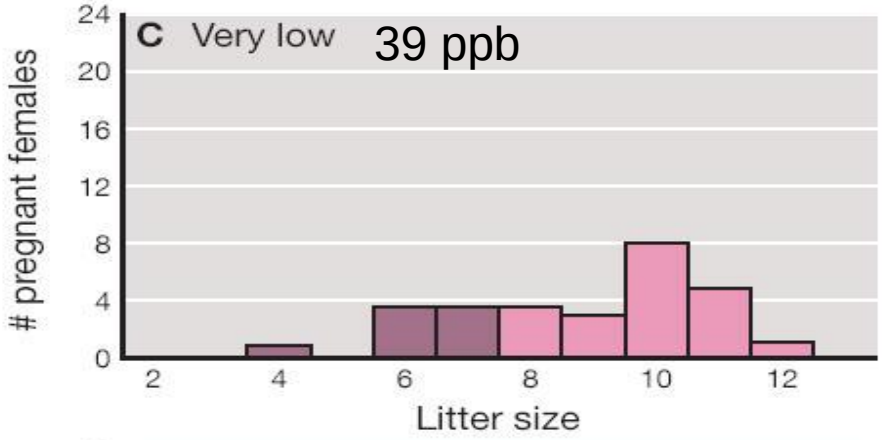
Dicamba

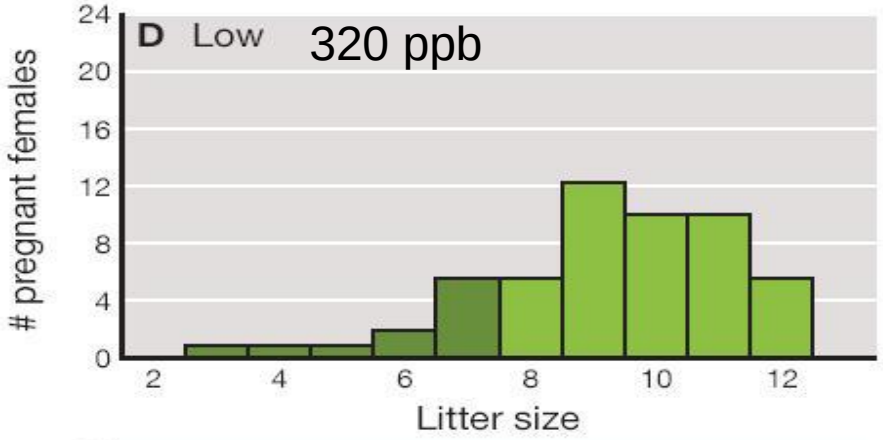
Notice ring-shaped (*fat soluble*) structures and strong negatively charged Cl^- and $COOH^-$ groups (*water soluble*)

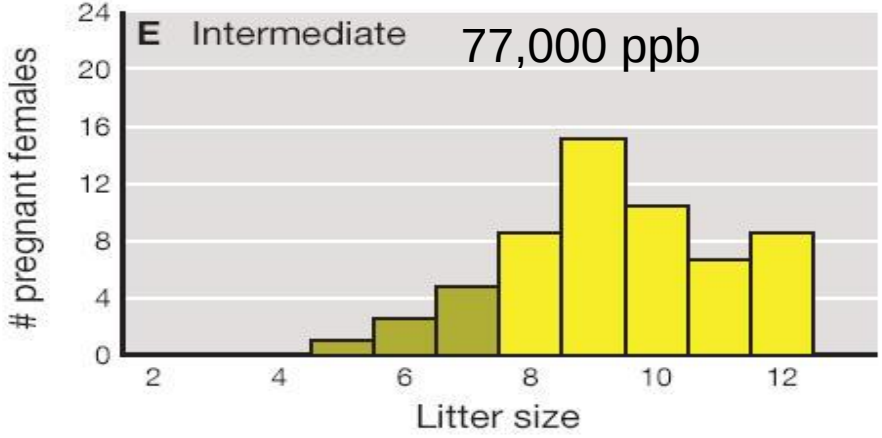
Endocrine 'inverse' dose response

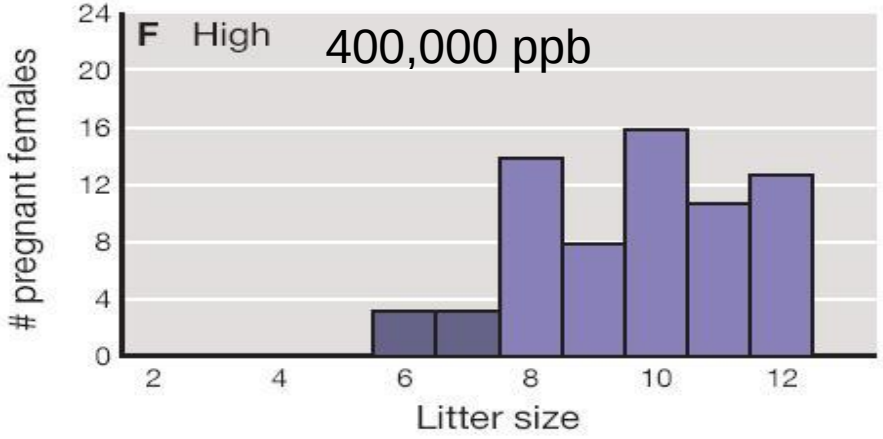












Cavieres et al. 2002. Env. Health Persp. 110(11)

Compromised immune function at low concentrations

Immune Inverse Dose

Response 34 days Exposure

Olson et al, 1987. Arch Env Contam Toxicol. 16: 433-439.

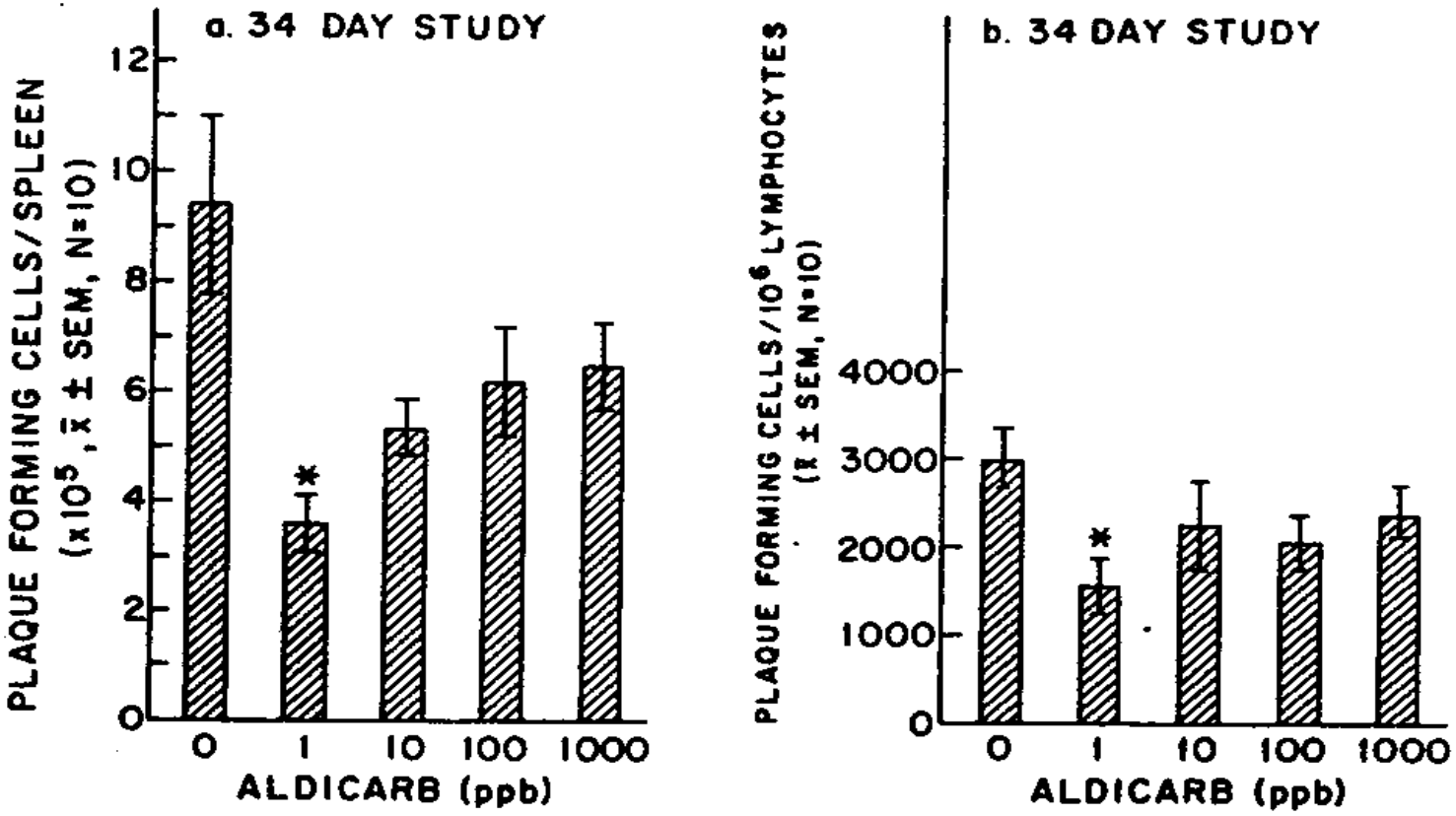


Fig. 2. (a) Plot of PFC's/spleen for four aldicarb drinking water doses given for 34 days (Exp. 2). (b) Plot of PFC's/10^6 viable spleen lymphocytes for four aldicarb doses in the 34 day experiment (Exp. 2) (* indicates significant difference [$p < 0.05$] from distilled water controls)

Immune insult is associated with many serious chronic health problems

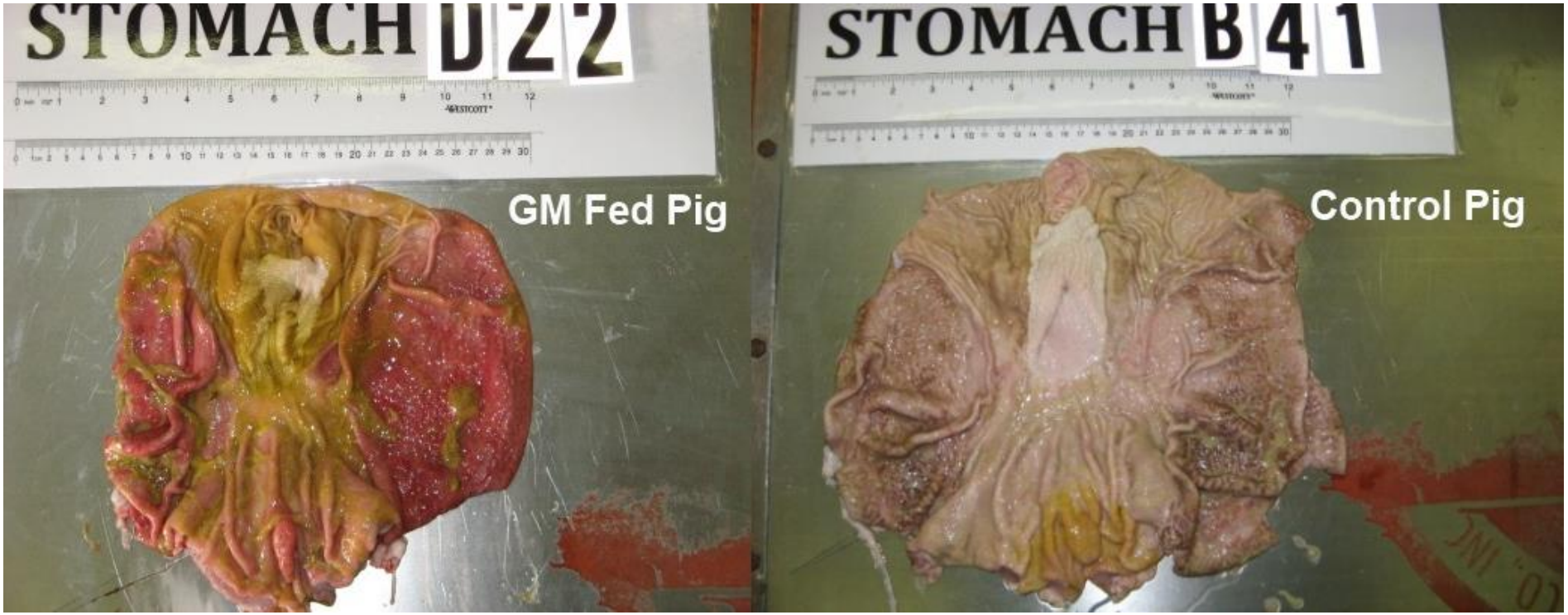
Table 1. Diseases Associated with Early-Life Immune Insult and DIT and Potential for Restorative Immunotherapy

Target Diseases And Conditions	DIT/Cytokine Dysfunction	Environmental Risk Factors	Key References
Asthma & Allergic Diseases	Hyper-IgE, Th2 Bias With Skewed Cytokine Production, Dendritic Cell Maturation Block	Maternal Smoking, ETS, Heavy Metals, Genistein, Prenatal Antibiotics, Ascaris vs. TB Exposure, Allergen Exposure Without LPS, Caesarian Delivery, Diesel Exhaust, Drugs Of Abuse	[3,7,12,42,57-59,64, 65,68, 71,184-187]
Autoimmunity	Thymic Selection Interference, Regulatory T Cell Dysfunction, Hyper-Inflammation	DES, Xenoestrogens, Complicated Delivery, Heavy Metals, TCDD, Atrazine, Maternal Infection	[26,28,73-75,188-190]
Infectious Diseases, Ineffective Vaccine Responses	Skewed Th Balance, Hyper-Inflammation, Dendritic Cell Interference; Reduced Leukocyte Migration	PCBs, TCDD, Heavy Metals, Maternal Smoking, ETS, Maternal Alcohol, Prenatal Antibiotics, Prenatal Malnutrition, Nonyphenol, Drugs of Abuse	[4, 22, 29, 56, 58,77, 78, 191-195]
Cancer	Th1 Cytokine Suppression, NK Cell Suppression, CTL Suppression	Maternal Smoking, ETS, Metals, Maternal Alcohol, PAHs, Nonyphenol	[15, 18, 20, 29, 80]
Neurodegen. Disease & Neurocognitive Loss	Inflammatory Cell Dysfunction, Hyper-Production of Proinflammatory Cytokines	Maternal Infection/Stress, Lead and other Metals, PAHs	[32,82,83,85, 88, 196-199]
Cerebral Palsy	Cytokine Network Disruption	Maternal Infection/Stress	[89]
Atherosclerosis	Macrophage Dysfunction, Hyper-Inflammation	Lead, Cadmium	[200]
Hypertension	Prenatally-Programmed Immune Cell Infiltration Of The Kidney	Maternal Low Protein Diet, Prenatal IL-6 Exposure	[92,201]
Male Sterility	Macrophage Homeostatic Disruption And Hyper-Inflammation	Lead	[91]

Dietert, R.R. and J.M.Dietert. 2007. Curr. Medicinal Chem. 1075-1085

Gut inflammation with PR-&PP-GMO foods

Inflammation indicates strong immune activity, ‘leaky’ connections between cells, opportunity for bacteria and other substances to escape the gut and enter the blood stream.



Carman, J. A., et al. (2013). A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet. Journal of Organic Systems 8(1): 38–54.

July 2013 | Volume 8 | Issue 7 | e69805

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PLOS ONE

Complete Genes May Pass from Food to Human Blood

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Abstract

Our bloodstream is considered to be an environment well separated from the outside world and the digestive tract. According to the standard paradigm large macromolecules consumed with food cannot pass directly to the circulatory system. During digestion proteins and DNA are thought to be degraded into small constituents, amino acids and nucleic acids, respectively, and then absorbed by a complex active process and distributed to various parts of the body through the circulation system. Here, based on the analysis of over 1000 human samples from four independent studies, we report evidence that meal-derived DNA fragments which are large enough to carry complete genes can avoid degradation and through an unknown mechanism enter the human circulation system. In one of the blood samples the relative concentration of plant DNA is higher than the human DNA. The plant DNA concentration shows a surprisingly precise log-normal distribution in the plasma samples while non-plasma (cord blood) control sample was found to be free of plant DNA.

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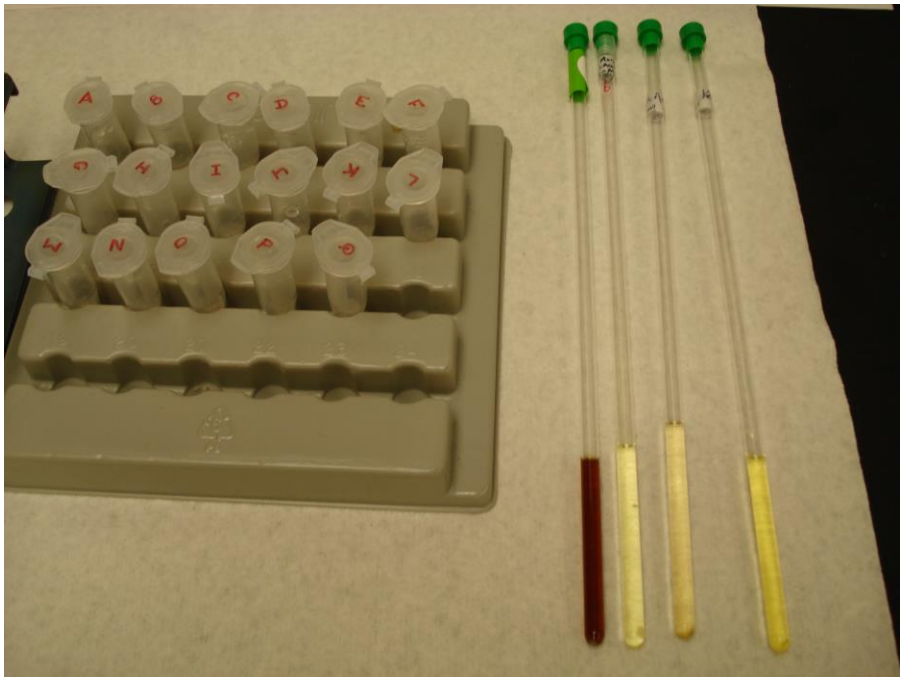
compromise epigenetic function <-> metabolome changes

New technologies developed by
Dr. Fariba Assadi-Porter

Metabolome Dynamics Platform (MDP)- from 30 uL of cell culture, tissue samples or biofluids of whole organisms -unbiased detection of biomarkers that change in response to perturbations, e.g. stress, infection, disease, toxicants, hibernation, substrate utilization, gut microbiome functions, etc.

Stable Isotope Assisted Labeling (SIAL)- biochemical pathway shifts and *flux measurements in real time non-invasively (breath)* in response to perturbations, e.g. stress, infection, disease, toxicants, hibernation, etc.

Samples in NMR tubes

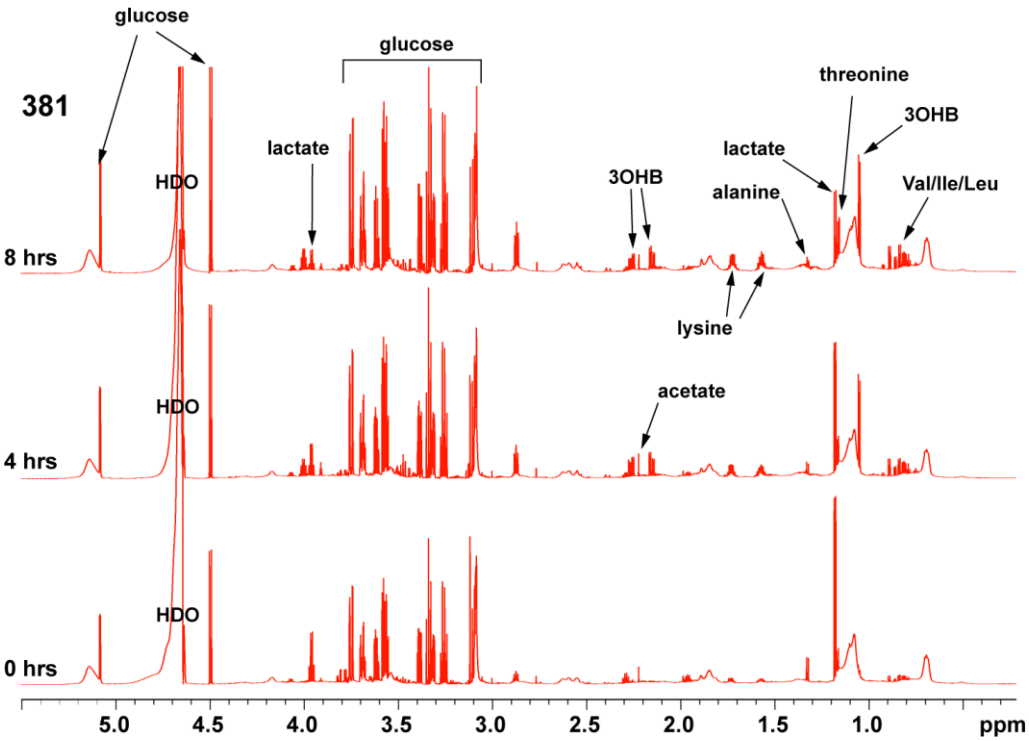


NMR machine

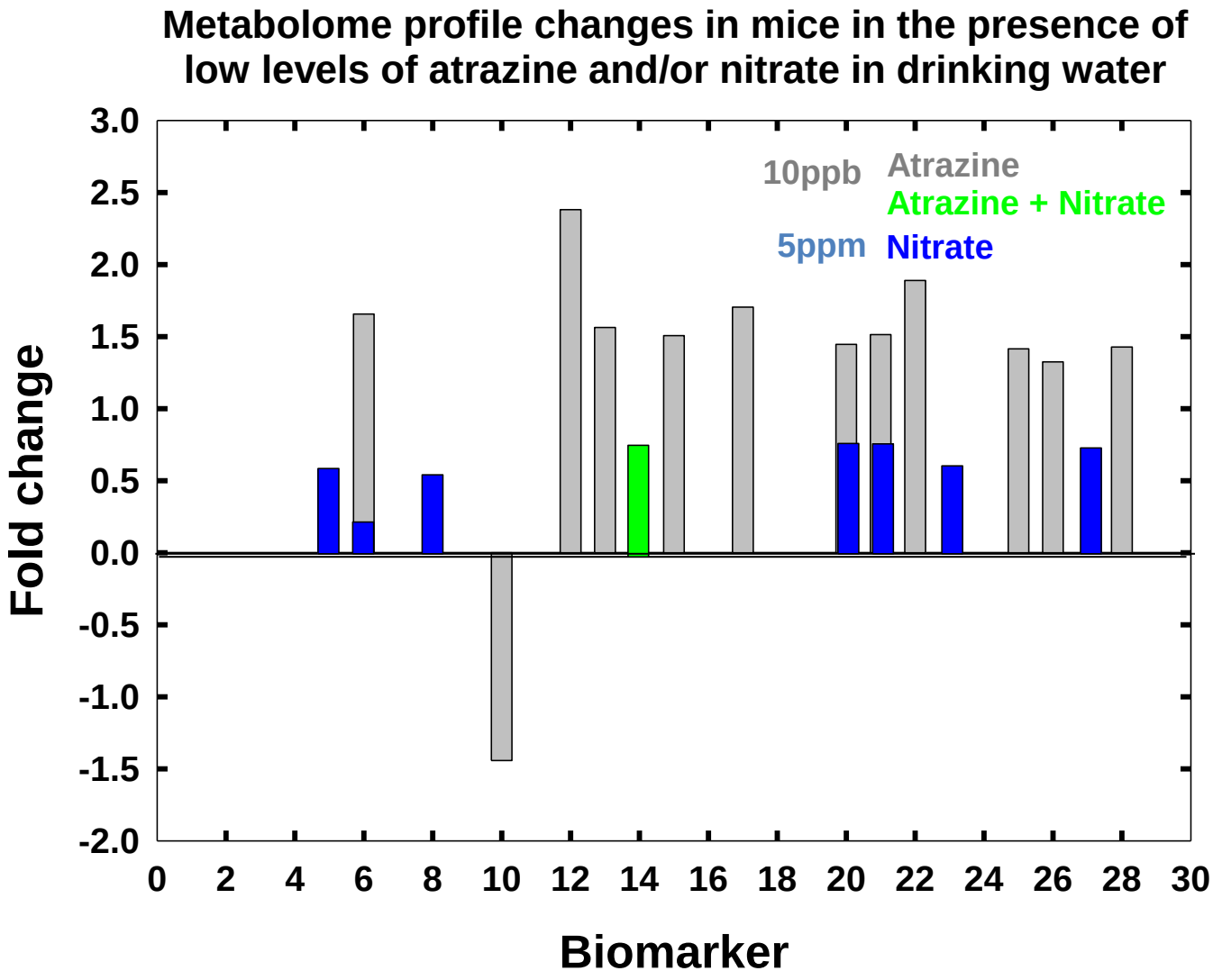
Output from NMR spectroscopy

Onset of infection in mice

- **Complex signals**
- **Signals translated to identify and quantify metabolites**
- **Fast data collection (15-120 min)**
- **Non-destructive** NMR Spectra
- **Patterns and temporal Changes of signals at the individual level**
- **Rapid analysis**
- **Allows identifying healthy from disease**



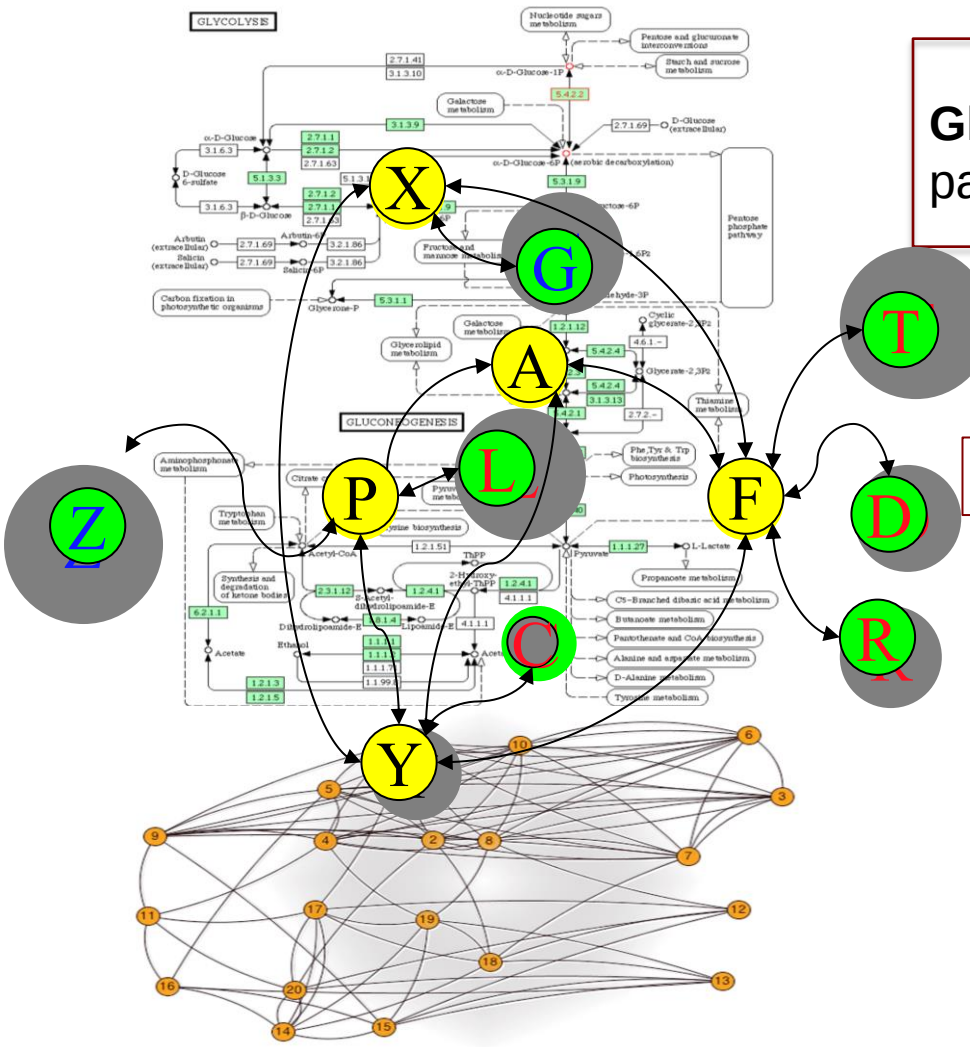
One dimensional proton (1H) spectrum



key lipid, amino acid and TCA cycle pathways altered

Haviland, J., Assadi-Porter, F., Porter, D.R., Velasco, J, and W.P. Porter . 2015. Metabolome profiles and reproductive changes due to common registered agrochemicals in drinking water. (In review)

Metabolome Dynamics Platform (MDP)



Glycolysis/Gluconeogenesis
pathway (complex network)

A net effect in a complex system

ID a reduced subset of metabolites

Patterns of metabolites

Computational platform (MDP)

Opportunity for solution =
CHANGE MARKET SHARE

1. Educate the buying public What GMO labeling is all about!
2. Invest in

   a. ***product purchases*** - the lifeblood of all companies. A change in market share of 0.5% elates or depresses CEOs & Boards of Directors depending on change direction

   b. ***company stocks*** - stock market position is important to all CEOs and companies

   c. ***research support*** - National Science Foundation currently flunks out 85% of all research proposals in the biological sciences, though program directors & review panels deem 85% worth of funding. 'Those who innovate & implement fastest win' - Adam Smith, Charles Darwin

How do you control population size? (The ecology of ecotoxicology)

1) Decrease births

- **a) lower fertility**
- **b) reduce mating success**
- **c) increase birth defects (greatest source of infant mortality)**
- **d) change fetal gene expression**

2) Increase deaths

- **a) enhance aggression**
- **b) contaminate air, food, water**
- **c) compromise neurological, hormone, immune and epigenetic functions (none tested in EPA registration)**
- **d) alter the metabolome (biochemical pathways)**

Summary – what have we learned?

Data suggest that we may be sexually assaulting our children *in utero*, possibly altering their sexual preferences or aborting them prematurely.

Virtually no marketed pesticide formulation has *ever* been registered by the EPA.

Registrations do *NOT* include tests for neurological, endocrine, immune, developmental or epigenetic (DNA methylation) tests.

Data for registrations come from the companies that *make* the chemicals -a clear *conflict of interest*.

Can we afford to raise generations of children that are neurologically, endocrinologically, immunologically and reproductively impaired?

Can we afford to induce chronic, long term subtle diseases and alter genetic expression that may be passed to subsequent generations?

The co-pilot