

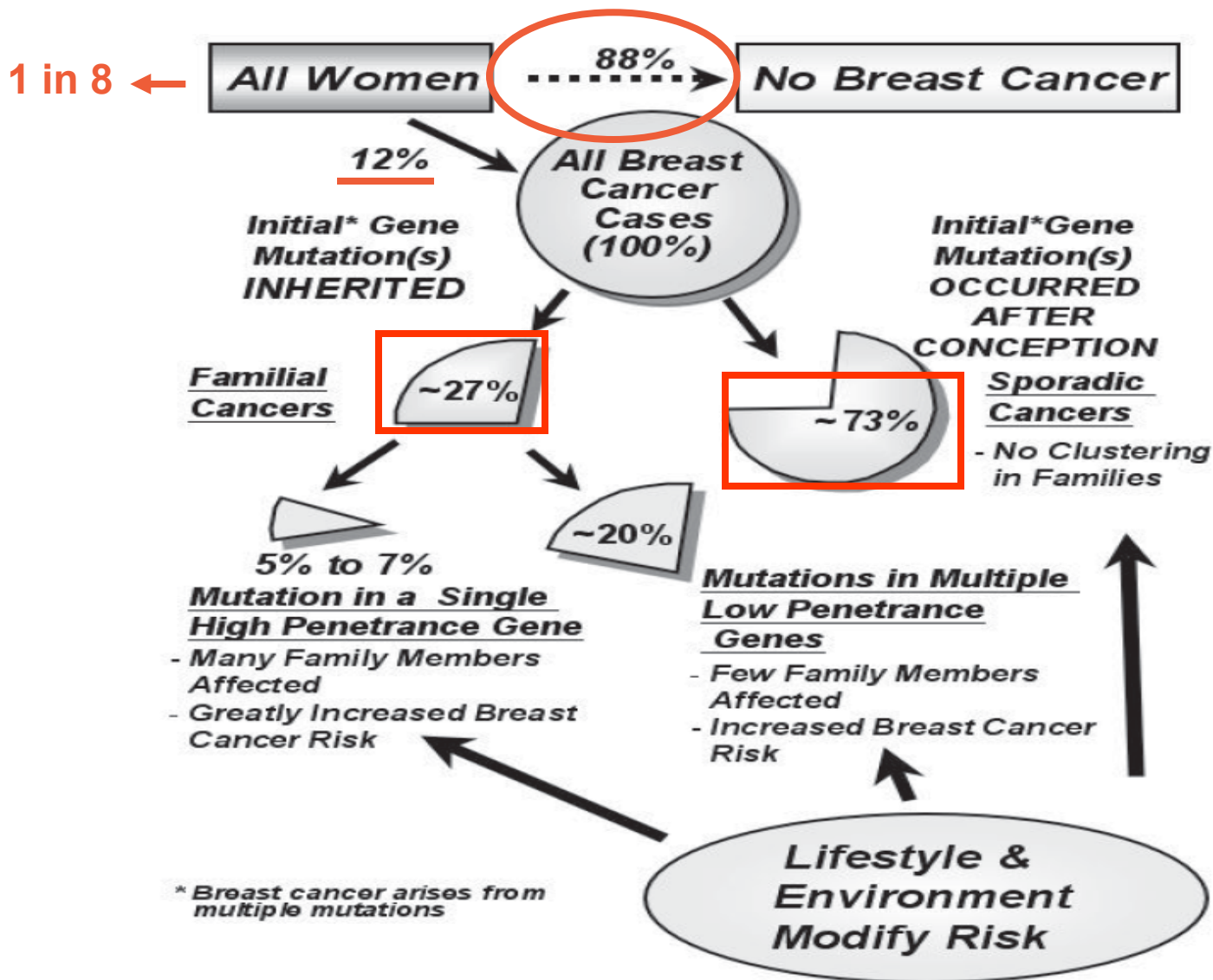


NTP
National Toxicology Program

Early Life Environmental Exposures: Lifelong Impact on Mammary Gland Development and Function

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Early Investigation of Environmental Exposures and Breast Cancer Risk:

Billions of dollars spent globally to investigate:

- 1. Circulating chemical levels in women recently diagnosed with breast cancer**
- 2. Potential exposure to a chemical applied to fields or roadsides near home address of women diagnosed with breast cancer**
- 3. Potential adult occupational exposures linked with recent breast cancer diagnoses**
- 4. Few tangible leads using these approaches**

Early Life Environmental Exposures:

A few studies evaluated longitudinal breast cancer risk or retrospective study of women with breast cancer and early life chemical exposure:

- 1. DDT exposure increased from 1945 through 1959, when DDT use peaked (with dietary exposure peaking in 1965) (Wolff et al., 2005. *Cancer Epidemiol Biomarkers Prev* 14:2224-36)**
- 2. Breast cancer odds ratio risk estimates by period of birth for the highest tertile of *p,p'*-DDT exposure were 0.6 for women born in 1931 or earlier (i.e., ≥ 14 years of age in 1945), 3.9 for women born in 1932–1937 (i.e., 8–13 years of age in 1945), 9.6 for women born in 1938–1941 (i.e., 4–7 years of age in 1945), and 11.5 for women born in 1942 or later (i.e., < 4 years of age in 1945). (Cohn et al., 2007 *EHP* 115:1406-14)**
- 3. 1976 industrial explosion in Seveso, Italy: Highest known population exposure to TCDD (dioxin). Nearly 1000 women in longitudinal cohort, with blood draw immediately after explosion, and intervals since then. Women were infants to 40 yr old at the time of explosion. The hazard ratio for breast cancer associated with a 10-fold increase in serum TCDD levels was significantly increased to 2.1 (95% confidence interval, 1.0-4.6). (Warner et al., 2002. *EHP* 110:625-8)**
- 4. Studies evaluating early life exposures to dioxins/PCBs have found delayed breast development in adolescents with the highest circulating (Seveso, Italy; denHond et al., 2002. *EHP* 110:771-6) or prenatal/lactational dioxin levels (The Netherlands; Leijds et al., 2008. *Chemosphere* 73:999-1004).**



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**
 - **Mammary gland is a simple structure with few branches at birth**
 - **Tissue matures after birth and grows rapidly**
 - **Distinct phenotypic structures are similar in rodent and human**



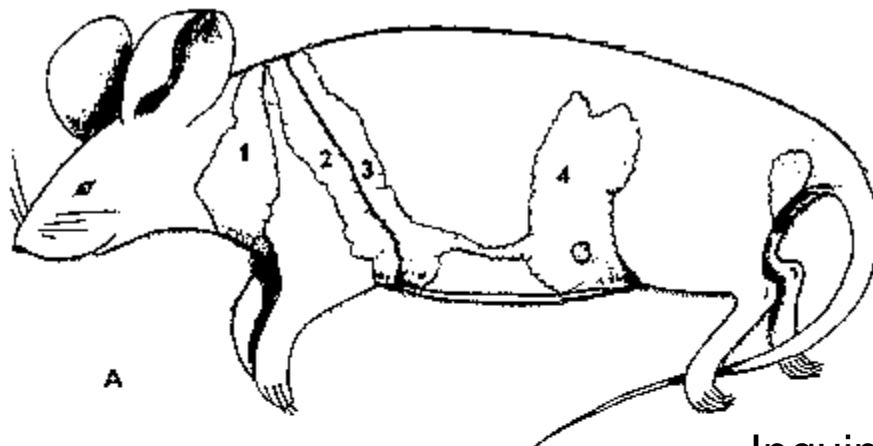
Developmental Events in Human and Rodent Mammary Tissue

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 (rodents)
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.

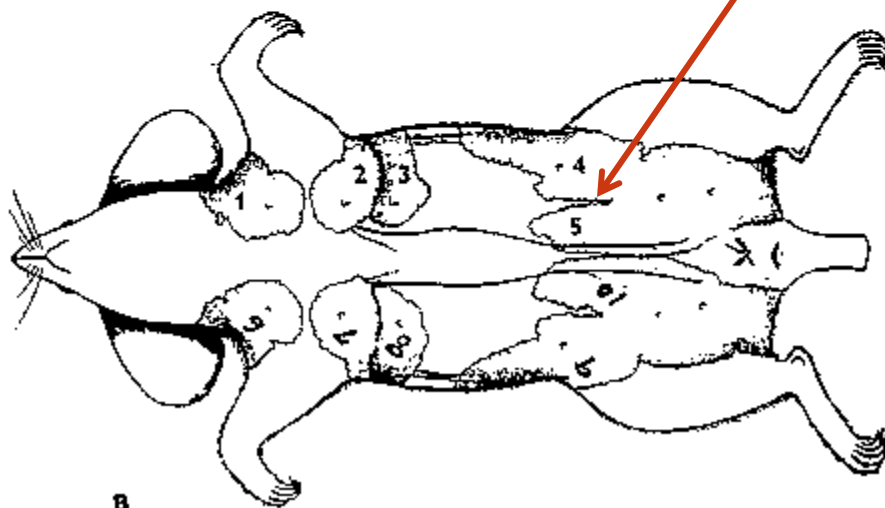


Location of Mouse Mammary Glands



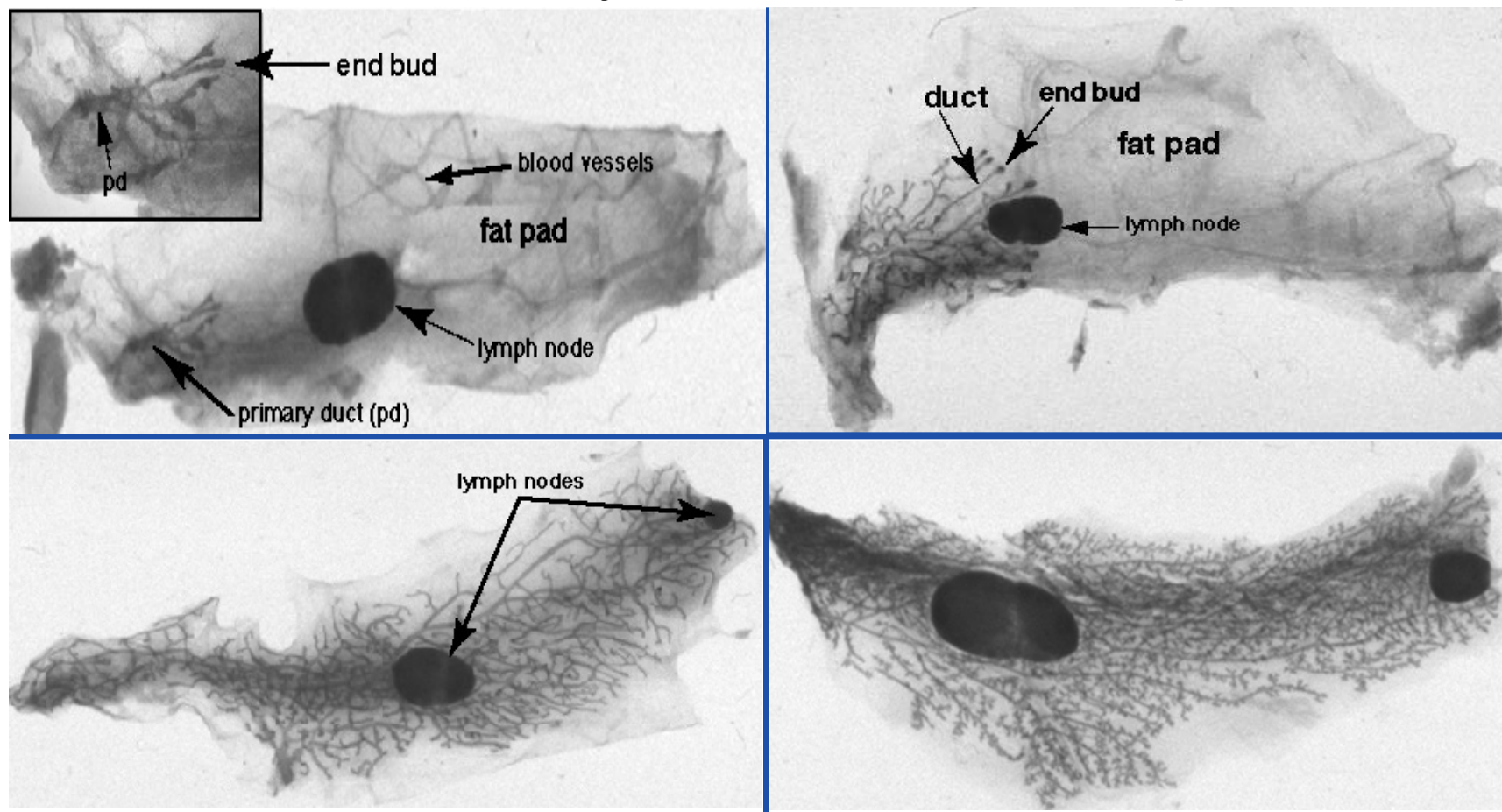
A

Inguinal lymph node

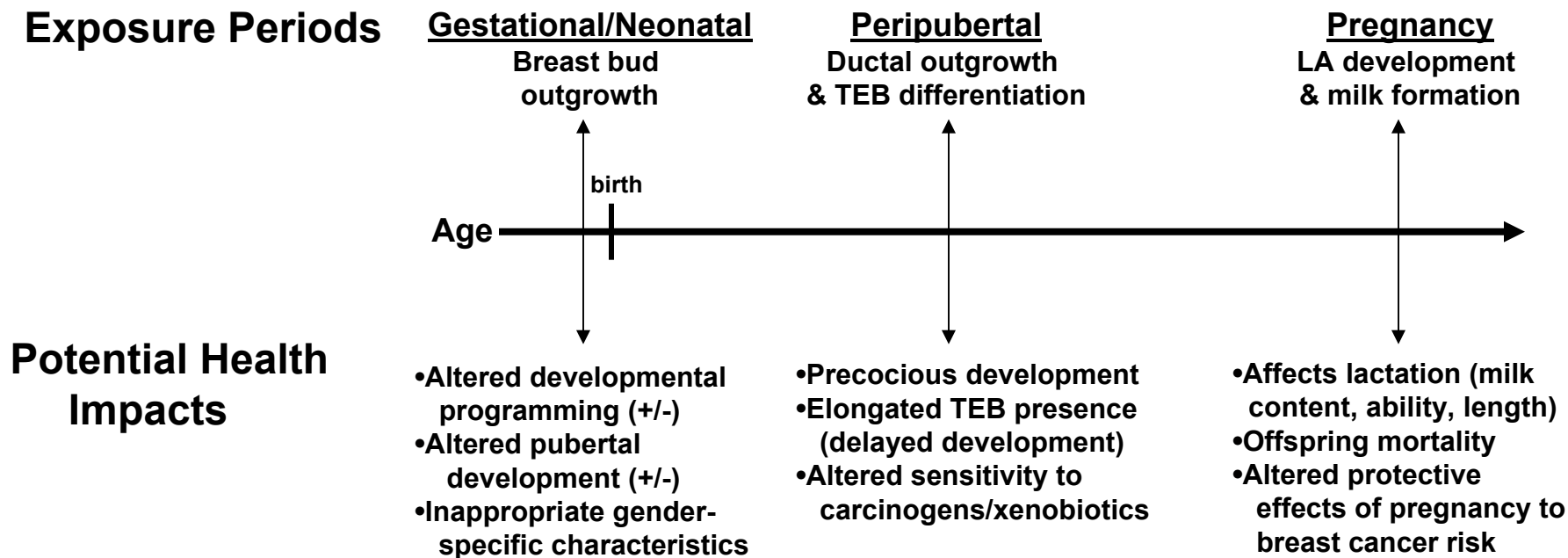


B

Utility of Whole Mounts for Assessing Rodent Mammary Gland Development



Identified Critical Periods in Mammary Gland Development



from Fenton, 2005. *Endocrinology*

Three Environmental Examples:

- 1. Dioxin – single developmental exposure on GD 15 in Long Evans rats.
Long half-life, lipophilic (and mice)**
- 2. Atrazine – 100 mg/kg, 5 day exposure in late pregnancy (GD15-19) in LE rats
Short half-life (about 10-15 hr)**
- 3. PFOA – 1 to 5 mg/kg during last half of pregnancy (GD10-18) in CD-1 mice
Long half-life (2 wk) and protein binding**

Developmental exposure to the pregnant dam has effects in two generations.

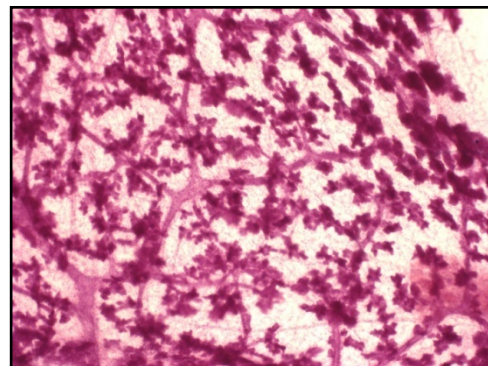
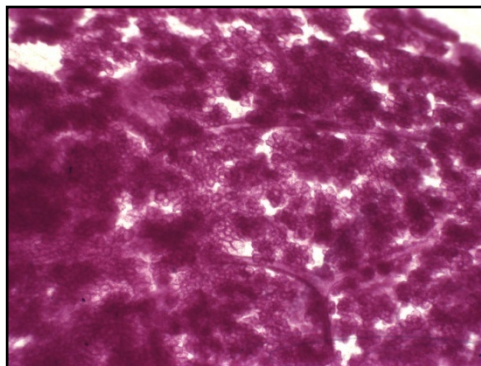


Effects of 5 mg/kg PFOA on Lactating Mouse Mammary Glands Following Gestational Exposure

A
GD 18

CONTROL

GD 1-17



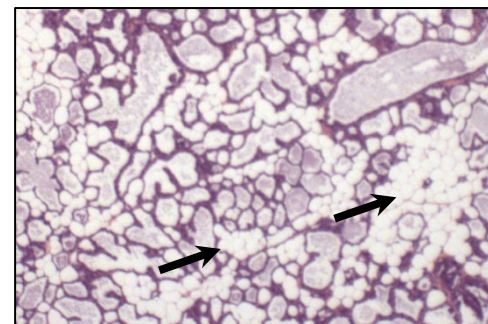
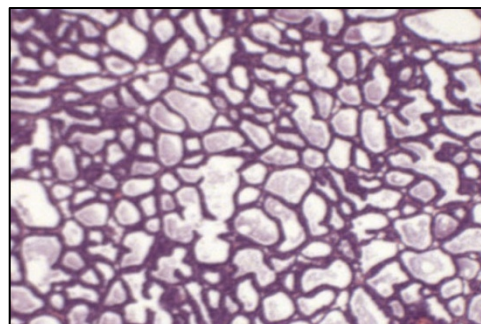
From White et al., Toxicol Sci 2007

CONTROL

GD 1-17

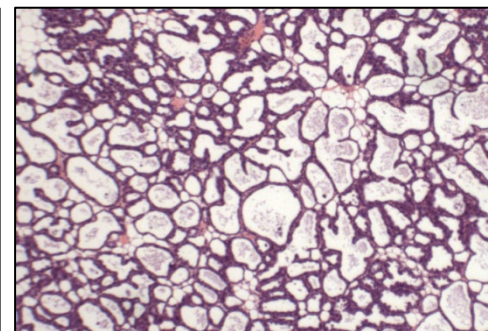
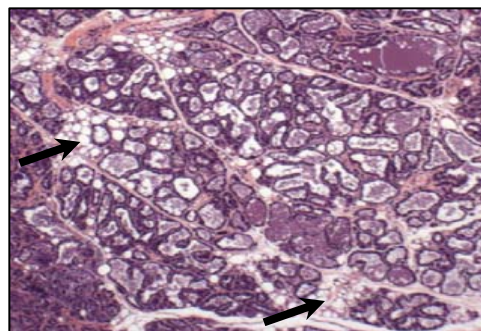
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PND 10



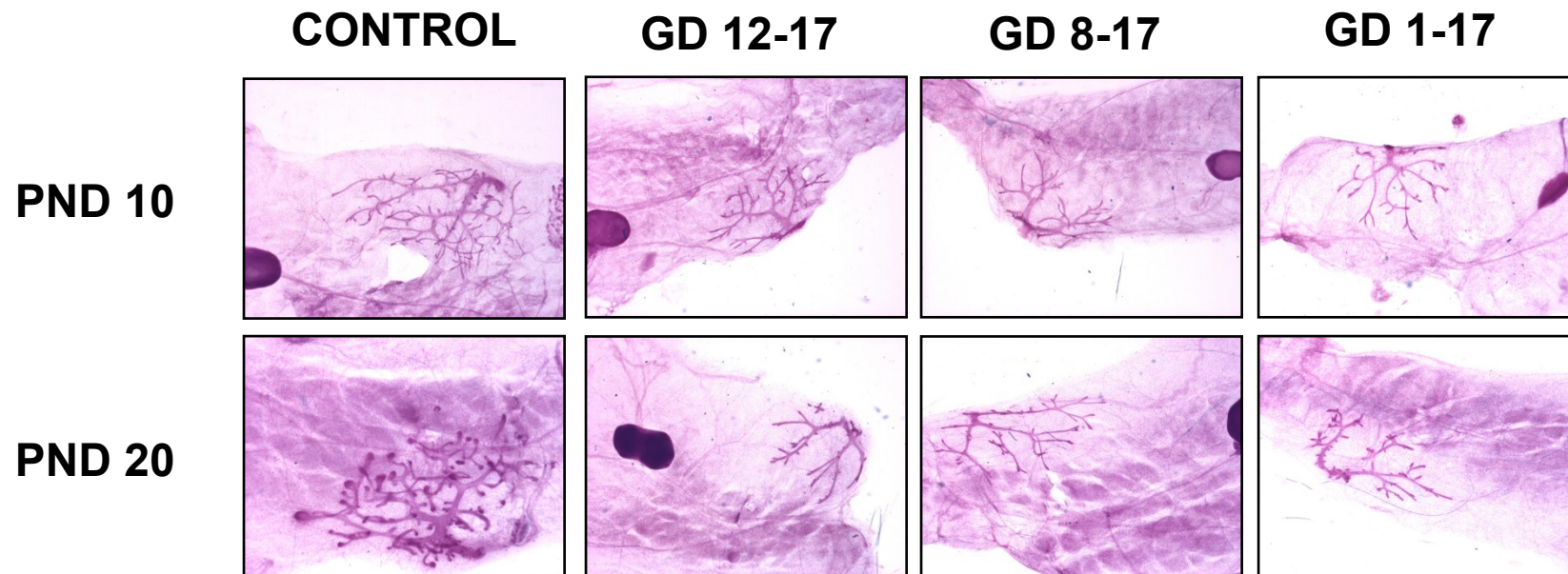
Observed as early postnatal offspring mortality, significant decrease in offspring weight gain, and/or lethargy. ←

PND 20



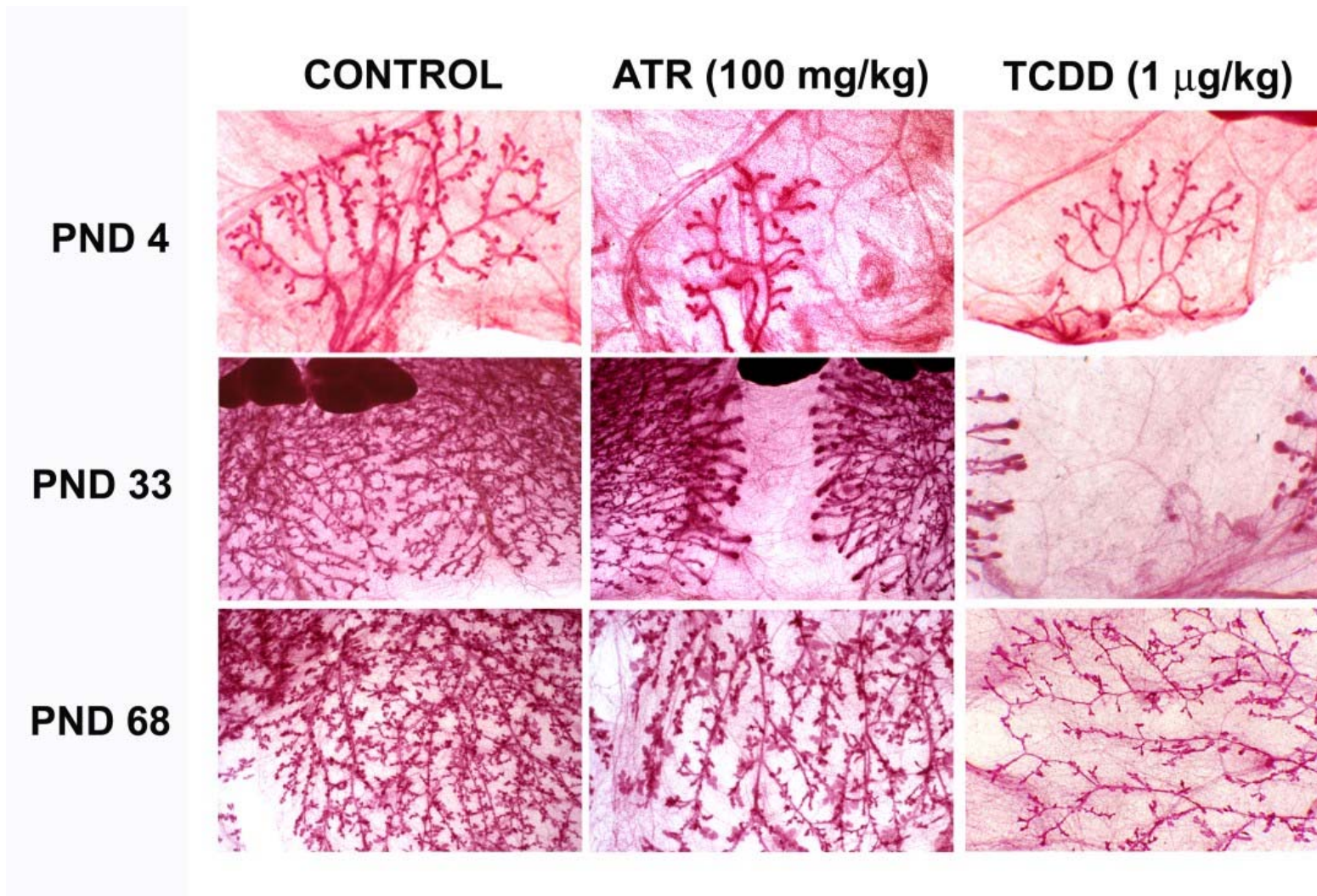


Effects of Varying Exposure Length to PFOA (5 mg/kg)



From White et al., Reprod Tox 2009

Toxicant Comparison in MG



Mammary Gland Evaluation in Screening & Testing:

- **As of February 1, 2010, there were slightly more than 85,000 chemicals registered for commercial use on the market [Toxic Substances Control Act (TSCA) Chemical Substances Inventory]**
- **Chemical specific health hazards have been identified for somewhere between 1-2% of those chemicals.**
- **Most guideline studies expose adult animals to chemicals. There are NO screening & testing protocols that require evaluation of the mammary gland. It is collected and evaluated IF a tumor is present or lactation is perturbed.**
- **In a 2007 report by Silent Spring Institute, 216 chemicals have been identified to cause mammary tumors in a rodent bioassay (in at least one lab). (Rudel et al., 2007. *Cancer* 109(S12):2635-66). Potency factors have been developed for only 3 of those chemicals (acrylamide, 2,4-dinitrotoluene, and 3,3-dichlorobenzidine)**
- **Conclusion: We are not identifying chemicals that may affect breast cancer risk very well. One likely reason is that the typical 2-yr bioassay includes none of the “critical periods” of mammary gland development in it.**



Identified Critical Periods in Mammary Gland Development

Exposure Periods

Gestational/Neonatal

Breast bud
outgrowth

Peripubertal

Ductal outgrowth
& TEB differentiation

Pregnancy

LA development
& milk formation

Age

birth

Potential Health Impacts

- Altered developmental programming (+/-)
- Altered pubertal development (+/-)
- Inappropriate gender-specific characteristics

- Precocious development
- Elongated TEB presence (delayed development)
- Altered sensitivity to carcinogens/xenobiotics

- Affects lactation (milk content, ability, length)
- Offspring mortality
- Altered protective effects of pregnancy to breast cancer risk



2006 National Toxicology Program (NTP) Workshop on Hormonally Induced Reproductive Tumors—Relevance of Rodent Bioassays

- **Focused on cancers of the ovary, mammary gland, prostate, and testis**

Specific to the mammary gland:

- **Expert panel recommended considering experimental designs that address relevant exposure windows (e.g., *in utero exposures*, *exposures during puberty*, *exposures before a first full-term pregnancy*). NTP has since incorporated early life exposures in long-term studies.**
- **NTP foresees more routine assessment of *in vivo endocrine endpoints*, such as whole mounts of mammary glands, when preliminary data suggest an endocrine mode of action or effect.**
- **NTP and NCTR (National Center for Toxicological Research) are currently working to develop standard operating procedures for mammary gland whole mounts that can be used in contract labs, and potentially validated for incorporation into any screening and testing regime that includes an early life environmental exposure.**



Summary:

- 1. Developmental windows of exposure may be critical in identifying chemicals involved in altering early mammary gland development and the sensitivity of the gland to other environmental stressors... two hits required.**
- 2. Critical windows – bud formation, pubertal development, and pregnancy- should be the exposure targets.**
- 3. Mammary gland whole mounts allow an early evaluation of the chemical's effects on mammary gland development. Protocols abound, but NTP is a leader in the field.**

Myself and others are now busy setting the precedent, instead of avoiding or specifically not including end points because there is no precedent.



Thanks for your attention.....

Contributors to the work shown:

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