Environmental Influences on Mammary Gland Development and Function

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Developmental Basis of Disease: Disease Focus in Animals

Reproductive/Endocrine

Breast/prostate cancer
Endometriosis
Polycystic Ovary Syndrome
Fertility
Diabetes/Metabolic Syndrome
Puberty
Obesity
Lactation

Pulmonocardiovascular

Atherosclerosis
Asthma
Chronic Obstructive Pulmonary Disease (COPD)
Heart disease/Hypertension

Brain/Nervous System

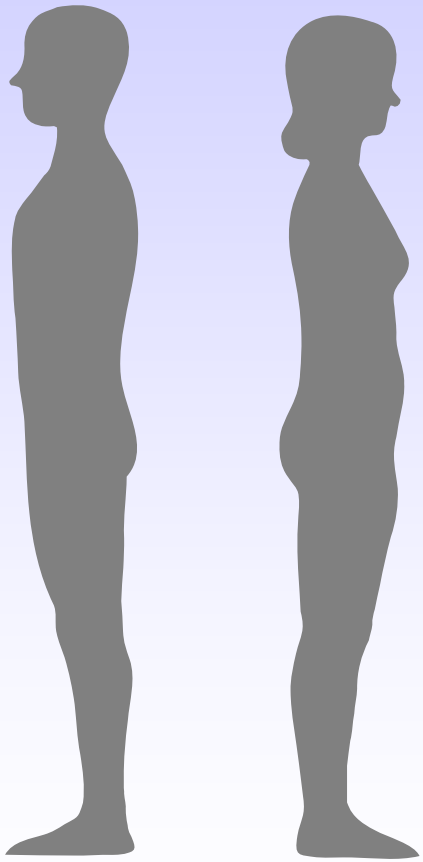
Alzheimer's Disease
Parkinson's Disease
Attention Deficit Hyperactivity Disorder (ADHD)

Immune/Autoimmune

Systemic/tissue specific Autoimmune disease
Immunosuppression

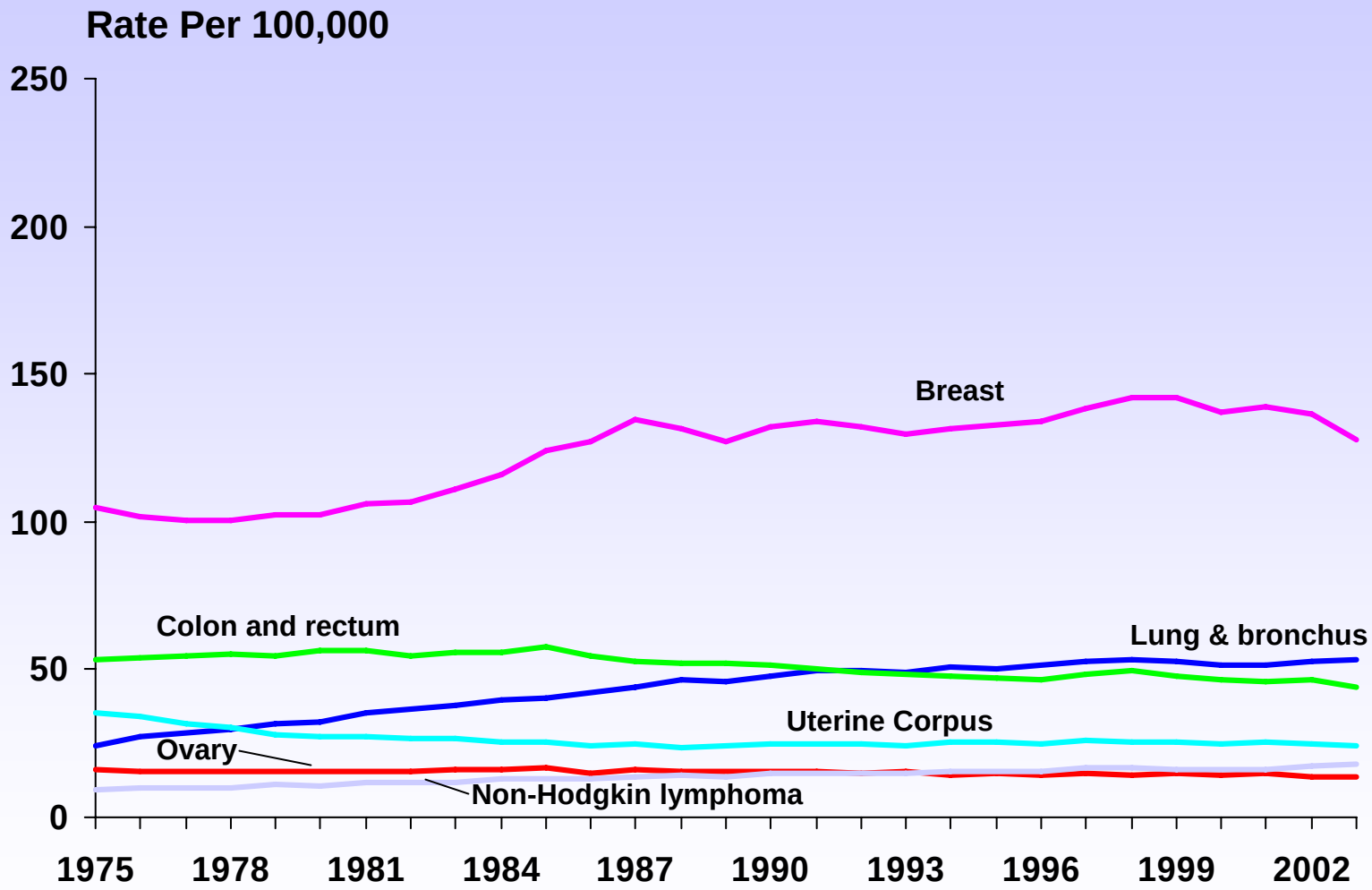
2007 Estimated US Cancer Cases*

Men 766,860		Women 678,060	
Prostate	29%	26%	Breast
Lung & bronchus	15%	15%	Lung & bronchus
Colon & rectum	10%	11%	Colon & rectum
Urinary bladder	7%	6%	Uterine corpus
Non-Hodgkin lymphoma	4%	4%	Non-Hodgkin lymphoma
Melanoma of skin	4%	4%	Melanoma of skin
Kidney	4%	4%	Thyroid
Leukemia	3%	3%	Ovary
Oral cavity	3%	3%	Kidney
Pancreas	2%	3%	Leukemia
All Other Sites	19%	21%	All Other Sites

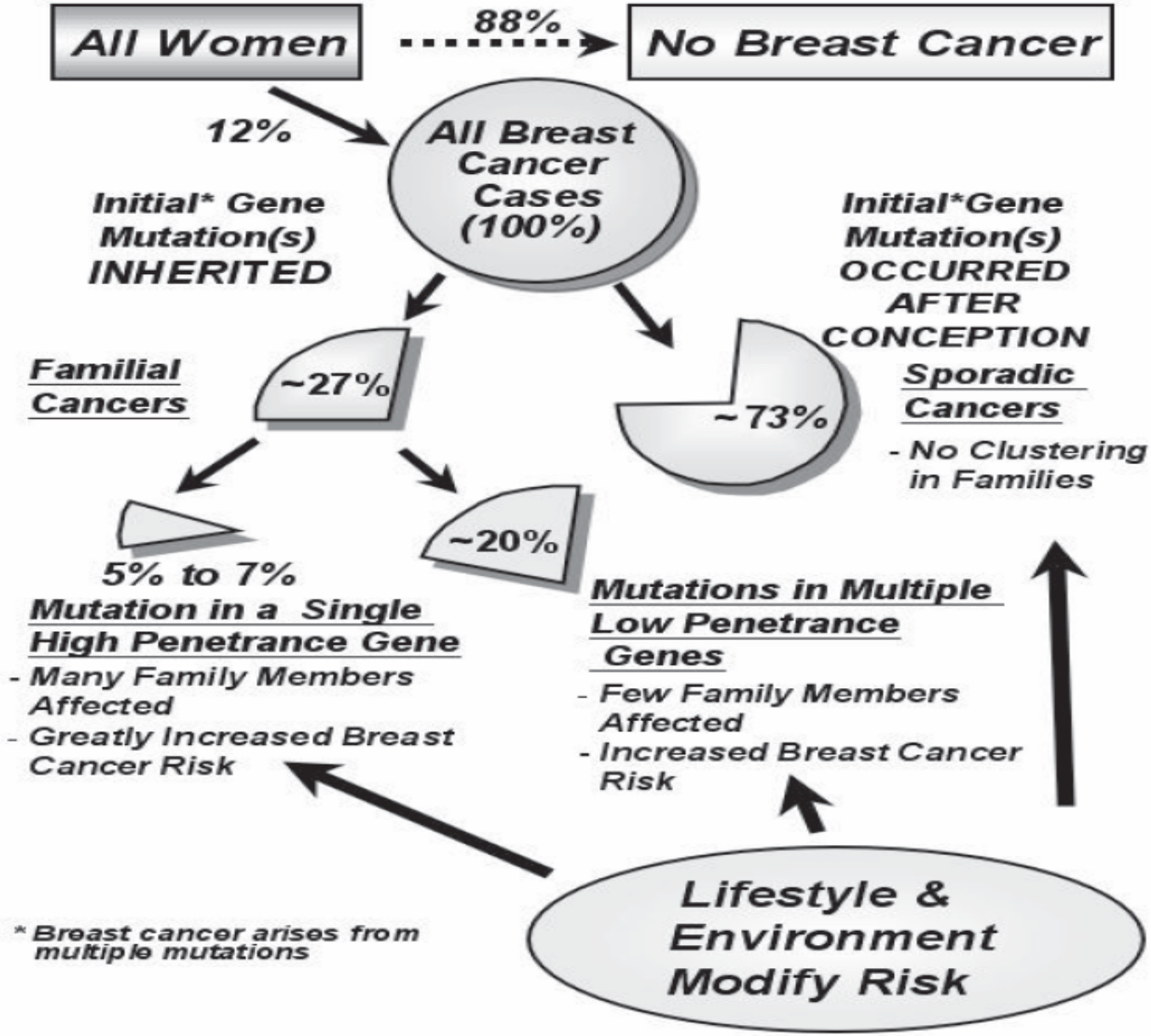


*Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.
Source: American Cancer Society, 2007.

Cancer Incidence Rates* for Women, 1975-2003



*Age-adjusted to the 2000 US standard population and adjusted for delays in reporting.
Source: Surveillance, Epidemiology, and End Results Program, 1975-2003, Division of Cancer Control and Population Sciences, National Cancer Institute, 2006.


All Women
88%
No Breast Cancer
12%
All Breast Cancer Cases (100%)
Initial* Gene Mutation(s) INHERITED
Initial*Gene Mutation(s) OCCURRED AFTER CONCEPTION
Familial Cancers
~27%
~73%
Sporadic Cancers
- No Clustering in Families
~20%
5% to 7%
Mutation in a Single High Penetrance Gene
- Many Family Members Affected
- Greatly Increased Breast Cancer Risk
Mutations in Multiple Low Penetrance Genes
- Few Family Members Affected
- Increased Breast Cancer Risk
Lifestyle & Environment Modify Risk
* Breast cancer arises from multiple mutations

Endocrine Disrupting Compounds

EDCs:

Exogenous agents that "interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis (normal cell metabolism), reproduction, development, and/or behavior."

Developmental Basis of Disease: Disease Focus in Animals

Reproductive/Endocrine

Breast/prostate cancer
Endometriosis
Polycystic Ovary Syndrome
Fertility
Diabetes/Metabolic Syndrome
Puberty
Obesity
Lactation

Pulmonocardiovascular

Atherosclerosis
Asthma
Chronic Obstructive Pulmonary Disease (COPD)
Heart disease/Hypertension

Brain/Nervous System

Alzheimer's Disease
Parkinson's Disease
Attention Deficit Hyperactivity Disorder (ADHD)

Immune/Autoimmune

Systemic/tissue specific Autoimmune disease
Immunosuppression

Increased Susceptibility of the Developing Organism

1. Development is a highly integrated process
2. Rapid growth & extensive differentiation
3. Opportunities for initiation of lesions and promotion of altered cells
4. "Critical periods" of development
   - Is fetal programming occurring? (persistence)
   - Is there an adverse outcome?

Puberty and Breast Development:

- Limited human data is available on the increased breast cancer risk related to exposure to environmental estrogens or to EDCs.

- Percentage of girls with precocious puberty (signs of puberty prior to age 8) rose from 2.5% in 1969 to 10% in the 1990's (Chalumeau et al., 2002). Not changed for boys.

- PROS and NHANES III study data estimate that between 11-12% (white) or 28-38% (African-American) 8 year-old girls have Tanner stage II or higher breast development (Herman-Giddens, 2003).

- Review of pertinent literature at 2003 Puberty Expert Panel Workshop
  - Panel voted that evidence in literature supports change in timing of puberty onset. However, mean age of menarche unchanged (12.2 yr).

- My goal: Identify environmental exposures alter rodent mammary gland development following prenatal/lactational exposures to high use and/or persistent environmental contaminants that are known or suspected to be Endocrine Disrupting Compounds (EDCs).

Environmental compounds proven to alter mammary gland (MG) development following early life exposure.

Compound	Rodent Model Tested	Accelerated MG Development	Delayed MG Development	Reported Putative Long-term Consequences	Reference(s)
Atrazine	Rat		X	MG Tumors Lactation Transgenerational Effects	Rayner et al., 2004. *Toxicol Appl Pharmacol* Fukamachi et al., 2004. *Cancer Sci* Rayner et al., 2005. *Toxicol Sci* Ueda et al., 2005. *Cancer Sci* Enoch et al., 2007. *Environ Health Perspect*
Bisphenol A	Mouse & Rat	X	X	Neoplasia Leukemia MG Tumors Development effects limited to early exposure	Colerangle & Roy, 1997. *J Steroid Biochem & Mol Biol* Markey et al., 2001. *Biol Reprod* Huff, 2001. *Odontology* Nikaido et al., 2004. *Reprod Toxicol* Munoz de-Toro et al., 2005. *Endocrinology* Yin et al., 2006. *Ind J Exp Biol* Murray et al., 2006 *Reprod Toxicol* Vandenberg et al., 2007. *Endocrinology*
Cadmium	Rat	X		Lactation	Johnson et al., 2003. *Nature Med* Ohrvik et al., 2006. *Toxicol Lett*
Dieldrin	Rat		X	Lactation	Tarrafet et al., 2003. *Food Addit Contam*
Dioxin or TCDD	Rat		X	MG Tumors Prenatal exposure needed for effects Lactation	Fenton et al., 2002. *Toxicol Sci* Brown et al., 1998. *Carcinogenesis* Lewis et al., 2001. *Toxicol Sci* Vorderstrasse et al., 2004. *Toxicol Sci* Desaulniers et al., 2001. *Environ Health Perspect*
Dietylstilbesterol (DES)	Rat & Mouse	X		Spontaneous MG tumors	Too many to list – date back to 1950's
Genestein Zearalenone Resveritrol	Rat	X	X	MG Tumors? Effects depend on time and route of exposure	Hilakivi-Clarke et al., 1999. Oncol Rep Lemartiniere, 2000. *Am J Clin Nutr* Delclos et al. 2001. *Reprod Toxicol* Padilla-Banks et al., *2006 Endocrinology*

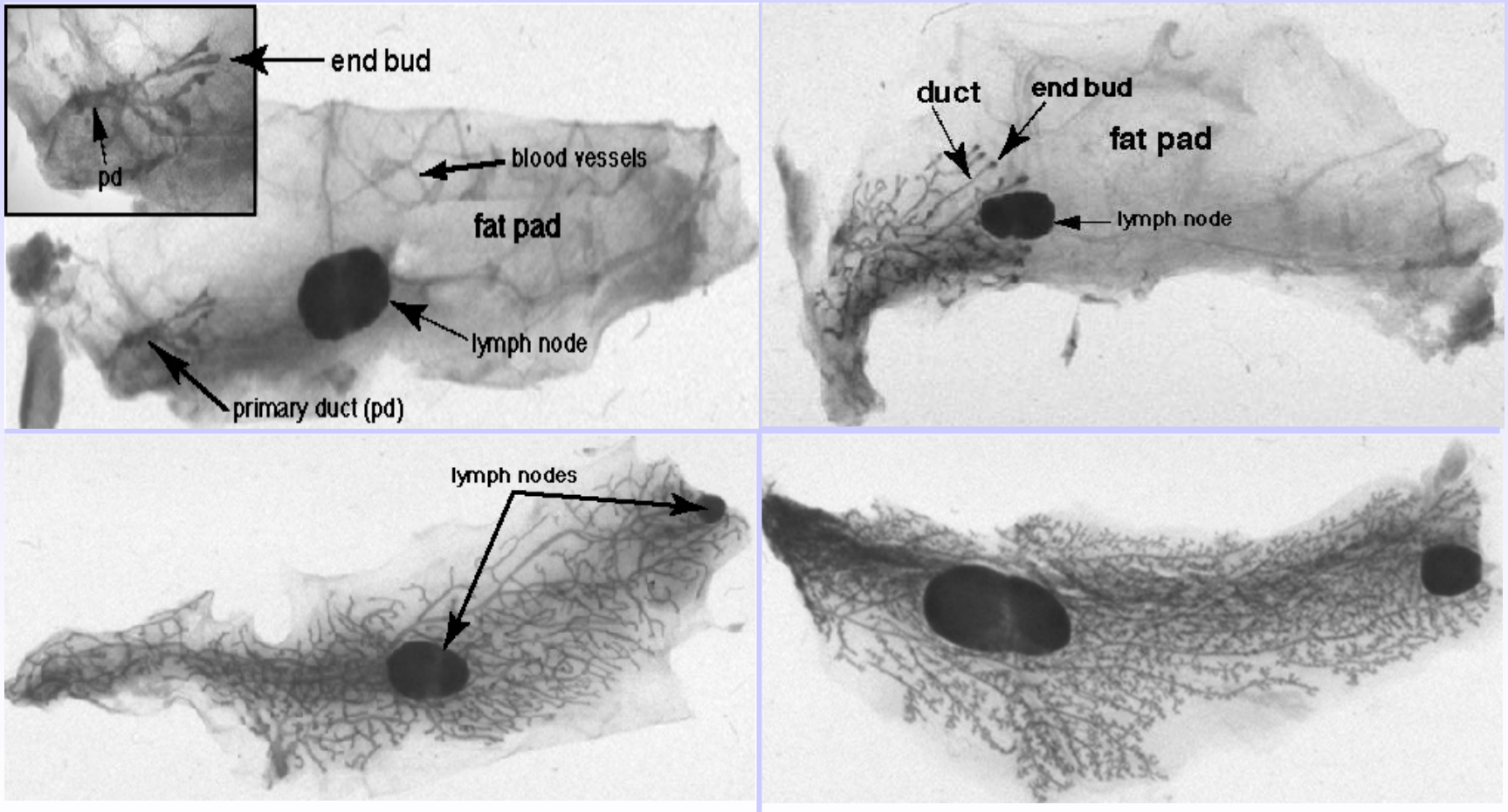
Environmental compounds proven to alter mammary gland (MG) development following early life exposure (con't.)

Compound	Rodent Model Tested	Accelerated MG Development	Delayed MG Development	Reported Putative Long-term Consequences	Reference(s)
Nonylphenol	Rat	X		Unknown	Colerangle & Roy, 1996. *Endocrine* Fukamachi et al., 2004. *Cancer Sci* Moon et al., 2007. *J Reprod Devel*
Organochlorine mixtures	Rat		X	Increased MG tumor incidence if carcinogen early in development.	Desaulniers et al., 2004. *J Toxicol Environ Health A* Desaulniers et al., 2001. *Environ Health Perspect*
Polychlorinated Biphenyls (PCBs)	Rat	X	X	Dose dependent development and tumor outcomes	Muto et al., 2002. *Toxicology*
PhIP (2-amino-1-methyl-6-phenylimidazol [4,5-b]pyridine)	Rat		X	MG tumors Transgenerational Effects	Ito et al., 1997. *Mutat Res* Snyderwine et al., 1998. *Carcinogenesis* Snyderwine et al., 2002. *Mutat Res*
PFOA	Mouse		X	Lactation	White et al., 2007. *Toxicol Sci*
High Fat diet (PUFA)	Rat & Mouse	X		Increased MG tumor incidence	Walker, 1990. *J Natl Cancer Inst* Hilakivi-Clarke et al, 1997. *Proc Natl Acad Sci USA* Hilakivi-Clarke et al, 1997. *J Natl Cancer Inst* Hilakivi-Clarke et al, 1999. *Nutrition* Uijten et al., 2006. *Reprod Toxicol*

Note – Only positive effects are reported; this does not mean there are no reports in contrast to these.

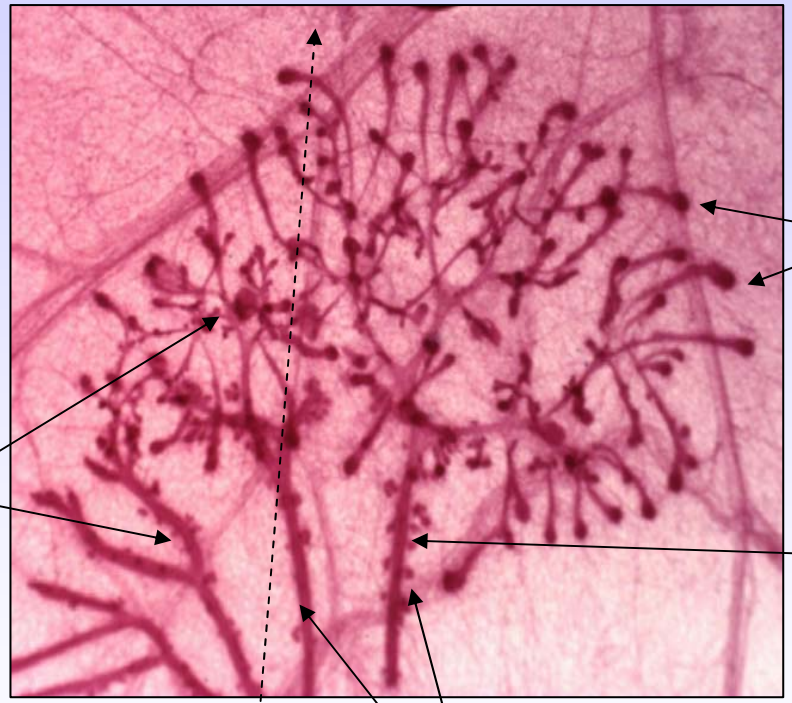
taken from S.E. Fenton, 2007 *The Ribbon*, Volume 12(1).

Rodent Mammary Gland Development

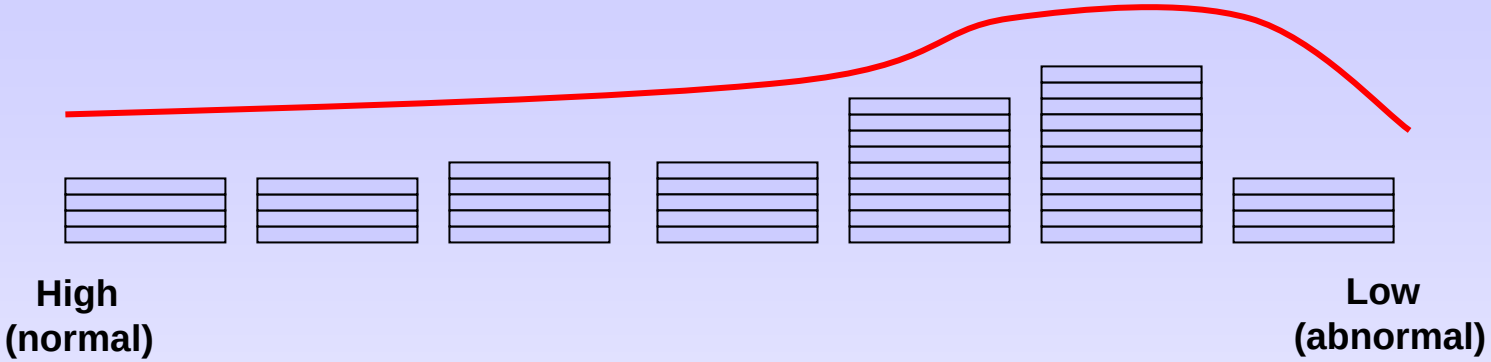


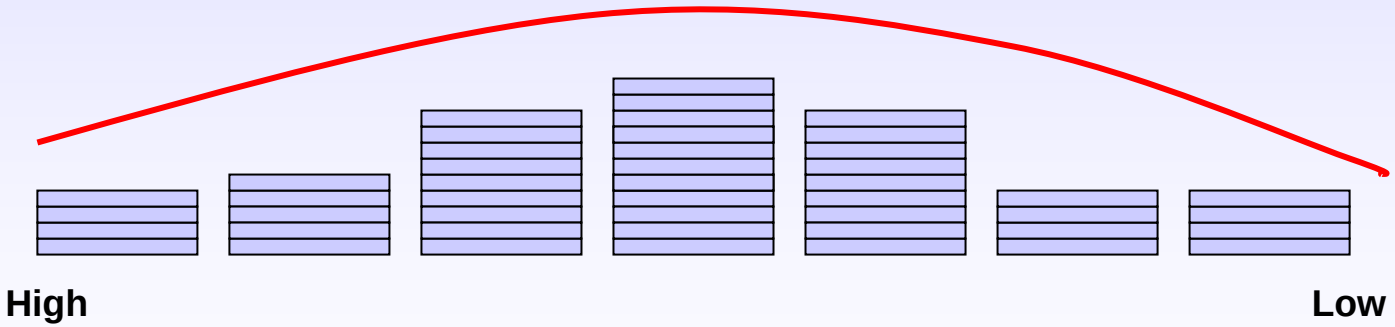
http://mammary.nih.gov/atlas/wholemounts/normal/index.html

SCORING CRITERIA:



Morphology Distribution

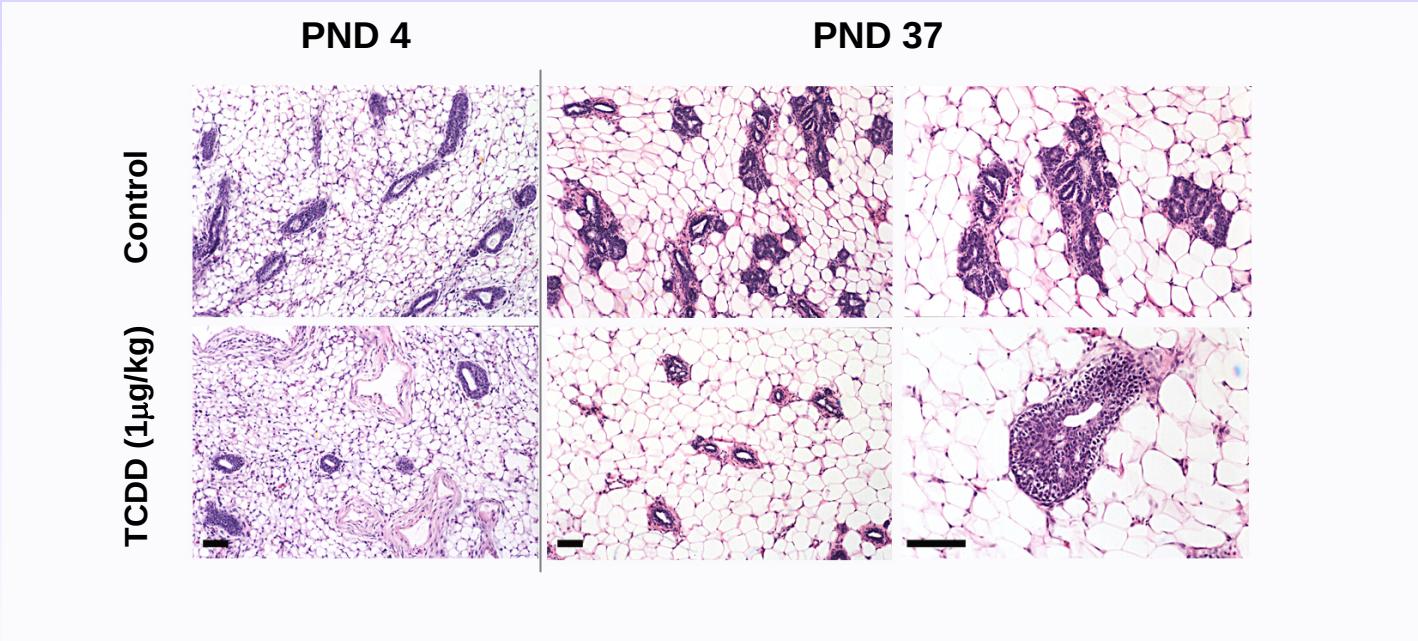




DIOXIN

- Structurally related class of chemicals
  - Dioxins, dibenzofurans, biphenyls, naphthalenes, etc…
  - Chlorinated, brominated, mixed Br-Cl
- Effects mediated via binding to AhR (aryl hydrocarbon receptor)
- Persistent and bioaccumulative
- Common spectrum of responses
- 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the prototype

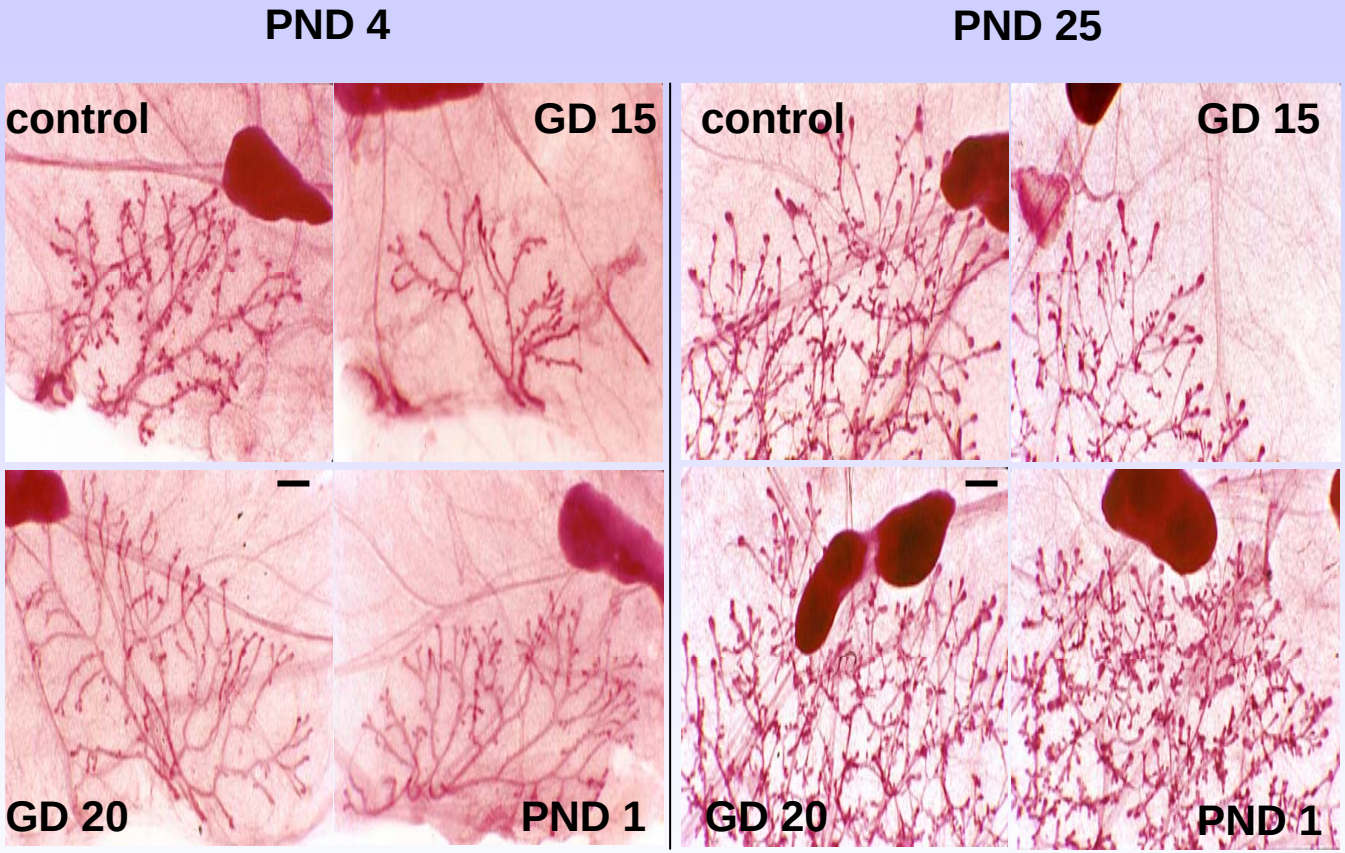
TCDD Decreased Ductal Branching and Delayed TEB Differentiation



Histological sections from rat MG on PNDs 4 or 37, exposed on GD15 to 1 μg/kg dioxin. Sections were stained with hematoxylin and eosin. Scale=100 um.

taken from Fenton et al., *Toxicol. Sci*. 2002

Critical Period for TCDD-Induced MG Development Delays



Timed pregnant LE were exposed to 1 μg TCDD/kg BW on GD 15, GD 20, PND 1, PND 3 (5 and 10, also) and female offspring euthanized on PND 4 for whole mount analysis of mammary glands.

Other Pubertal Changes Caused by a Single Prenatal Dioxin Exposure

Delayed Vaginal Opening at 1 μg/kg:

- 1995 TAAP Gray and Ostby – delayed VO in LE rats (GD15)
- 1997 TAAP Flaws et al. – no change in VO Holtzman rats (GD15)
- 1998 Carcinogenesis Brown et al. – delayed VO in SD rats (GD15)
- 2002 Tox Sci Fenton et al. – delayed VO in LE rats (GD15)

Cleft thallus or “thread” in the vaginal opening (across rat strain)

Inconsistent reports on estrous cyclicity depending on which part of the animal’s reproductive life was examined.

- Cyclicity immediately following the vaginal opening was normal.

Dioxin Dose Response

- **Time pregnant LE rats dosed on GD 15 by oral gavage with 0, 0.05, 0.2, or 0.8 μg/kg dioxin**
- **Evaluate dams and offspring on GD21(sex ratio of F1 offspring and gene expression in MG of females)**
- **Evaluate the remaining offspring for puberty and MG development post-puberty**

Puberty (VO) in F1 Female Offspring

Dose (μg/kg)	Age at VO (PND)	Weight at VO (g)
0	30.1	108
0.05	29.5	104
0.2	29.2	98
0.8	31.5*	110

Low Dose Mammary Gland Effects:

- Significant mammary gland delays in F1 females at 0.2 μg/kg
- Associated with > 100X increase in cyp1B1, > 10X increase in cyp1A1,> 2X increase in AhR, and > 3X increase in ARNT expression on GD21.
- Maximal gene changes at 0.2 μg/kg for AhR and ARNT, and 0.8 μg/kg for the cyp genes

Transgenerational Effects (single 1 μg/kg exposure) :

- F1 offspring have persistent changes in their mammary glands until PND110
- F1 dams have altered maternal behavior
- F2 pups – increased mortality (15% vs. 2.5% in controls)
  decreased milk ingestion in 30 min lactational challenge
  significantly altered mammary gland development

What is the significance of delayed vaginal opening?? We don't know!!

What is the significance of the mammary gland findings??

In rats:

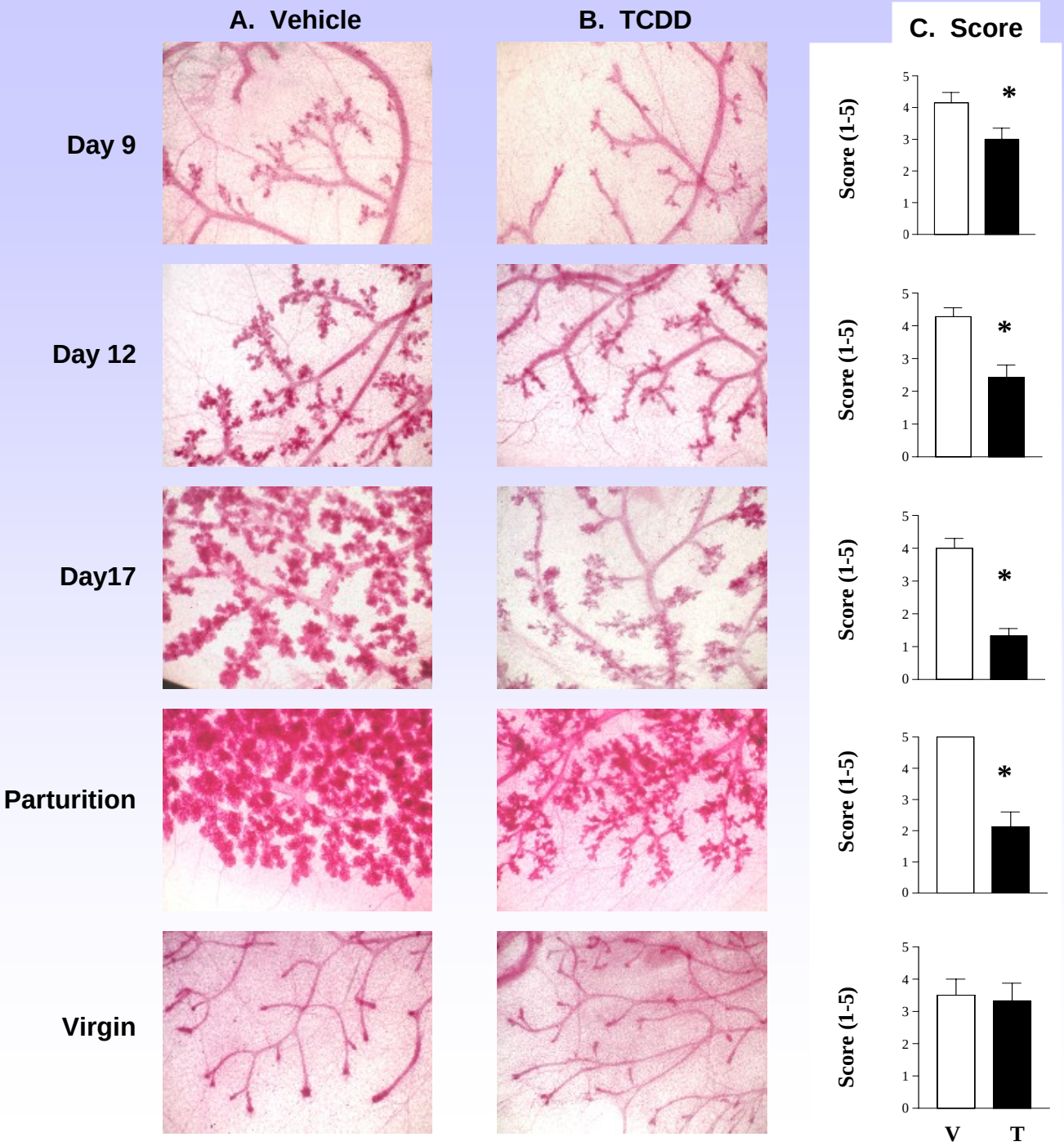
1. **1 μg/kg on GD 15 altered MG differentiation at PND 50, and associated increase in DMBA-induced tumor formation (Brown et al., 1998 Carcinogenesis)**
2. **2.5 μg/kg on GD 18 increased MNU-induced tumors (Desaulniers et al., 2001).**

In humans:

1. **Doubling of serum dioxin concentrations in girls more than doubled the odds of stunted breast development (Den Hond et al., 2002)**

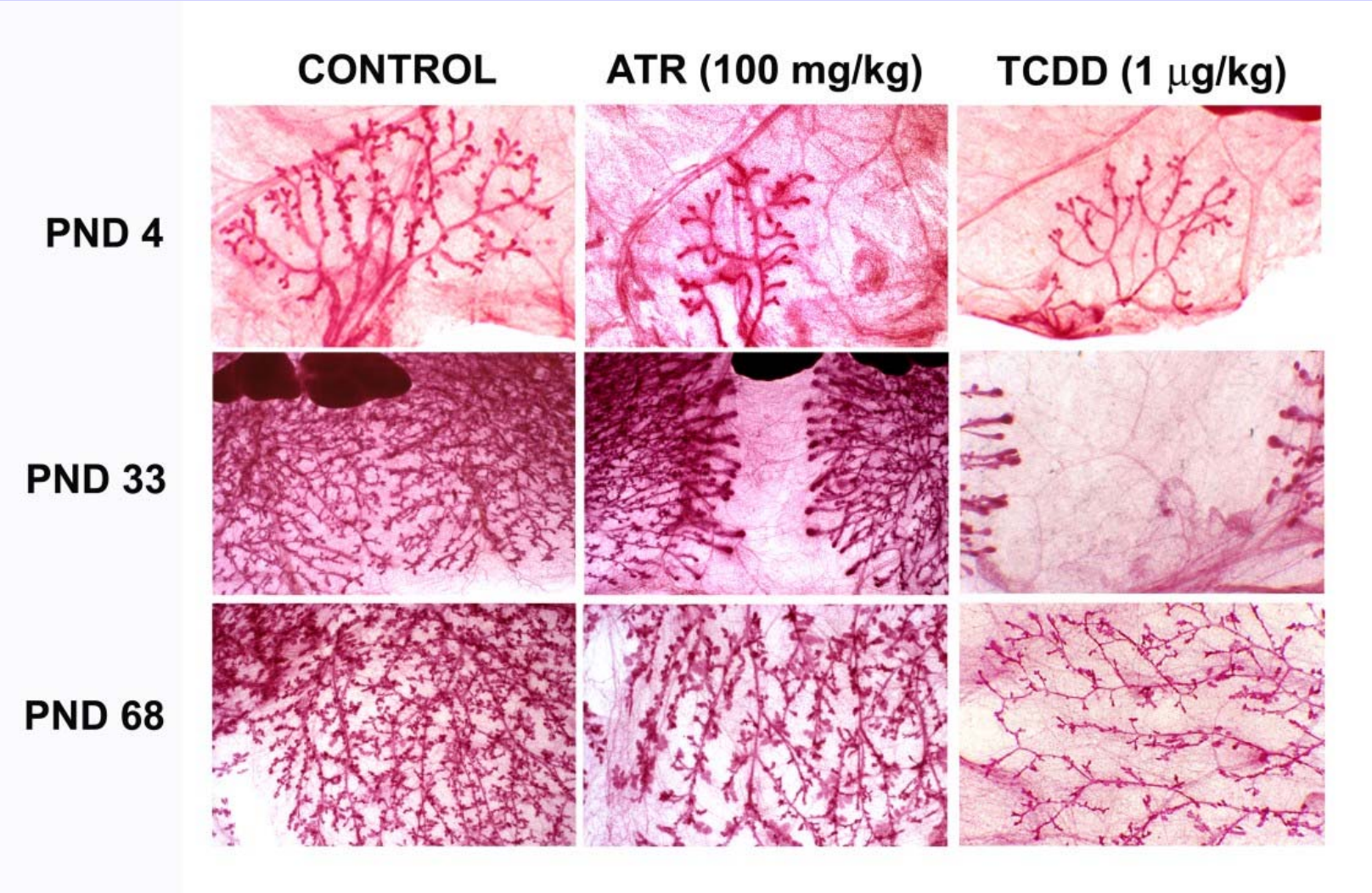
2. **Chemical plant workers exposed to TCDD contaminants >2 fold increase in breast cancer of females (Manz et al., 1991)**

3. **Chapaevsk, Russia – TCDD measurable in blood, milk, drinking water breast cancer OR 2.1 (C.I. 1.6-2.7) (Revich et al., 2001)**



taken from Vorderstrasse et al., 2004 *Toxicol Sci.*

Toxicant Comparison in MG



From Birnbaum and Fenton, 2003. *Environ Health Perspect 111:389-94.*

Atrazine and Reproductive Function in the Rat

- Chlorotriazines are documented endocrine disruptors
- Disrupt critical reproductive processes:

  Puberty, Pregnancy Maintenance, Mammary Development, Prostate Development, and Lactation
- Potential MOA via CNS control of hypothalamic-pituitary-gonadal axis
  - Pituitary hormone secretions, LH-surge
  - Cyclicity; hastens reproductive senescence
- Increases mammary tumor development in SD rats with chronic adult exposure via food
- Short half-life in the adult rat & reported health effects from chlorotriazine metabolites

Exposure Parameters Important for Developmental Effects

Cross-Foster Exposure Paradigm

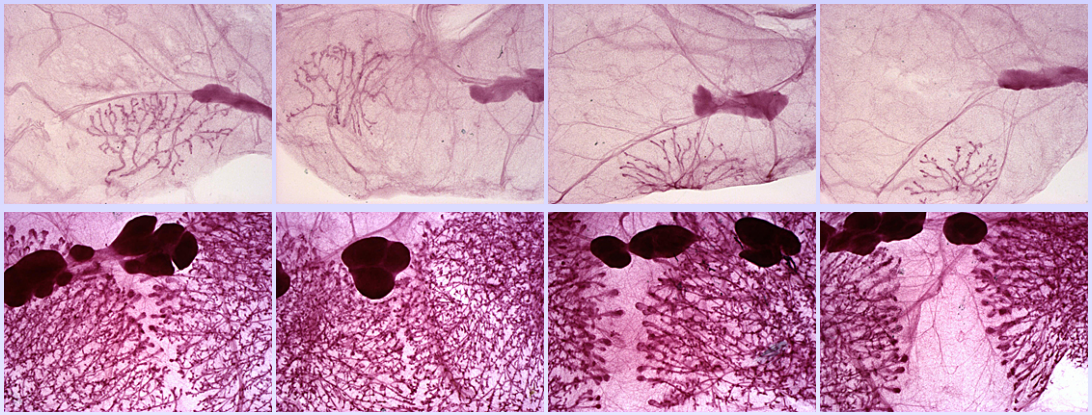
Control (20 dams)
1% Methyl Cellulose

Atrazine (20 dams)
100mg/kg

PND1
half of all litters crossed

Birth Dam:	C 10 dams	C 10 dams	ATR 10 dams	ATR 10 dams
Milk Source:	C 10 dams	ATR 10 dams	C 10 dams	ATR 10 dams
Offspring Exposure Groups	C-C	C-ATR	ATR-C	ATR-ATR

Female Offspring Effects – Cross Foster



	C-C*	ATR-C	C-ATR	ATR-ATR
VO (PND)	33±0.4	32.8±0.4	35.4±0.6[c]	37.1±0.5[c]
Aromatase	1.00±0.00	0.40±0.77	-0.41±0.76	-2.19±0.49[a]
EGF-R	1.00±0.00	-1.29±0.25	-2.75±0.77[b]	-5.25±1.86[c]

*Birth Dam-Milk Source
Gene expression data presented as fold induction value ±SE (performed by RT-PCR).
Significant treatment effect by ANOVA (LSM); a $p<0.05$ vs. C-C, ATR-C, b $p<0.01$ vs C-C, c $p<0.02$ vs. C-C, ATR-C
Dam N=2 to 5, with ≥ 5 offspring per exposure group at each time.

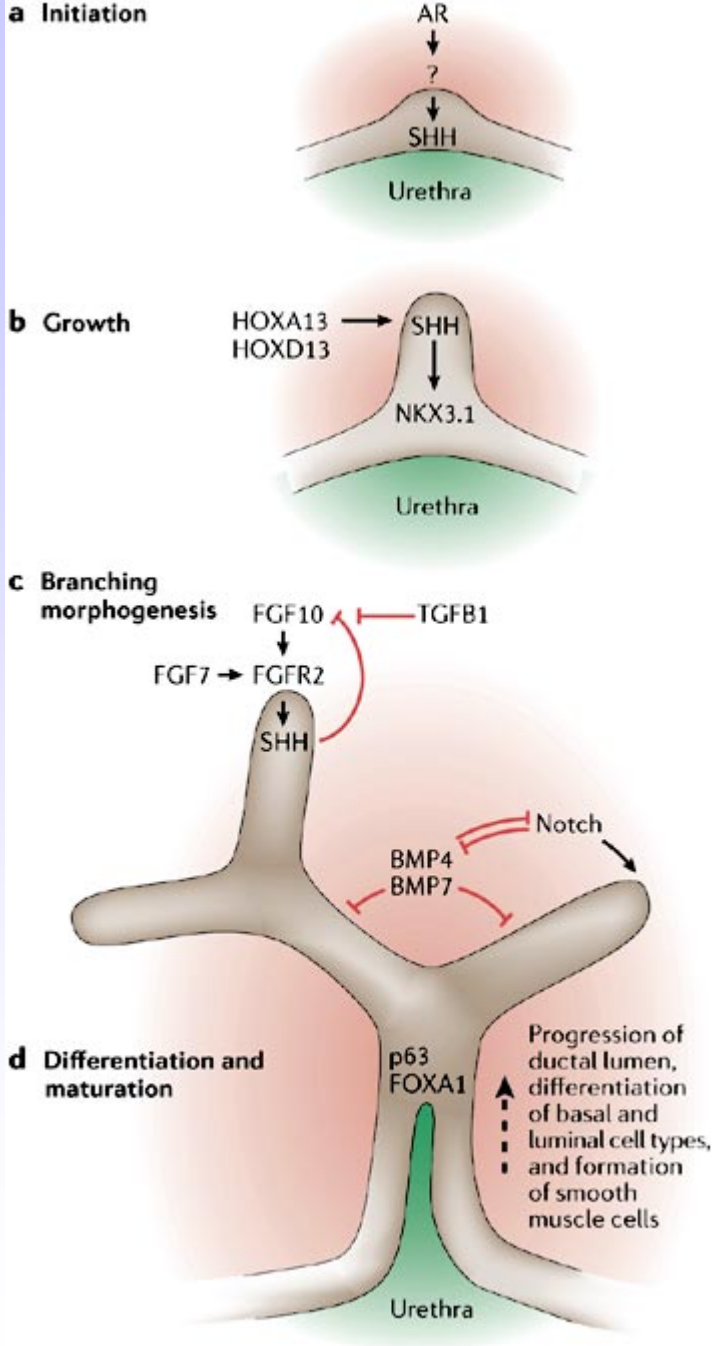
From Rayner et al., *Toxicol Appl Pharmacol. 2004 Feb 15;195(1):23-34.*

Rodent Prostate Bud Development

Similarities to the mammary gland:

- **Late developmental in fetus**
- **Budding of the epithelium just prior to birth**
- **Important role of androgens**
- **Branching morphogenesis occurs primarily after birth**
- **Differentiation of the epithelium**

From Wilhelm and Koopman, *Nature Reviews Genetics* 7, 620-631 (August 2006) | doi:10.1038/nrg1903



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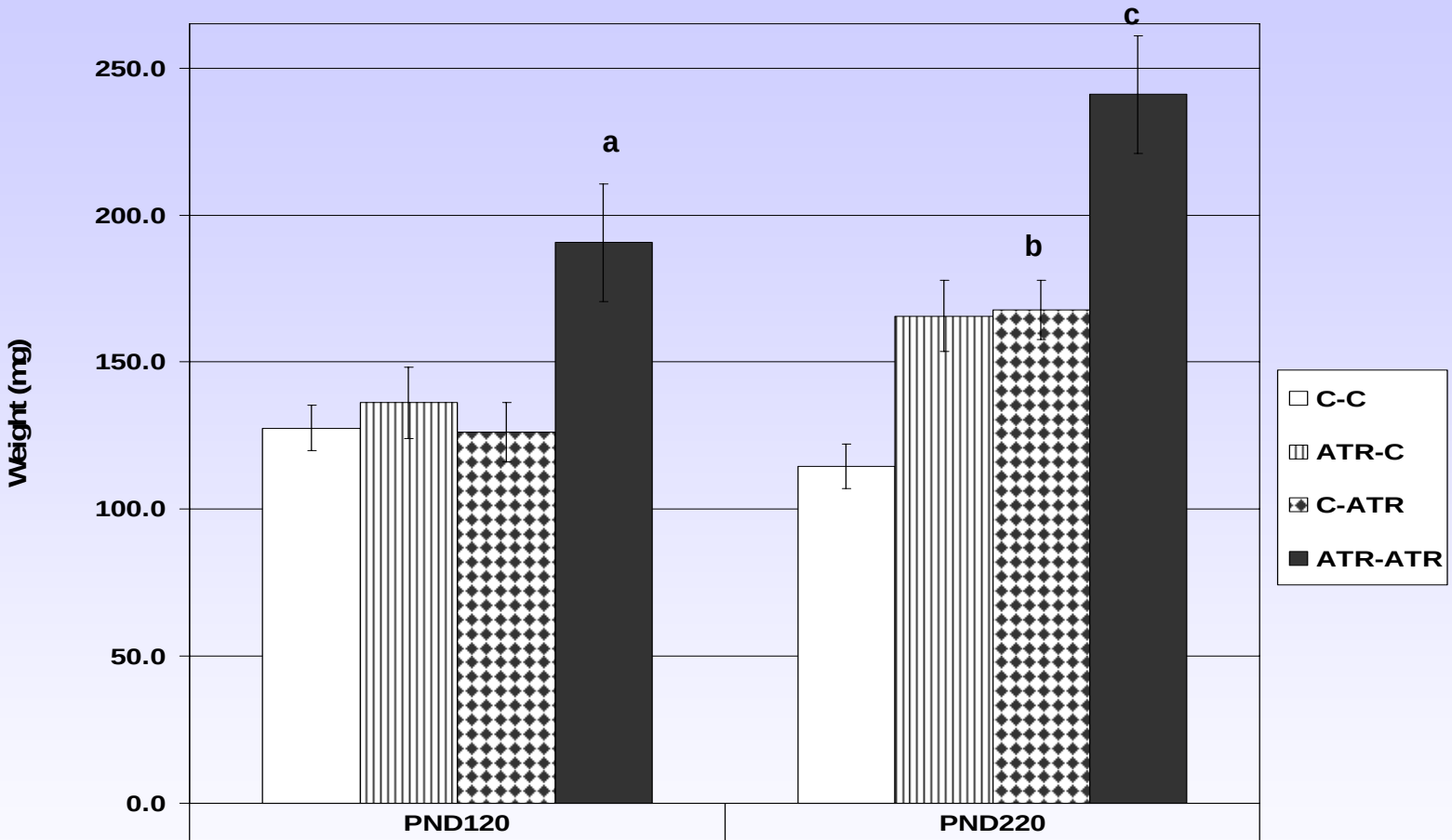
Male Offspring Effects – Cross Foster

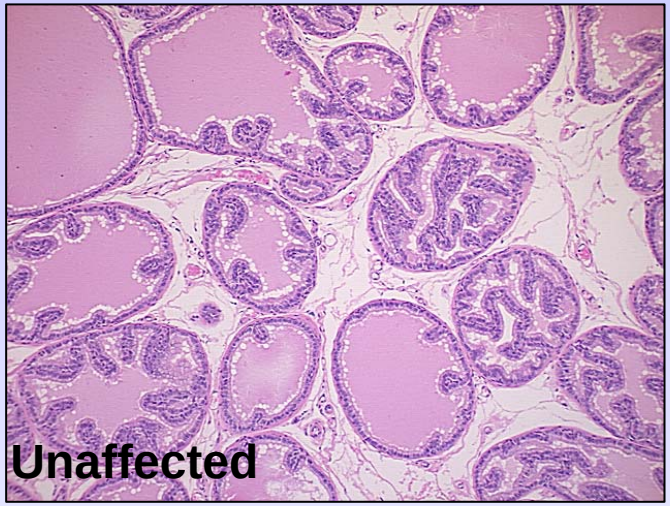
Lateral Prostate Weight (PND120 & PND220)

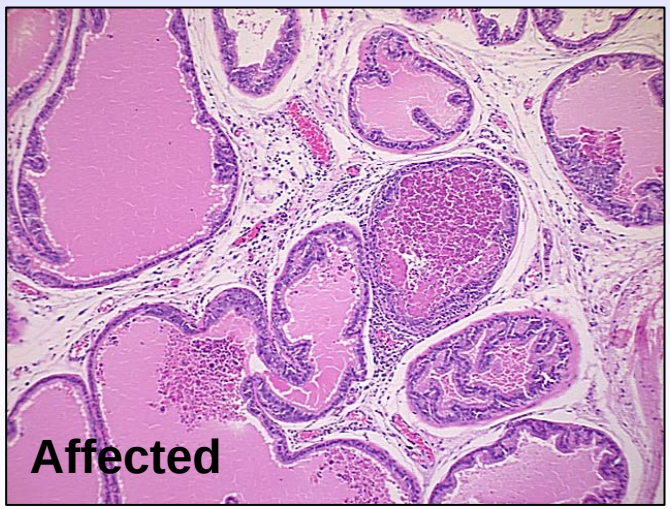


	PND120	PND220
C-C	127.6	114.6
ATR-C	136.2	165.7
C-ATR	126.1	167.8
ATR-ATR	190.6	241.0

From Rayner et al., *Toxicol Appl Pharmacol. 2007 Feb 1;218(3):238-48.*

Male Offspring Effects – Cross Foster

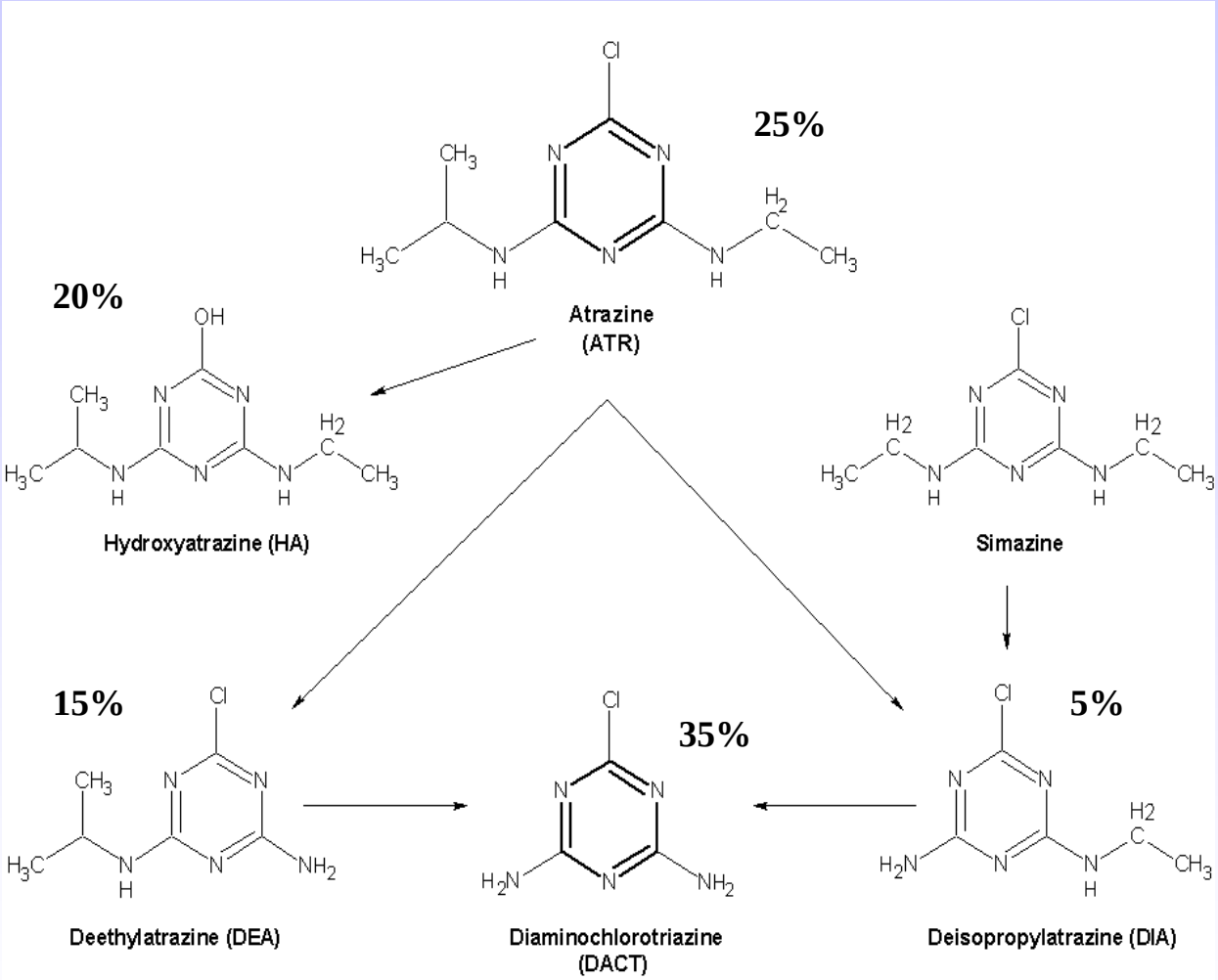




Histological sections of lateral prostates at PND120

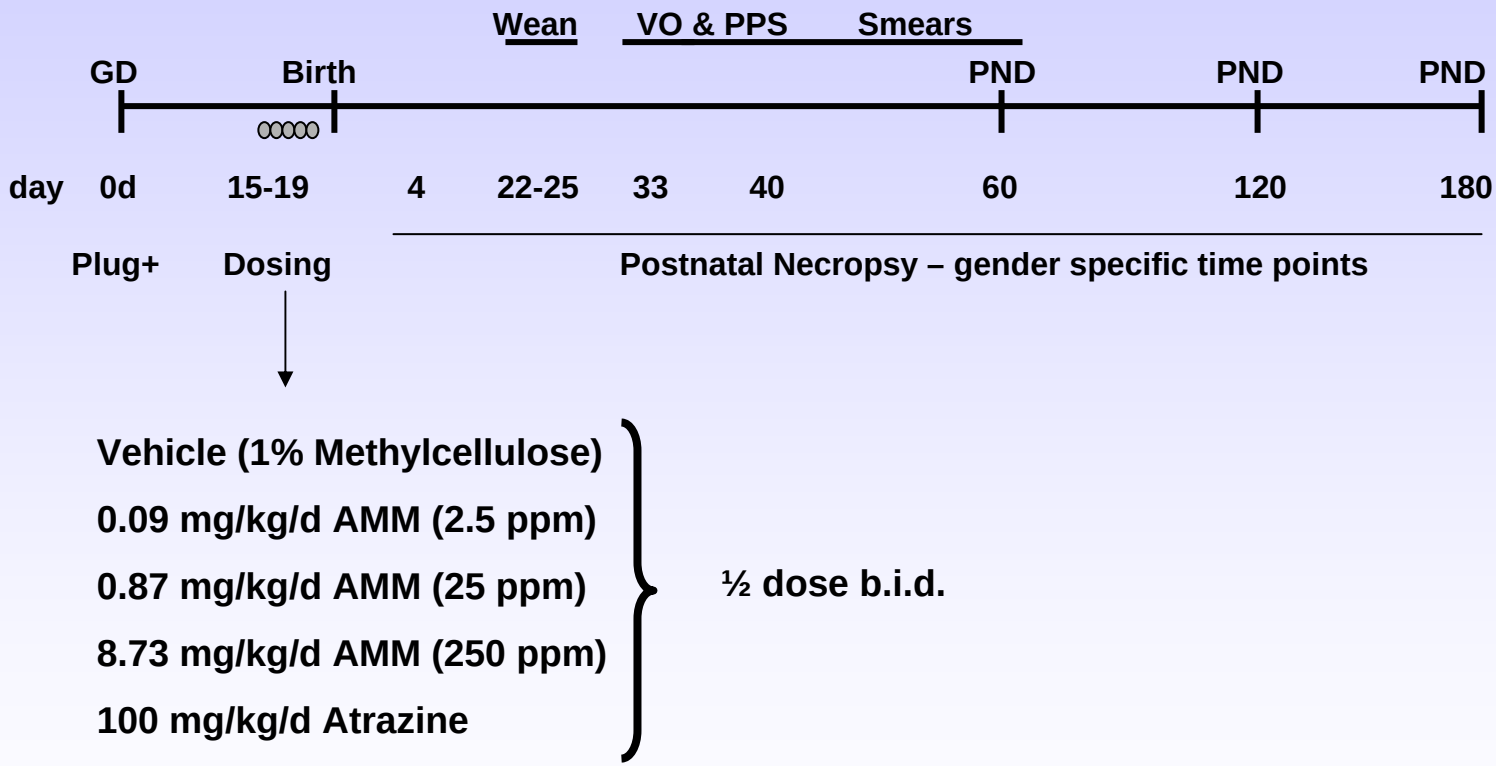
From Rayner et al., *Toxicol Appl Pharmacol. 2007 Feb 1;218(3):238-48.*

Atrazine Metabolite Mixture – AMM

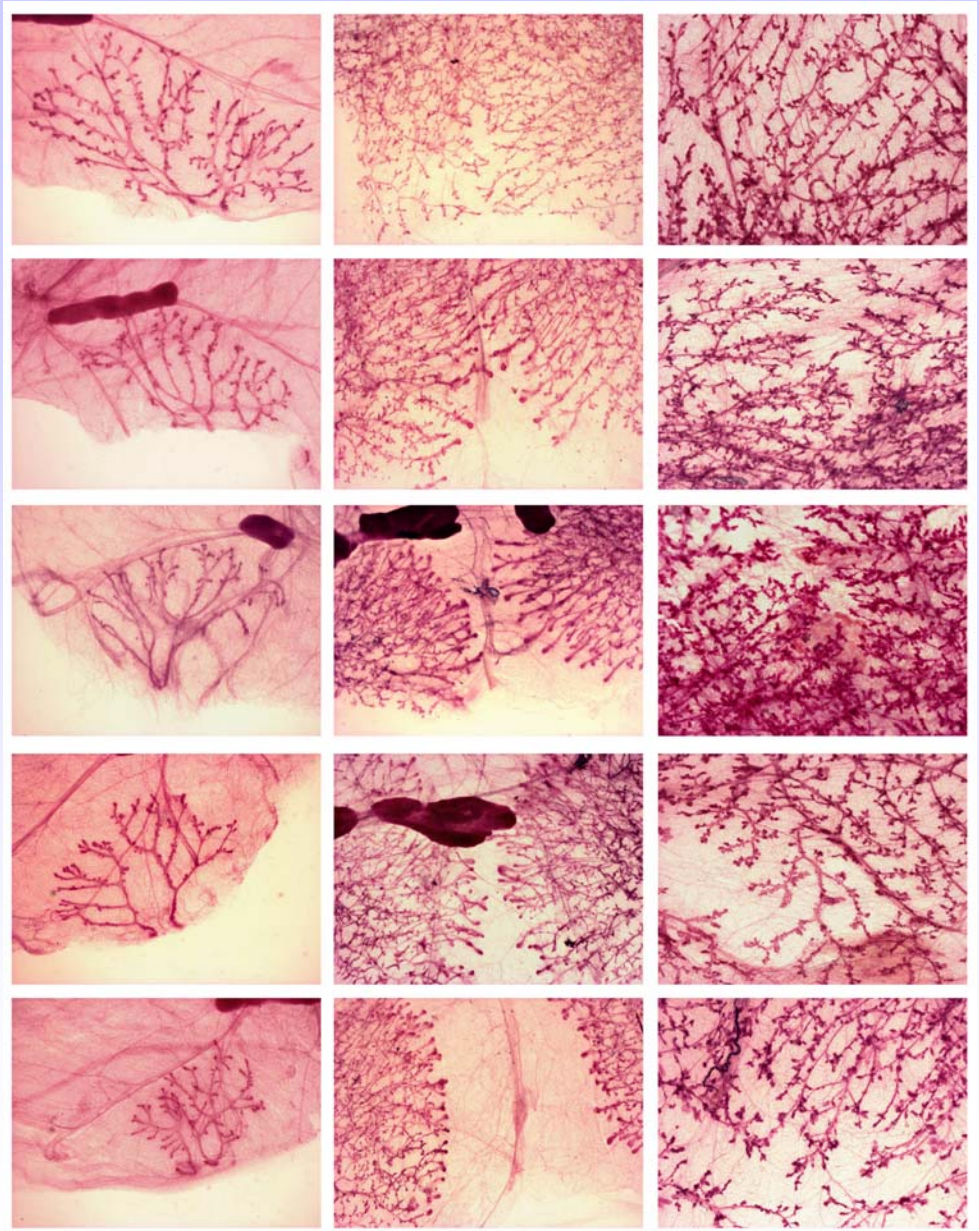


Percentages on a mass basis – estimated maximum cumulative level of 25 ppb
From three published sources as referenced in Enoch et al., 2007. *EHP*

Atrazine Metabolite Mixture (AMM) Study Design



AMM in Female Offspring



From Enoch et al., *Environ Health Perspect. 2007 Apr;115(4):541-7.*

AMM in Male Offspring

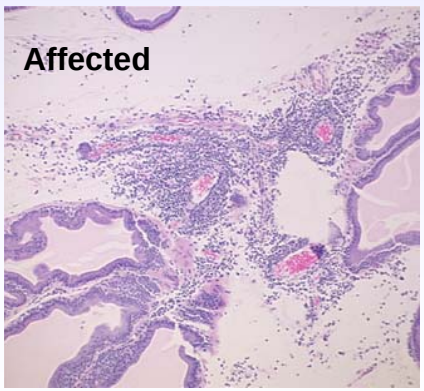
Lateral Prostate Pathology on PND 120					
	Incidence of inflammation*	Distribution (affected)	Severity (affected)	Incidence of microabscesses**	Severity (affected)
Lateral Prostate					
Control	1/10 (10%)	0.1 ± 0.3 (1)	0	1/10 (10%)	0.3 ± 0.9 (3)
0.09 AMM	2/12 (17%)	0.3 ± 0.6 (1.5 ± 0.7)	0	2/12 (17%)	0.7 ± 1.6 (4)
0.87 AMM	4/13 (31%)	0.5 ± 1.0 (1.8 ± 0.9)	0	4/13 (31%)	1.2 ± 1.8 (3.8 ± 0.5)
8.73 AMM	6/13 (46%)	0.5 ± 0.7 (1.2 ± 0.4)	0.4 ± 0.7[t] (1.3 ± 0.5)	4/13 (31%)	1.5 ± 2.1 (3.3 ± 2.0)
100 ATR	1/8 (13%)	0.1 ± 0.4 (1)	0.1 ± 0.4 (1)	1/8 (13%)	0.6 ± 1.8 (5)

Note: PND, postnatal day; AMM, atrazine metabolite mixture; ATR, atrazine. Data represent incidence of alteration followed by mean severity score ± SD for that alteration and of the subset of animals with the alteration. *Significant dose-dependent increase in the incidence of inflammation in the lateral prostate by chi-square analysis ($p<0.05$). **Significant dose-dependent increase in the incidence of microabscesses in the prostate by chi-square analysis ($p<0.05$).

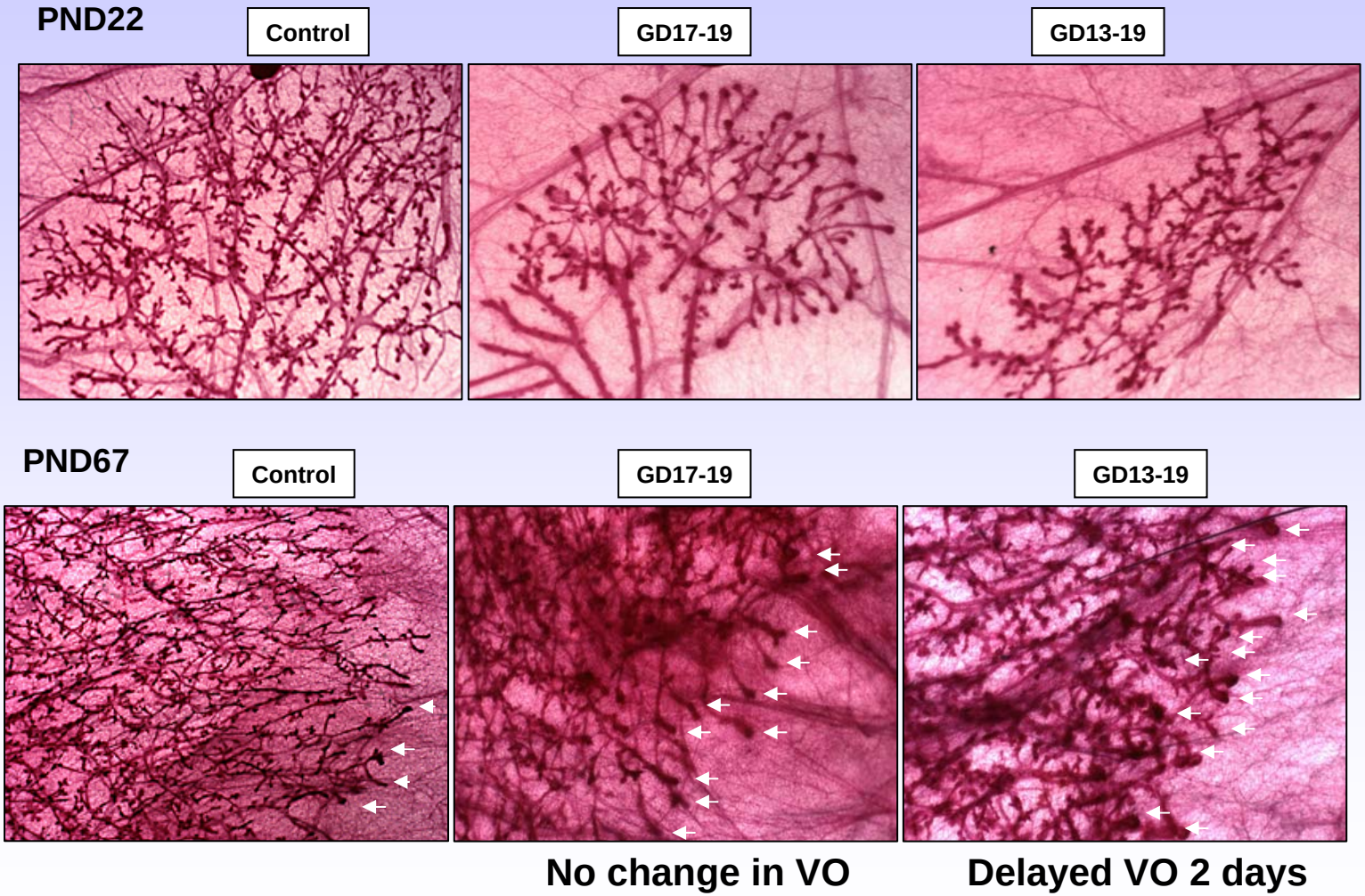
From Stanko et al., *(submitted 2/08)*

Ventral Prostate Pathology on PND 180					
	Incidence of inflammation*	Distribution (affected)	Severity (affected)	Incidence of Microabscesses	Severity (affected)
Ventral Prostate					
Control	3/11 (27%)	0.6 ± 0.9 (2.0 ± 0)	0.6 ± 0.9 (2)	1/11 (9%)	0.8 ± 1.6 (3.0 ± 1.7)
0.09 AMM	0/11 (0%)	0	0	0	0/11
0.87 AMM	7/13 (54%)	0.9 ± 0.9 (1.6 ± 0.5)	0.9 ± 0.9 (1.6 ±0.5)	2/13 (15%)	1.3 ± 1.8 (2.4 ± 1.8)
8.73 AMM	5/12 (42%)	0.6 ± 0.8 (1.2 ± 0.8)	0.6 ± 0.8 (1.4 ±0.6)	0	0.8 ± 1.3 (2.0 ± 1.2)
100 ATR	2/8 (25%)	0.4 ± 0.7 (1.5 ± 0.7)	0.4 ± 0.7 (1.5 ±0.7)	1/8 (13%)	0.4 ± 0.7 (1.5 ± 0.7)

Note: **PND, postnatal day; AMM, atrazine metabolite mixture; ATR, atrazine. Data represent incidence of alteration followed by mean severity score ± SD for that alteration and of the subset of animals with the alteration.**
***Significant dose-dependent increase in the incidence of inflammation in the ventral prostate by chi-square analysis (*p*<0.05).**

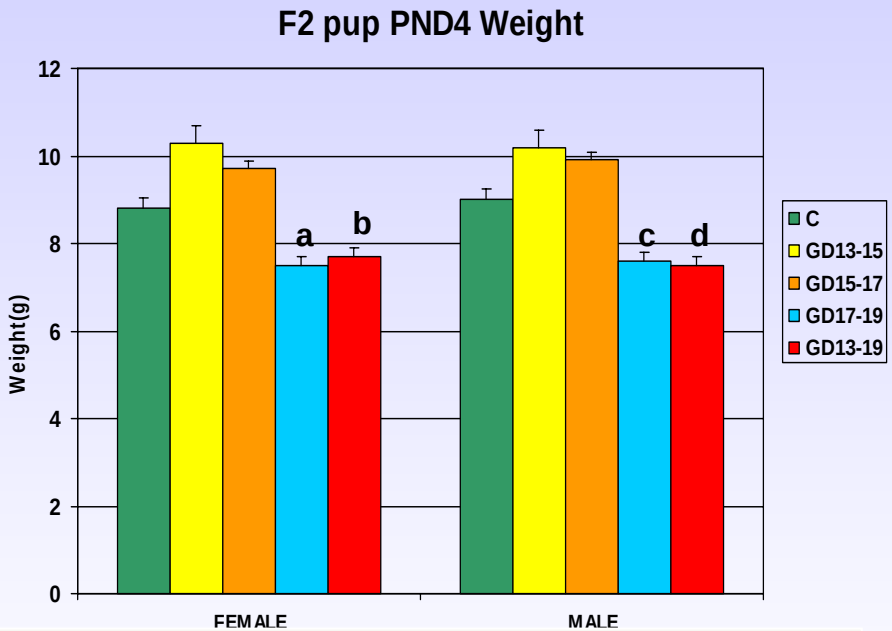


Brief Prenatal Atrazine Alters Rat Mammary Gland Development

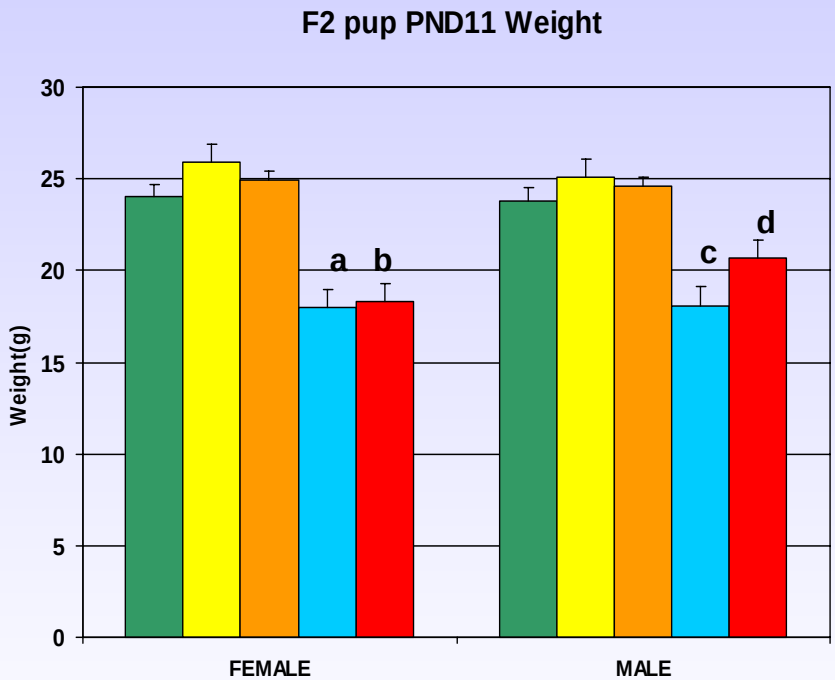


From Rayner et al., *Toxicol Sci. 2005 Sep;87(1):255-66.*

Significant effect of atrazine on F2 pup weight gain



F_2 BW on PND4. Data presented as mean ±SE. Significant treatment effect by ANOVA (LSM) and reduction in BW; different from C: a= p<0.003 (14.8%), b=p<0.02 (12.5%), c=p<0.002 (15.6%), d=p<0.0001 (16.7%).



F_2 BW on PND11. Data presented as mean ±SE. Significant treatment effect by ANOVA (LSM); different from C: a=p<0.0001 (25%), b=p<0.0003 (23.8), c=p<0.0001 (23.9%), d=p<0.002 (13%).

From Rayner et al., *Toxicol Sci. 2005 Sep;87(1):255-66.*

Atrazine Critical Period Study Summary

- **Mammary gland developmental delays evident in offspring of 2 generations following 3 day atrazine exposure (GD17-19 = sensitive window). There is no effect on VO due to the brief exposure.**

- **First generation – no effect of BW on MG effect Second generation- MG effect appears due to lack of sufficient nourishment of F2 offspring**

- **Malnourishment potentially stems from underdeveloped mammary glands in F1 dams.**

From Rayner et al., *Toxicol Sci. 2005 Sep;87(1):255-66.*

In Summary…..

1. Effects of atrazine due to residual lactational exposure only
   a. Females have persistent changes in mammary gland
   b. Males have nodules on prostate and inflammation by 120 days
   c. Significant delay in VO with lactation only exposure, not PPS in males.
2. Atrazine metabolite mixture exposure may be more potent than parent compound
   a. Females- delayed MG development at the lowest doses tested
   b. Males- novel prostate inflammation in ventral prostate (not ATR)
   c. No pubertal delays in females, significant delays in males.
3. Critical periods of exposure
   a. Females- 3 d late gestational exposure on mammary gland - multigenerational
   b. Males- not tested
   c. What happens in late life??
4. Disposition of atrazine and metabolite mixture
   a. OH-ATR present in serum of ATR exposed dams, but not AMM exposed
   b. ATR present in the milk of all dams tested 6 days after dosing ended
   c. Accumulation in mammary gland of treated dams

Perfluoroalkyl acids (PFAAs)

- PFAA are hydrocarbons with the carbon backbone (4-15) fully substituted by fluorine, and a functional group of either sulfonic, carboxylic or phosphonic acid.
- The most common PFAAs are the C8 chemicals, perfluorooctane sulfonate (PFOS) and perfluorooctanic acid (PFOA). PFOAs estimated half-life in humans is 3.8 yr.
- These chemicals are man-made, very stable, hydrophobic and oleophobic, and are terminal metabolites of their derivative products.
- Their surfactant properties lend themselves to wide (> 200) industrial and consumer applications. In 2000, global production of PFOS was estimated at 3,500 metric tons/year and PFOA at 500 metric tons/year.
- Because of environmental concerns, 3M phased out production of PFOS by the end of 2002, but replacement PFAA such as PFOA are poised to take up the market void left by PFOS.
- Major routes of exposure in humans include drinking water, inhalation, inhalation (dust), oral intake (food contamination and hand to mouth), and dermal absorption

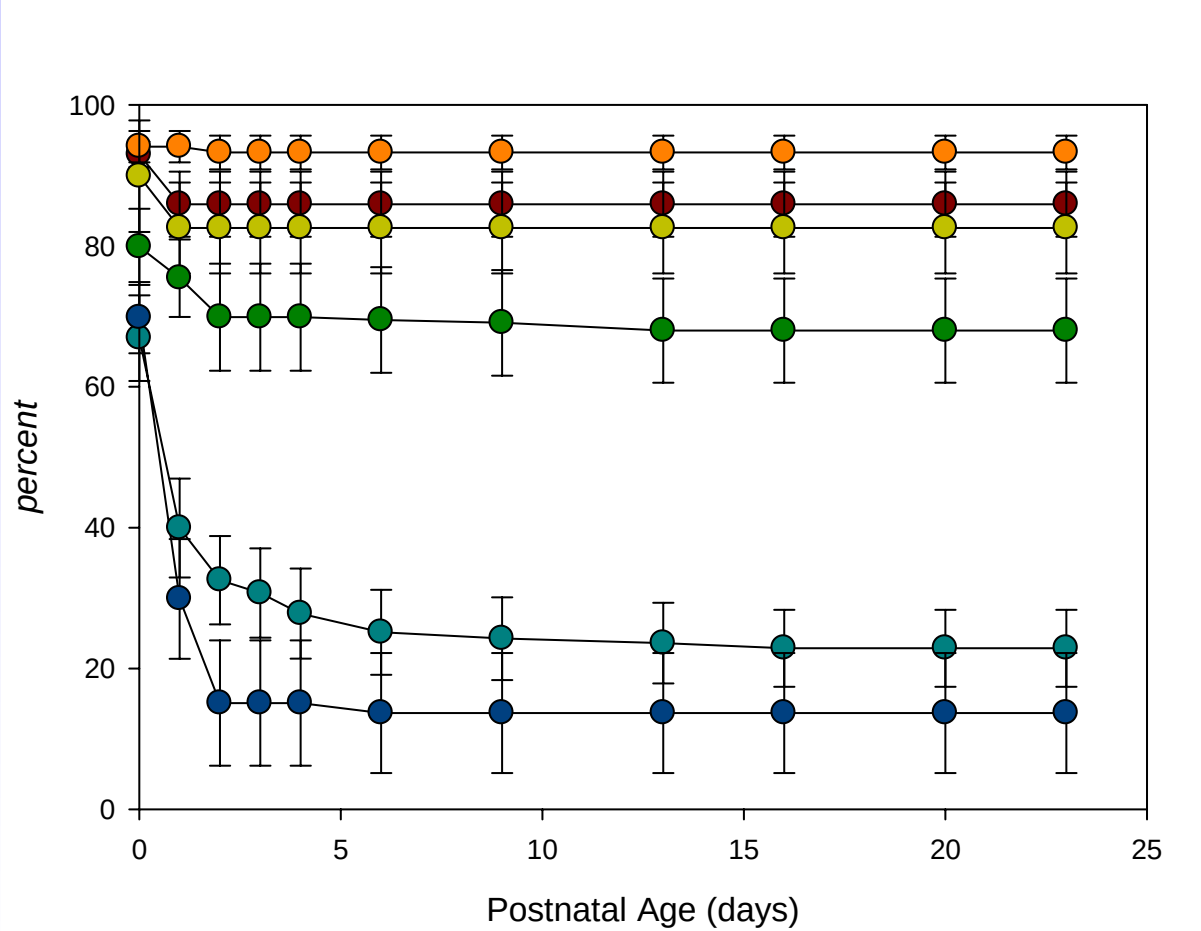
Animal Model/Dose of Choice for Perfluorinated Alkyl Acids

Female rat efficiently excretes PFOA – half-life in hours, compared to the extended and non-gender dependent half-life of weeks in the mouse.

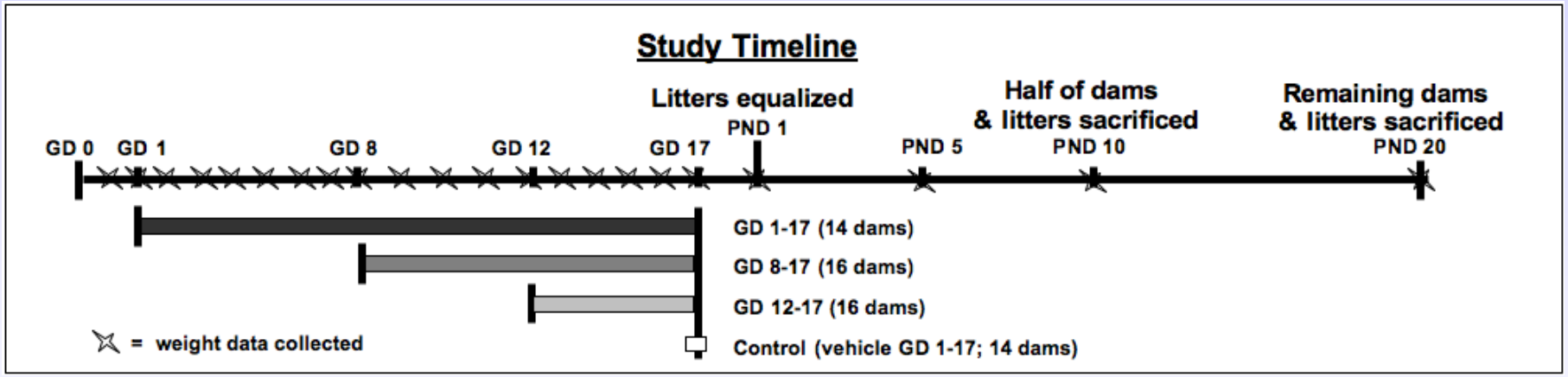
Outbred CD-1 mice – chosen model.

Doses over 10 mg/kg/d prenatally cause increased mortality of offspring.

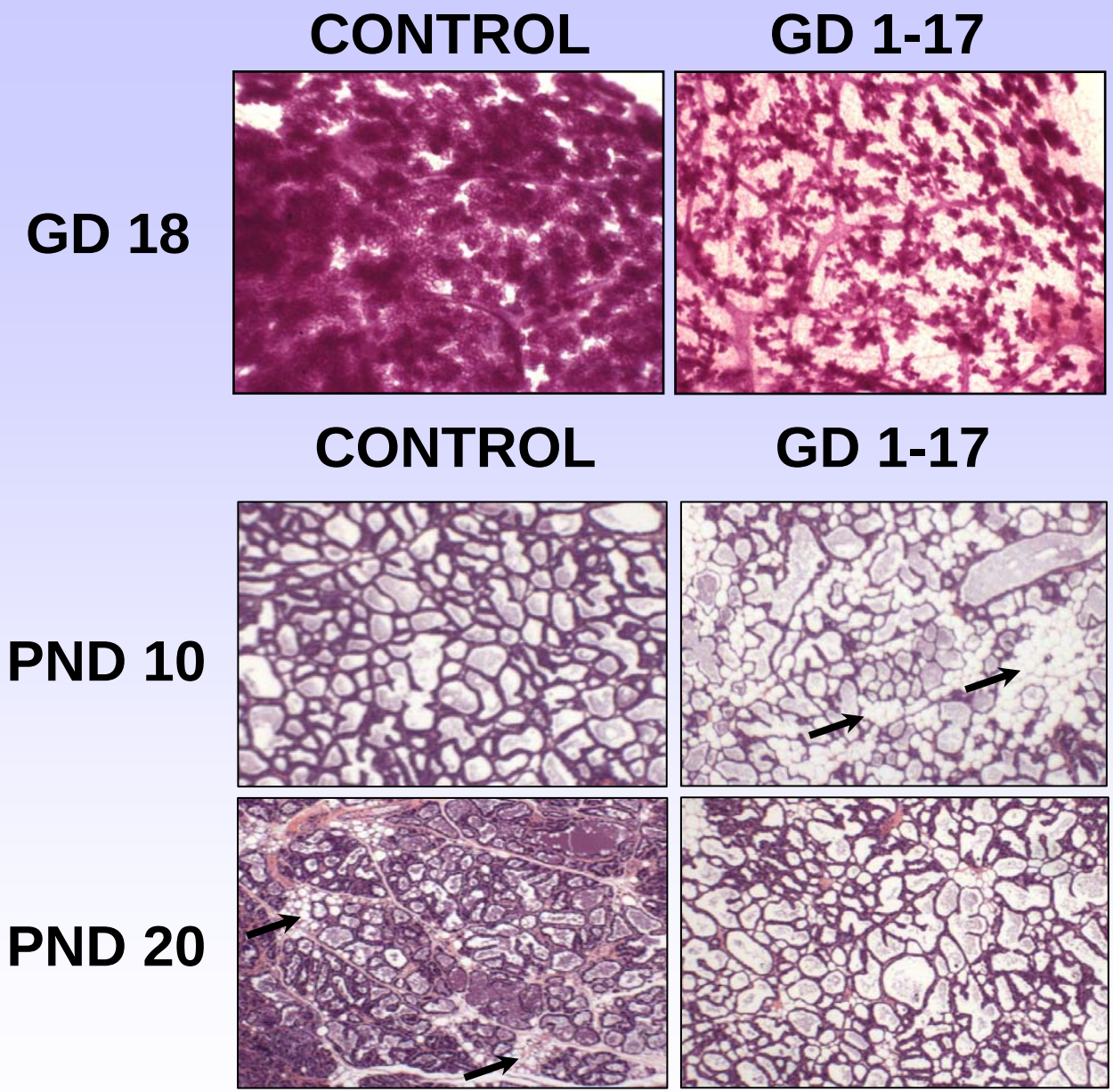
Postnatal survival



taken from Thibodeaux et al., 2006 *Toxicol Sci*

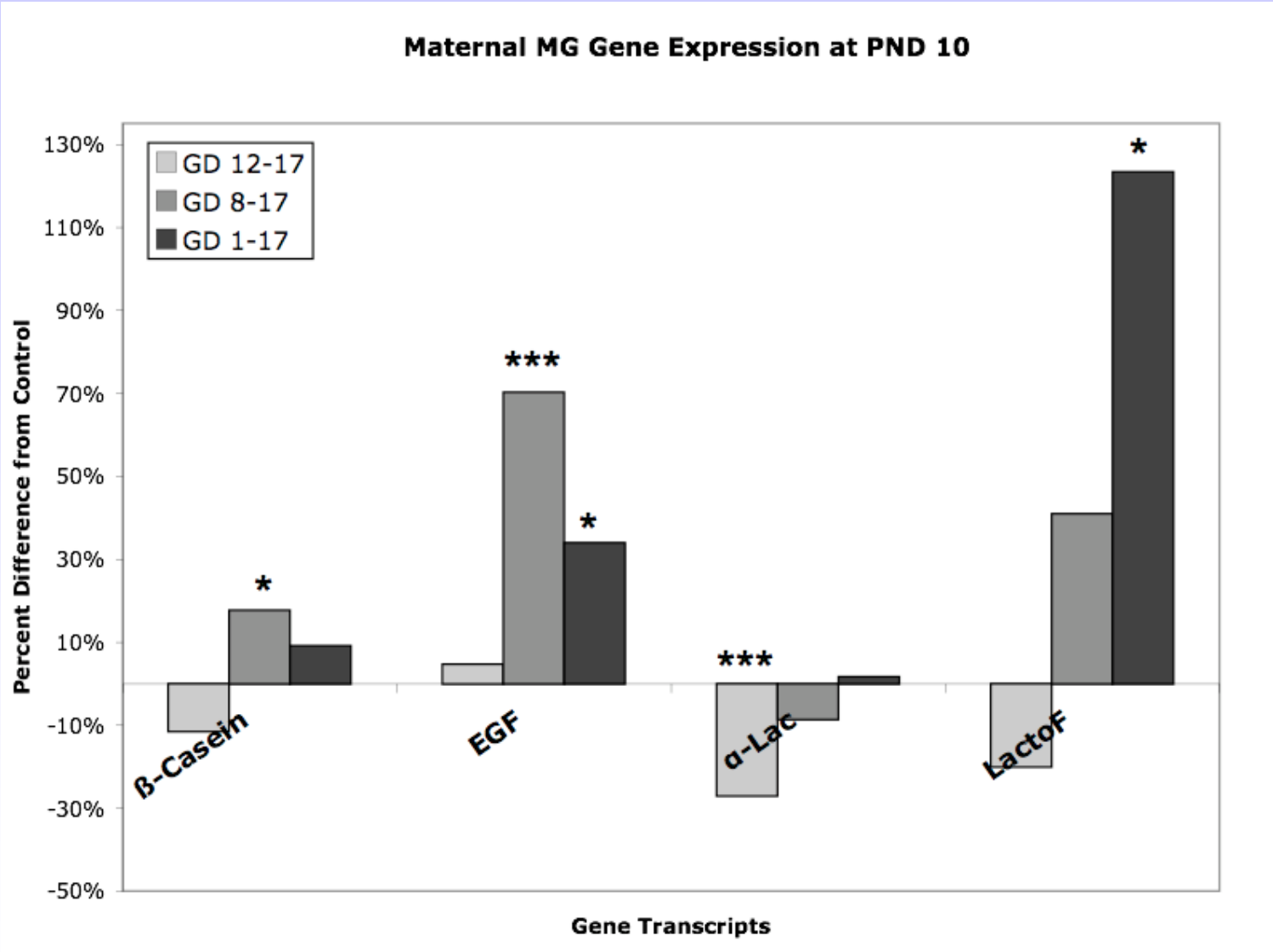


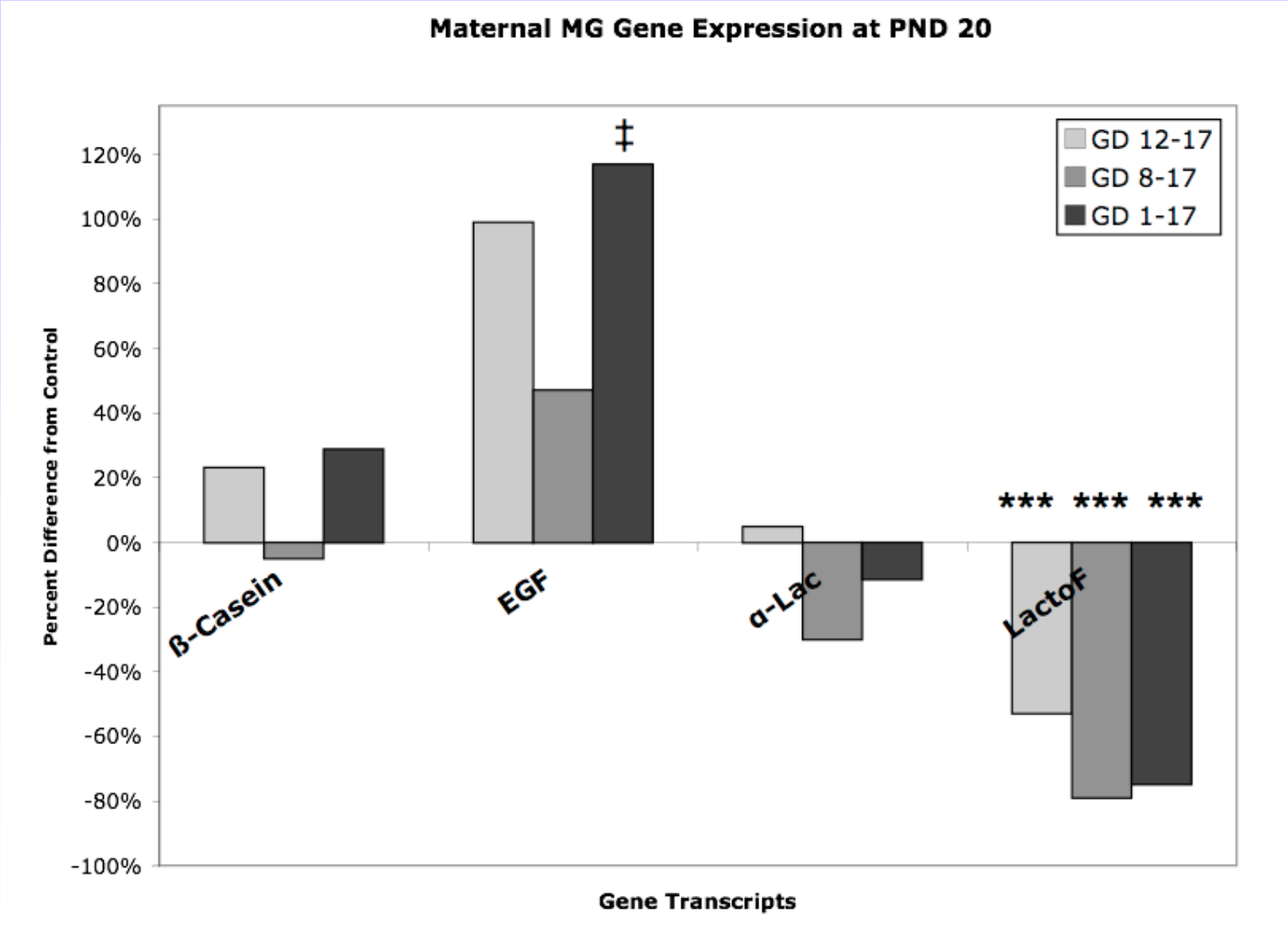
taken from White et al., 2007 *Toxicol. Sci.*



PFOA in Pregnancy Affects Lactation:

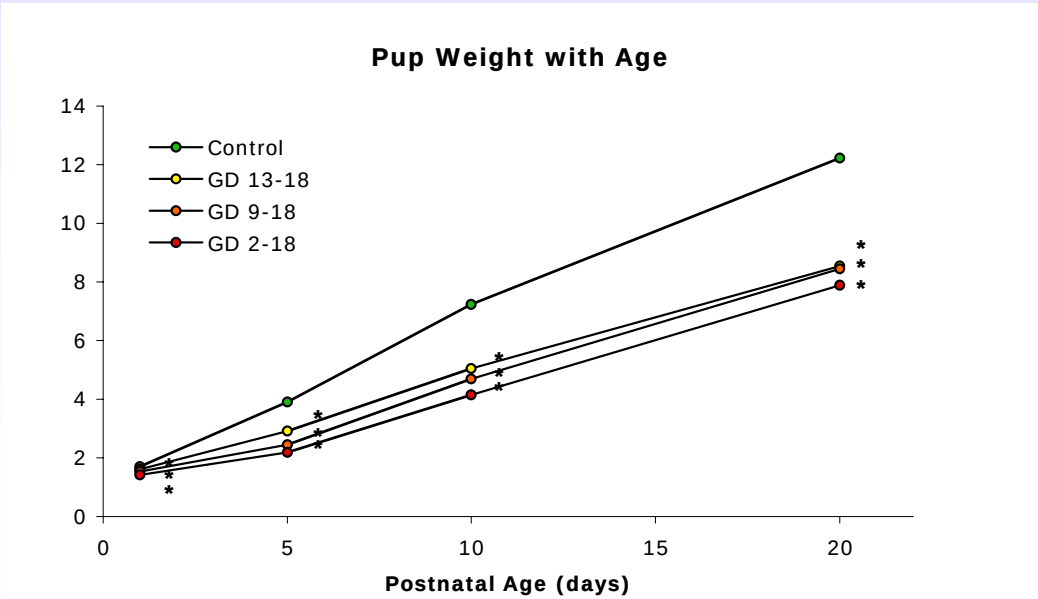
1. **Early effects**
2. **Delayed LAD**
3. **Delayed weaning**
4. **Affected weight of pups**


Maternal MG Gene Expression at PND 10
GD 12-17
GD 8-17
GD 1-17
130%
110%
90%
70%
50%
30%
10%
-10%
-30%
-50%
Percent Difference from Control
*
***
*
***
*
ß-Casein
EGF
α-Lac
LactoF
Gene Transcripts


Maternal MG Gene Expression at PND 20
GD 12-17
GD 8-17
GD 1-17
‡
***
***
***
120%
100%
80%
60%
40%
20%
0%
-20%
-40%
-60%
-80%
-100%
Percent Difference from Control
ß-Casein
EGF
α-Lac
LactoF
Gene Transcripts

Does duration of exposure affect pup weight gain?

5 mg/kg PFOA-exposed mean pup weights were significantly decreased compared to controls at all timepoints (*=p < 0.05); N=78-120 pups per timepoint through PND 10 (4-6 litters/dose group evaluated on PND10 or 20).



Offspring Mammary Gland Development

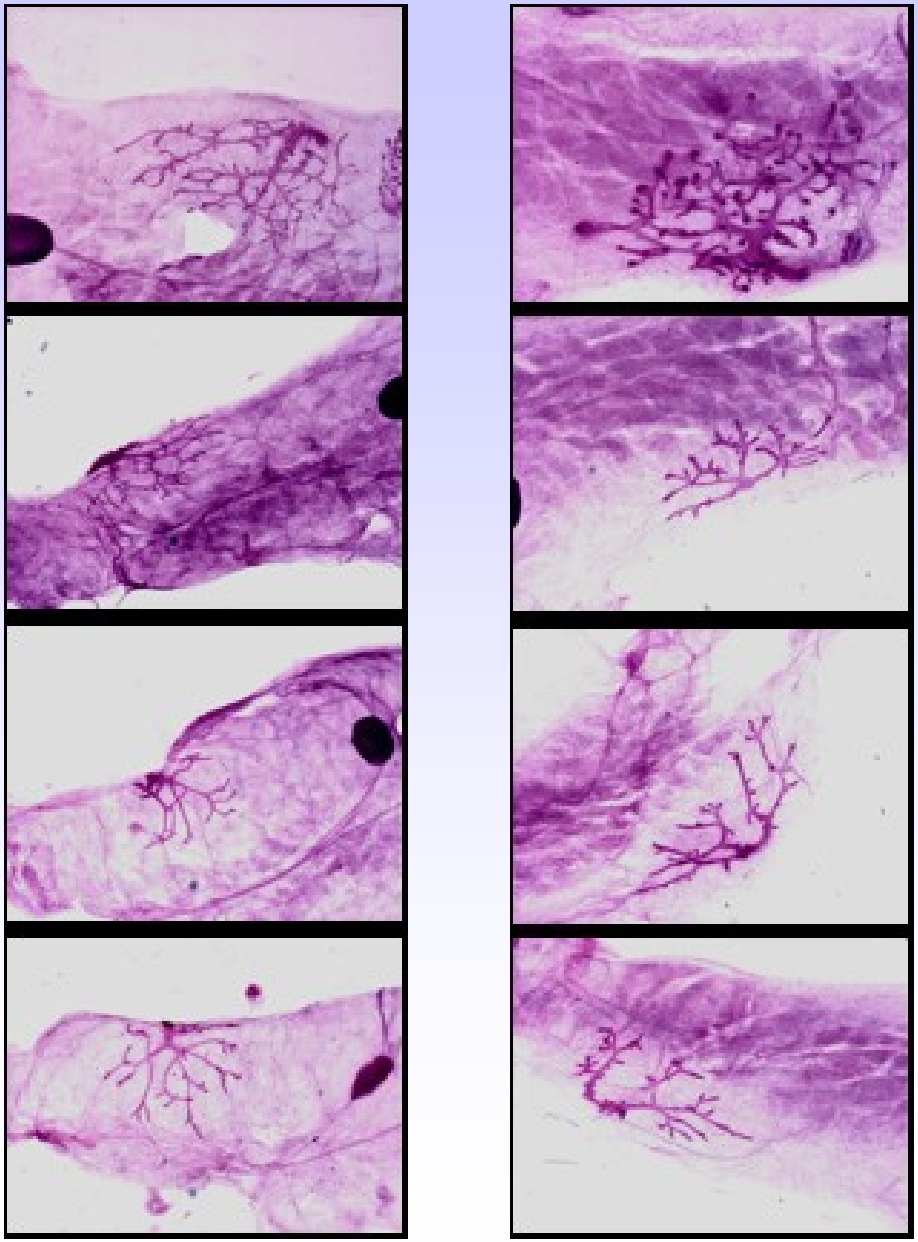
PND 10 | PND 20

Control

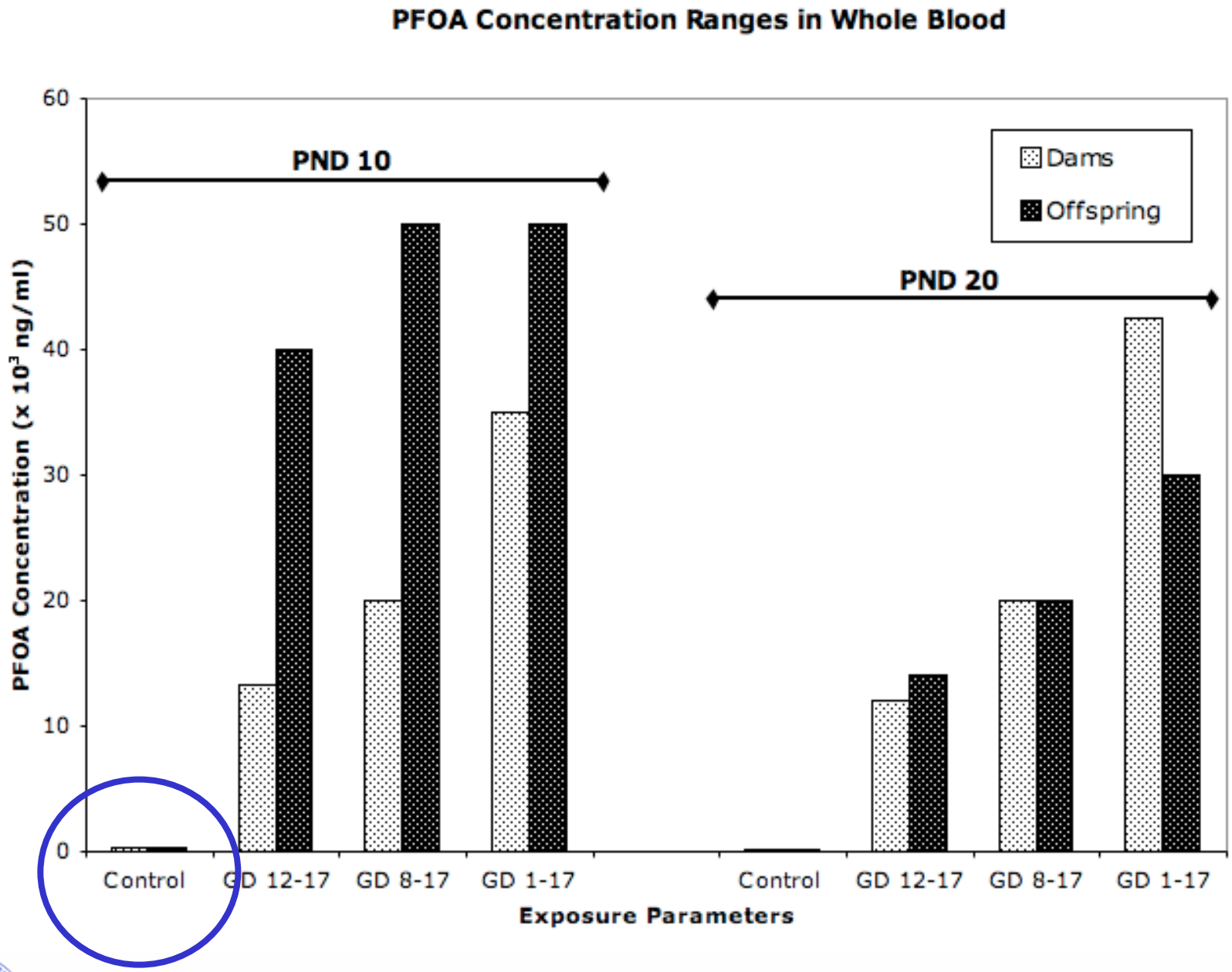
GD 12-17

GD 8-17

GD 1-17



20x lens mag.


PFOA Concentration Ranges in Whole Blood
PND 10
PND 20
Dams
Offspring
PFOA Concentration (x 10³ ng/ml)
60
50
40
30
20
10
0
Control
GD 12-17
GD 8-17
GD 1-17
Control
GD 12-17
GD 8-17
GD 1-17
Exposure Parameters

Summary of PFOA Effects

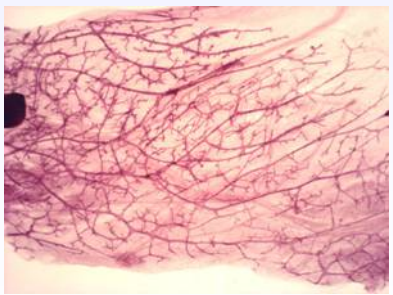
- Survival at birth, postnatal growth and development were compromised by chemical exposure during pregnancy (major effects) and lactation (lesser effects).
- Cross-fostering studies demonstrate majority of growth retardation effects from in utero exposure even though we have demonstrated effects on lactation.
- Transgenerational effects are being examined.
- Body burdens of PFOA in this animal model should continue to be determined so that they either reflect the exposure levels or can be correlated with human levels (CDC/NHANES) for MOE estimation.
- What happens in old age??

Mammary Glands at 18 months

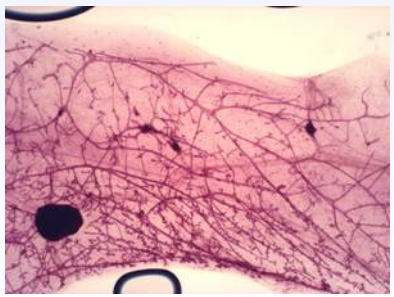
- **Hyperplastic alveolar nodules (HAN) do not represent normal growth and development of the mammary glands.**
- **In CD-1 mice, HANs may occasionally develop when the animals reach old age.**

Mammary Gland HAN

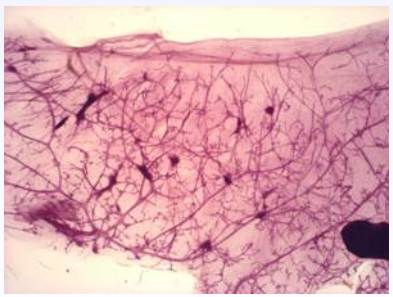
0-4



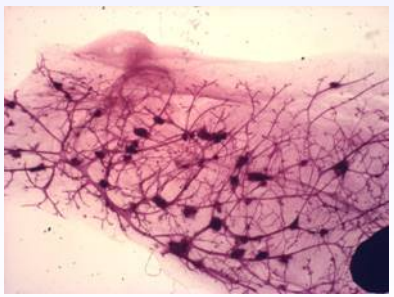
10-14



20-29



50+



Incidence of Hyperplastic Nodules in Mammary Glands

	Control	PFOA (1 mg/kg)	PFOA (3 mg/kg)	PFOA (5 mg/kg)
0 HAN	4/10	1/19	0/14	0/10
1 – 4 HAN	4/10	6/19	3/14	0/10
5 – 9 HAN	0/10	5/19	1/14	3/10
10 – 14 HAN	0/10	2/19	4/14	4/10
15 – 19 HAN	0/10	1/19	0/14	0/10
20 – 29 HAN	0/10	2/19	4/14	0/10
30 – 39 HAN	0/10	0/19	2/14	1/10
40 – 49 HAN	1/10	0/19	0/14	1/10
50+ HAN	1/10	2/19	0/14	1/10

What does this all mean?

- Mammary gland is a sensitive tissue to effects of EDCs
- EDCs can cause delayed or precocious mammary gland development
- Dose and timing dependent – critical period early in life
- Not dependent or correlated with timing of “puberty” in rodents
- EDCs affect branching, growth, and differentiation
- Results in “adverse” effects: altered F1 and F2 pup growth, changes in milk protein expression, maternal behavior is altered. Development is altered in a permanent fashion – persistent into adulthood for the examples shown.
- Prostate is also affected by EDCs – mode of action and reason for effects are not well understood yet. More difficult to assess due to required histopathology and focal nature of lesions.

- Can this be translated to human health?
- What about risk for breast cancer?

Developmental events in human and rodent mammary tissue

Developmental Event	Human	Rodent
milk streak evident	EW4-6	GD10-11 (mice)
mammary epithelial bud forms	EW10-13	GD12-14 (mice), GD 14-16 (rat)
female nipple and areola form	EW12-16	GD18 (mice)/GD20 (rat)
branching and canalization of epithelium	EW20-32	GD16 to birth (mice), GD 18 to birth (rat)
secretion is possible	EW32-40 (ability lost postnatally)	at birth, with hormonal stimuli
isometric development of ducts	birth to puberty	birth to puberty
TEBs present (peri-pubertal)	8-13 year old girls	23 to 60 days old(rat)
formation of lobular units	EW32-40, or within 1-2 yr. of first menstrual cycle	puberty and into adulthood

TEB=terminal end bud, EW=embryonic week, GD=gestational day

taken from S.E. Fenton, 2006 *Endocrinology* 147(Supplement):S18-S24.

Moving Forward

- **Exposure during the neonatal period causes the most serious and persistent effects on mammary gland and prostate development for many EDCs.**

- **The mammary gland development is significantly altered at times when other pubertal end points in rodent models are not; i.e., body weight, VO (which has no biological correlate in girls) or first estrous cycle.**

- **Collection of mammary tissue in assays assessing pubertal endpoints following early life EDC exposure can facilitate determination of which EDCs alter developing breast tissue and the mechanisms by which they are altered.**

Further research is needed to determine:

- If there are late life effects of various EDC exposures (what are the adverse effects?)

- Is there altered risk for mammary tumor development? Using a standardized operating procedure, can mammary gland biologists make quicker progress (similar terminology and ability to interpret data)?

- If there are calculated changes in the metabolism or clearance of EDCs when administered to a pregnant rat (vs. male or non-pregnant).

- What role can early gene expression profiles have in determining which compounds to test for further mammary effects?

Many Thanks….

US EPA

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