NTP
National Toxicology Program

Weighing Evidence from National Toxicology Program Cancer Bioassays

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Breast Cancer and the Environment
IOM
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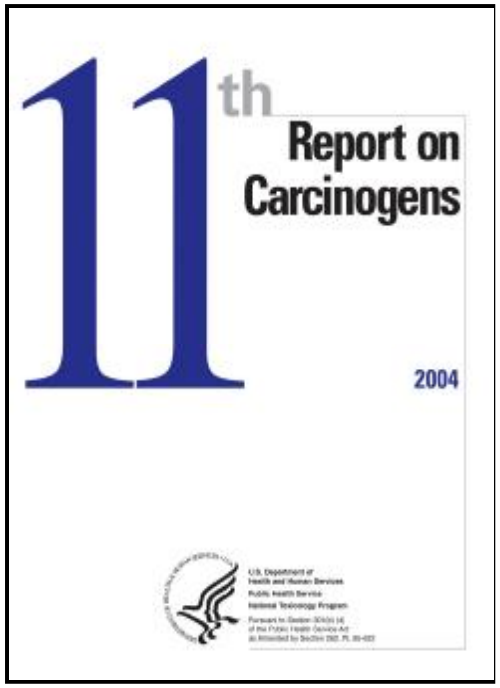
Topics

- Report on Carcinogens (RoC)
- Statistics on mammary cancer in NTP studies
- Chemical carcinogens
- Biology
- Genetically modified mouse models
- Applicability to humans

RoC

- Provides information about potential cancer hazards in our environment
- Hazard identification document
  - Identifies agents, substances, mixtures, or exposure circumstances that may pose a carcinogenic hazard for people in the United States
  - Lists “substances” as *known* or *reasonably anticipated human carcinogens*
- Congressionally mandated biennial report
  - Secretary, Health and Human Services, has responsibility for the report
  - 1st RoC published in 1980 had 26 listings
  - Current 11th RoC has 246 listings (58 *known* and 188 *reasonably anticipated*)



Substance profiles


Polychlorinated Biphenyls (PCBs)
CAS No. 1336-36-3

Carcinogenicity

Properties

Use

Production

Exposure

Regulations

- Identifies the listing
- Summarizes relevant information that supports the listing
  - Carcinogenicity, genotoxicity, and biologic mechanisms in humans and/or animals
  - Potential for human exposure
- Provides information on
  - Properties of the substance
  - Use and production
  - Current Federal regulations and guidelines to limit exposures

Listing criteria for the RoC

Known to be a Human Carcinogen:

- There is *sufficient evidence of carcinogenicity from studies in humans* which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be a Human Carcinogen:

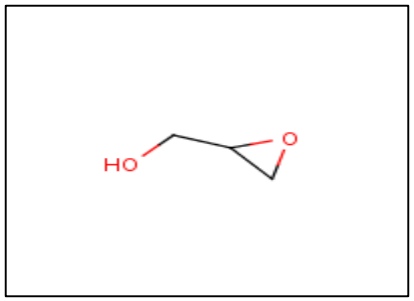
- There is *limited evidence of carcinogenicity from studies in humans* which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or
- There is *sufficient evidence of carcinogenicity from studies in experimental animals* which indicates there is an *increased incidence of malignant and/or a combination of malignant and benign tumors:* (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or
- There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen, or reasonably anticipated to be human carcinogen or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

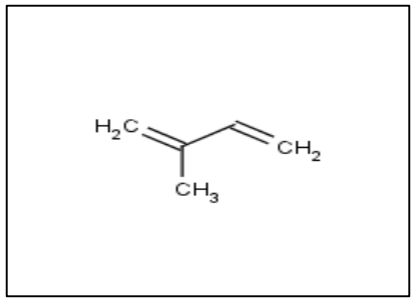
Listing criteria for the RoC (continued)

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Statistics on mammary cancer in NTP studies

- Database:
  - Of the 2-year rat and mouse studies of 555 substances
    - 7 (1.2%) positive for mammary tumors (all types) in male rats
    - 30 (5.4%) in female rats
    - 0 in male mice
    - 12 (2.1%) in female mice
  - Positive in male and female rats and female mice
    - (1) glycidol
  - Positive in male and female rats
    - (5) isoprene, methylene chloride, o-nitrotoluene, 2,2-bis-bromomethyl-1,3-propanediol, procarbazine
  - Positive in female rats and female mice
    - (4) chloroprene, 1,2-dibromoethane, 1,2-dichloroethane, sullfalate


HO
O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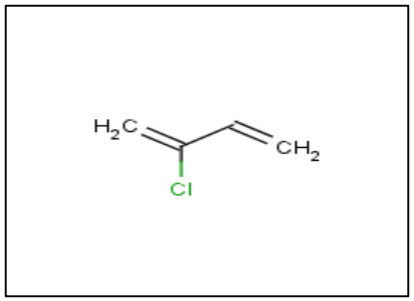
CH2
CH3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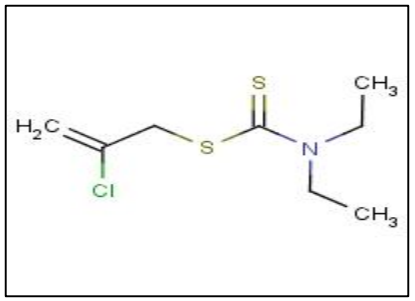
CH2
Cl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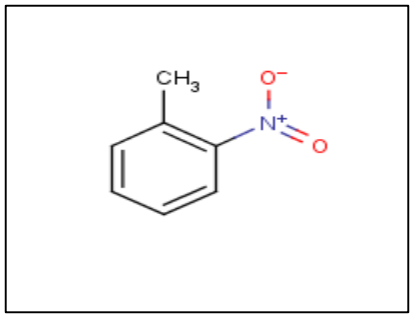
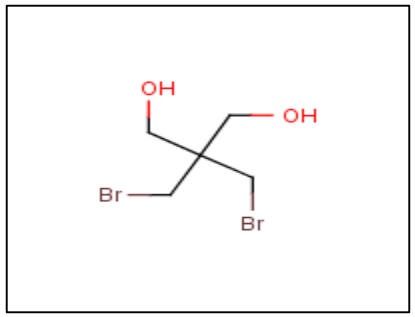
CH3
H2C
S
N
Cl
CH3


CH3
O−
N+
O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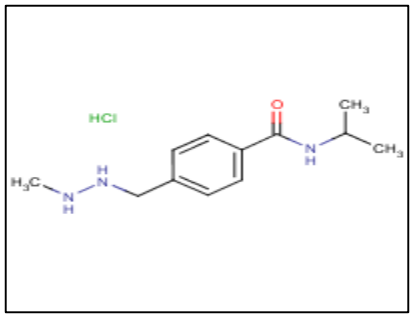
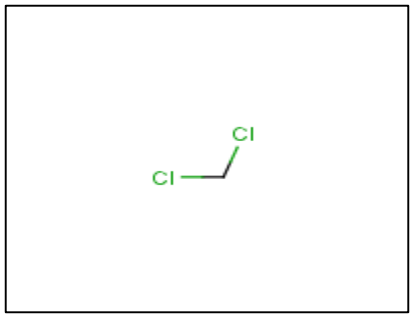
OH
Br
Br


HCl
O
CH3
N
H
CH3
H3C
N
H
N
H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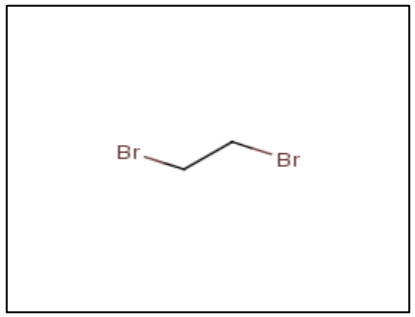
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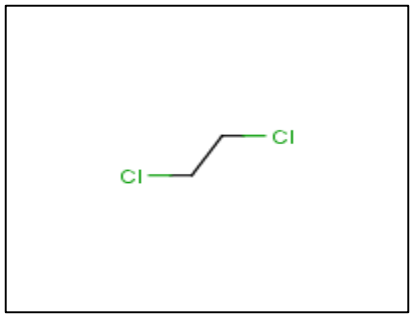

Cl
Cl

Biology of mammary cancer in NTP studies

- Control incidences:
  - Female Fischer 344 rat
    - Adenoma 2.1%
    - Carcinoma 5.2%
    - Fibroadenoma 52.4%
  - Male Fischer 344 rat
    - Adenoma 0.5%
    - Carcinoma 0.3%
    - Fibroadenoma 3.1%
  - Female Harlan Sprague Dawley rat
    - Adenoma 2.5%
    - Carcinoma 10.0%
    - Fibroadenoma 67.4%
  - Female B6C3F1 mouse
    - Adenoma 0.08%
    - Carcinoma 1.2%
    - Fibroadenoma 0.08%
  - Male B6C3F1 mouse
    - Adenoma 0.08%
    - Carcinoma 0.08%
    - Fibroadenoma 0%

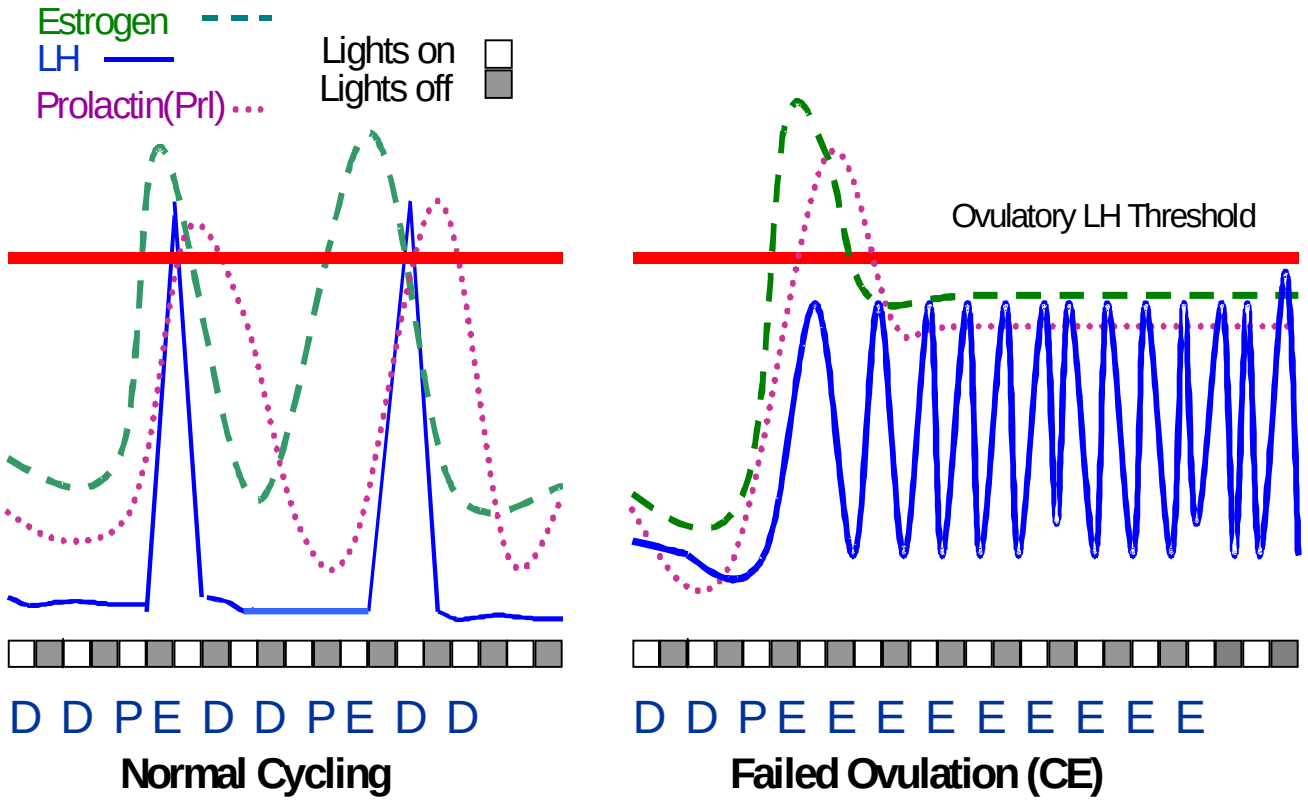
General mechanisms of mammary carcinogenesis

- Genotoxic
  - ~ 50% of NTP mammary carcinogens are mutagenic in Salmonella
  - More are positive in additional genotoxicity assays
  - Initiation promotion assays commonly used to study mammary carcinogenesis
- Hormonal
  - Reserpine - dopamine depletion (P female mice)
  - Genistein - estrogenic soy isoflavone (SE female SD rats- perinatal study)
  - Ethinyl estradiol - synthetic estrogen (EE male SD rats- perinatal study)
- Mixed
  - Phenestrin - a ”steroid alkylating agent” increases circulating E2 (P female rats)

"Strain specific" sensitivities for hormonal mammary carcinogens

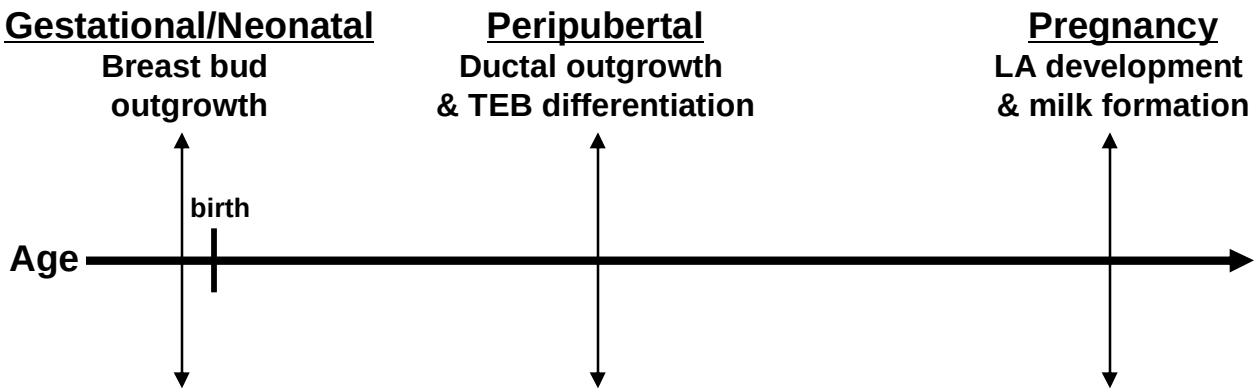
- Sprague Dawley ("susceptible")
  - Estrogenic agents
  - Agents that accelerate reproductive senescence
  - Agents that increase prolactin
- Fischer 344 ("susceptible")
  - Agents that increase prolactin
- Wistar Furth ("suceptible") --- prolactin
- Wistar Han ("susceptible")
- Wistar Kyoto ("resistant")
- Copenhagen ("resistant") --- low prolactin signaling (Ren *et al. Carcinogenesis* 28:177-185, 2008.
- Genetically intact mice, mmtv ("resistant")

Figure 6: Schematic of Normal and Constant Estrus in Sprague-Dawley Rats



Identified critical periods in mammary gland development

Exposure Periods



Potential Health Impacts

- **Gestational/Neonatal**
  - Altered developmental programming (+/-)
  - Altered pubertal development (+/-)
  - Inappropriate gender-specific characteristics
- **Peripubertal**
  - Precocious development
  - Elongated TEB presence (delayed development)
  - Altered sensitivity to carcinogens/xenobiotics
- **Pregnancy**
  - Affects lactation (milk content, ability, length)
  - Offspring mortality
  - Altered protective effects of pregnancy to breast cancer risk

Precocious puberty in girls – caused pediatricians to "re-write the book" on what precocious development really is….

from Fenton, 2006 *Endocrinology*

Developmental events in human and rodent mammary tissue

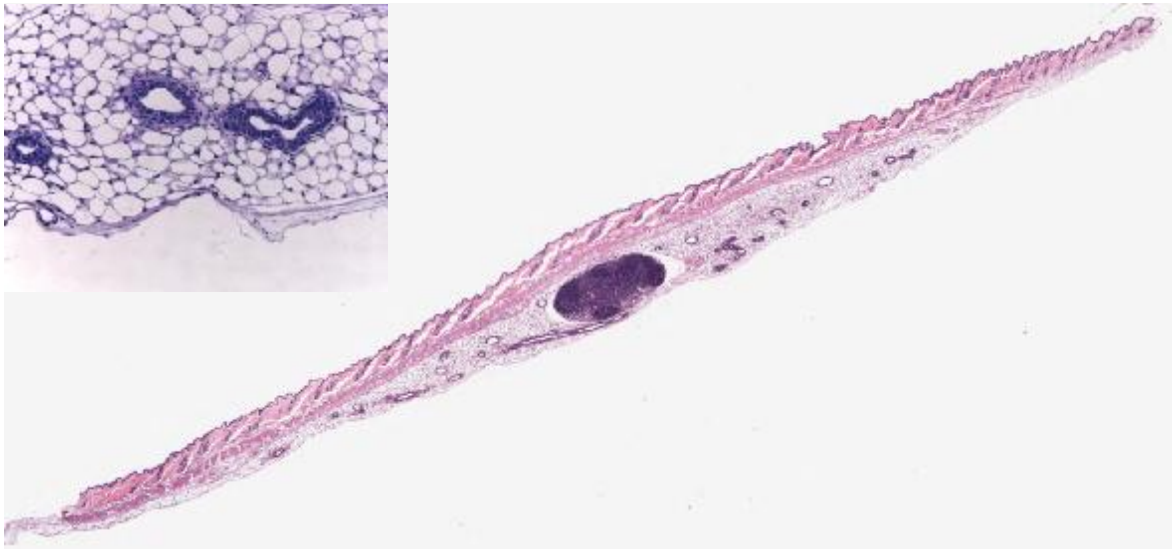
Developmental Event	Human	Rodent
milk streak evident	EW4-6	GD10-11 (mice)
mammary epithelial bud forms	EW10-13	GD12-14 (mice), GD 14-16 (rat)
female nipple and areola form	EW12-16	GD18 (mice)/GD20 (rat)
branching and canalization of epithelium	EW20-32	GD16 to birth (mice), GD 18 to birth (rat)
secretion 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

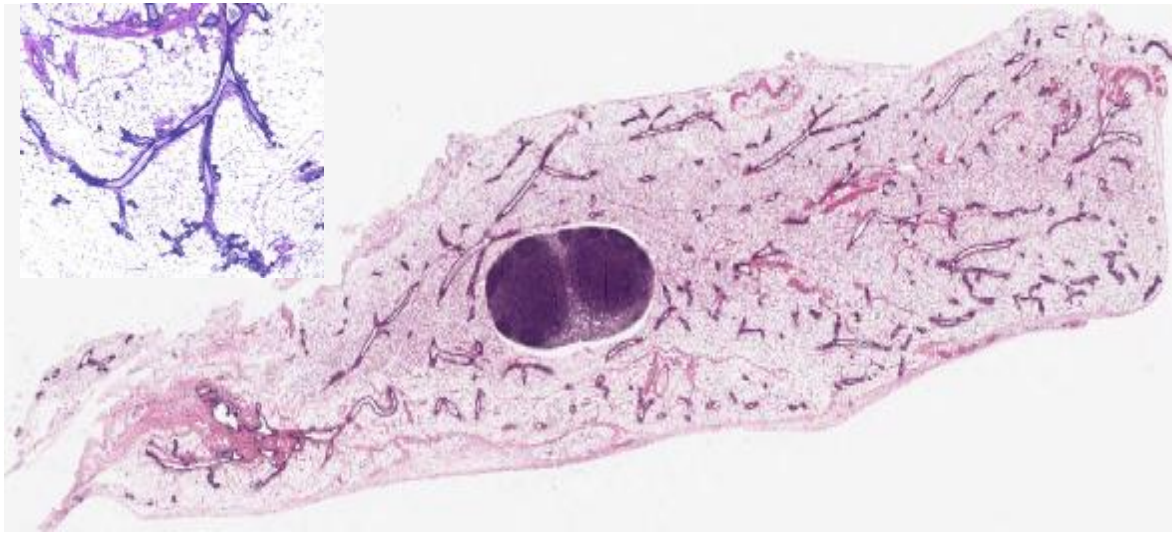
taken from S.E. Fenton, 2006 *Endocrinology* 147(Supplement):S18-S24.

Inguinal mammary gland sampling

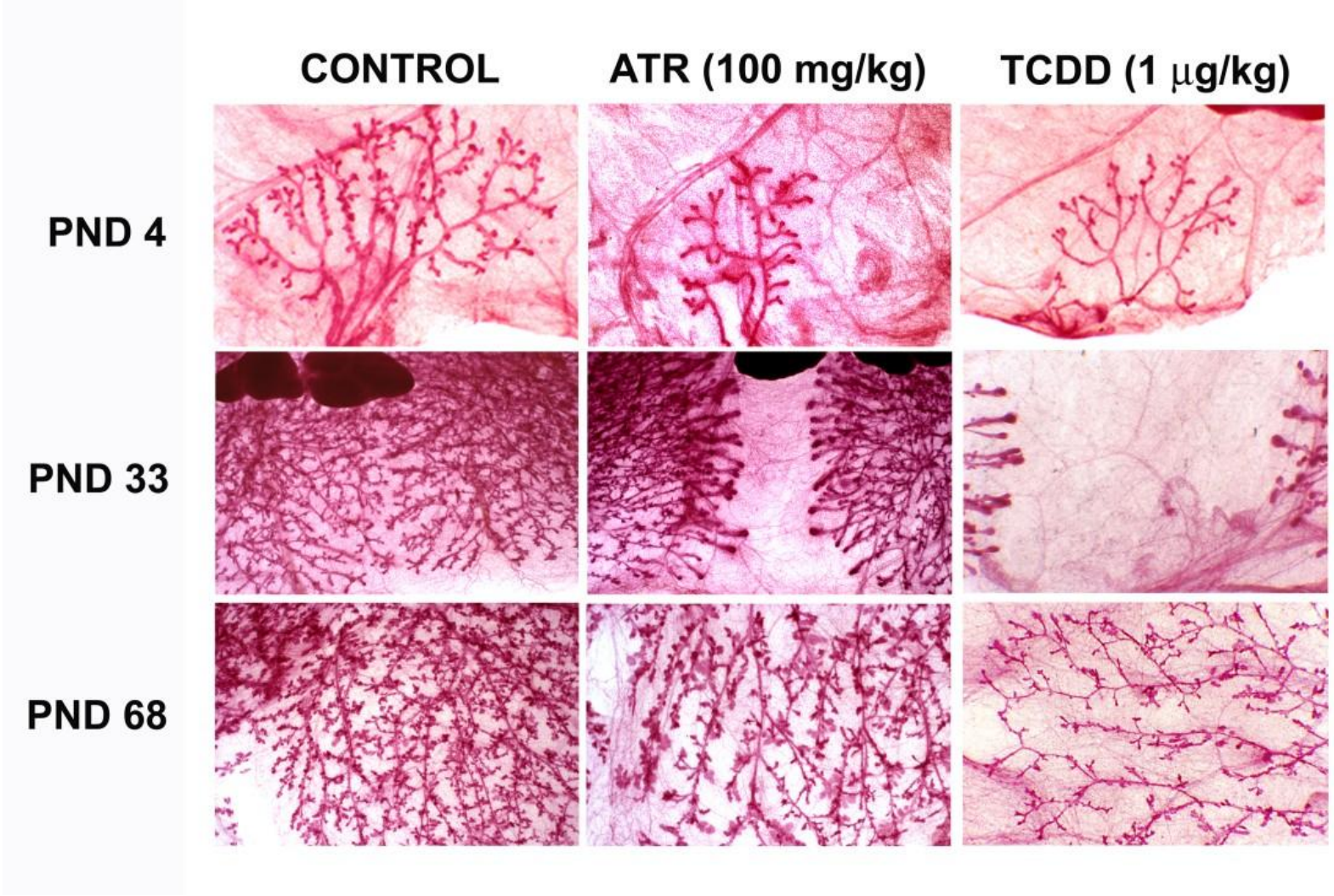
Cross Section



Horizontal Section



Toxicant effects on mammary gland development



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

Genetically modified mouse (GMM) models

- First model of breast cancer in 1984 by Stewart *et al.*
- >100 mouse models addressing breast cancer
  - Transgenes
  - Combinations of transgenes
  - Targeted mutations (site-directed, knock outs, knock ins)
- Valuable experimental systems for molecular analysis of the transforming activity of oncogenes in the mammary epithelium

Gene targets for transgenesis

- Growth factors
  - FGF3 (INT2), FGF7 (KGF), Heregulin (ligand to EGF receptor), HGF, IGFII, TGF-α, β
- Growth factor receptors
  - TGF-β, Erb-B2 (neu), RET, Tpr-MET
- Signal pathways
  - PyV-MT, Ras
- Cell cycle regulators
  - Cyclin D1, c-Myc, p53, SV40-Tag
- Differentiation mediators
  - Notch (INT3), WNT1, WNT10b, P-Cadherin
- Other transgenes
  - Stromelysin (MMP-3) (ECM)

Mouse models of breast cancer

Advantages

- Defined genetic background
  - Allows study of particular pathways without interference due to differences in genotype
- Evolution and progression of breast cancer
  - premalignant **à** metastatic end-stage disease
- Develop disease after predictable time period
  - Stage specific alterations in oncogenic pathways or responses to therapy translatable to humans
- Genes over expressed/mutated in human breast cancer cause mammary tumors in mice

Mouse models of breast cancer

Advantages

- Produce lesions that mimic human disease
  - Her2/neu (ErbB2) **à** lobular carcinoma, DCIS
    - § Over expressed in 30% of human breast cancer
  - PyV-MT, c-src, c-myc **à** scirrhous carcinoma
  - PyV-MT **à** papillary adenocarcinoma
  - Wnt-1 (int-2) **à** acinar adenocarcinoma
  - BRCA1, SV40-Tag **à** medullary carcinoma and poorly differentiated carcinomas

Mouse models of breast cancer

Important comparative similarities

- Molecular lesions causing breast cancer in humans cause mammary cancer in GMM
- Similar morphology occurs in both species
- Development of cancer consistent with multi-hit kinetics
- Breast cancers in both species are metastatic
- Frequently hormone independent

Applicability to humans

NTP Workshop on human relevance of hormonally-induced reproductive tumors

•Held May 22-24, 2006

•55 invited participants in endocrinology, cancer biology, reproductive toxicology, and statistics - over 100 in attendance

•Addressed ovary, testis, prostate, and mammary gland

•Fibroadenoma not considered a premalignant lesion in humans

•Premalignant lesions (e.g. atypical hyperplasia) similar in rats and humans, not mice

•Estrogenic stimulation of importance in humans and rodents

•Role of prolactin less clear (recent evidence stronger)

•Recommended extended exposures (*in utero* and during puberty)

•"In the absence of an ideal model, the existing rodent models are ….useful for identifying a biological change and serve a useful screening function to identify potential carcinogens"

– Thayer, KA and Foster, PMD (2007) *Environ Health Perspect* 115:1351-1356.

Summary

- Traditional 2-year rodent cancer studies with “sensitive strains” identify mammary carcinogens
- Exposure during mammary gland development may increase sensitivity
- Rodent mammary carcinogens are eligible for listing in the NTP Report on Carcinogens
- Mammary cancer in rodents and breast cancer in humans are polygenic
- Mice with a variety of genetic modifications develop tumors resembling those of humans

Acknowledgements

- Sue Fenton, NTP
- Mark Hoenerhoff, NTP
- Robert Sills, NTP
- Ron Herbert, NTP
- John Latendresse, Pathology Associates