



**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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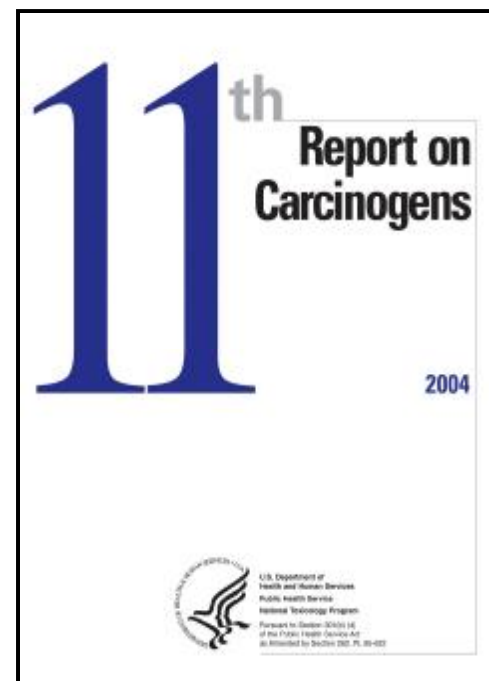


## Topics

- 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

**Substance Profile**

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

**Reasonably anticipated to be a human carcinogen**  
First listed in the *National Toxicology Program Carcinogen* (1985)

**Carcinogenicity**

Several mixtures of polychlorinated biphenyls, including Aroclor 1280 (1:1098-82-9), Aroclor 1254 (11097-69-1), and Kluveralon 500 (27319-11-2), are classified as potential human carcinogens based on a lifetime evidence of carcinogenicity in experimental animals (IARC 1979, 1981, 1987; Nishikawa and Waldman 1993). When administered in the diet, Aroclor 1267 induced liver tumors in young rats, and sarcomas in young rodents, single carcinomas, and cystic degeneration of the testis and hepatocellular carcinoma and liver adenocarcinoma in female rats. When rats were fed a diet containing a purely hepatotoxic and chlorinated Aroclor 1260 in an oral dose of 100 mg/kg body weight, hepatocellular carcinoma results in both sexes and benign and cystic thyroid adenomas, adenocarcinoma of the testis, and adenocarcinoma of the ovary. When administered in the diet, Aroclor 1254 induced hepatomas in male mice and Kluveralon 500 induced hepatocellular adenomas in male mice (Nishikawa and Waldman 1993; IARC 1979).

In one study, a lifetime exposure to the carcinogenicity of PCBs in humans (IARC 1993). A slight increase in the incidence of benign, primarily nodular, skin lesions has been reported in a small group of area residents occupationally exposed to Aroclor 1254. A study of 1,310 workers with a brief history of exposure to polychlorinated biphenyls in a cancer monitoring plant showed an excess of all cancers among men workers. The excess was mainly due to cancers of the digestive system and of the respiratory and hematopoietic systems (IARC 1993).

**Properties**

Theoretically, there are 203 possible polychlorinated biphenyl isomers, although not all are found in manufactured products. Polychlorinated biphenyls vary in appearance from mobile, oily liquids to white, crystalline solids to hard, noncrystalline resins. They are thermally stable, resistant to oxidation, acids, bases, and other chemical agents, and have excellent dielectric properties. Polychlorinated biphenyls are soluble in the pure form. Commercial products are liquids because of admixing with a dispersant when polychlorinated biphenyl are mixed. Polychlorinated biphenyls are practically insoluble in water and soluble in oil and organic solvents. Technical grade polychlorinated biphenyls have varying proportions of the different chlorinated isomers with small amounts of polychlorinated dibenzodioxins and polychlorinated dibenzofurans as contaminants (IARC 1979).

**Use**

Since 1974, at least 10 polychlorinated biphenyls in the United States have been commercial systems such as electrical capacitors and transformers, electrical insulators, and gas-insulated lines. Before 1970, polychlorinated biphenyls were used in transformer cooling liquids, heat-transfer and hydraulic fluids, vacuum pump fluids, ball bearings, plastic films, fillers in investment casting, paints, surface coating and sealants, pesticide containers, and cellulose cap paper (IARC 1979; Wood 1993). Currently, polychlorinated biphenyls are used by individual manufacturers for such applications as a mounting medium in microscopy, as an immersion oil in phase-contrast microscopy, as an optical liquid, and for sealants and dielectrics (IARC 2002).

**Production**

Polychlorinated biphenyls are no longer produced in the United States, except for limited research and development applications. Import and export of the compound have not been permitted since 1975. In 1974, the Monsanto Chemical Company, which manufactured 95% of the polychlorinated biphenyls used by U.S. industry, produced an estimated 40 million lb of polychlorinated biphenyl (775013-2-000). Domestic production reached a peak volume of 86 million lb in 1970 and decreased to approximately 11 million lb in 1979. Polychlorinated biphenyls were first produced commercially in the United States in 1929 (IARC 1979).

**Exposure**

The primary source of potential human exposure to polychlorinated biphenyls are ingestion, inhalation, and dermal contact. The routes of polychlorinated biphenyls from point industrial use and the persistence of the compounds in the environment have resulted in widespread contamination of waterways and, with a long and persistent history of its general population. Polychlorinated biphenyls have been listed as 16 hazardous air pollutants under the National Contingency Plan (NCP's) Toxic Chemical Release Inventory (TCRI) and under of federal law produced, processed, or otherwise used polychlorinated biphenyls in 1999. The health effects studies of polychlorinated biphenyls in humans, which were initiated to deal 10,000-227 lb (11,000-200) polychlorinated biphenyls have been found in meat, fat, milk, and in general diet. Concentration ranged from 6 to 462,000 µg/L. The National Organic Monitoring Survey conducted from 1975 to 1977 found polychlorinated biphenyls at 0% of ground water used for drinking water at levels of 0.1 µg/L. A major source of exposure is through the diet, fish, shell eggs, and commercial animal feed. The major U.S. commodities in which polychlorinated biphenyls have been found. Residues of polychlorinated biphenyls have been detected in human milk and fat samples collected from the general U.S. population (IARC 1979). In 1994, the average daily human food intake was estimated to be 0.027 µg/kg body weight, but declined to 0.021 µg/kg body weight at 1991 (ATSDR 2002).

Leakage in urban explosion or electrical explosions results in contamination of nearby areas. This occurrence can result in possible human exposure through inhalation of airborne polychlorinated biphenyls or contact with contaminated surfaces. Proper prevention and management of this fire can greatly reduce human exposure (IARC 1993). EPA estimates that approximately 18 million people within 16 miles of these facilities are at risk. Potential occupational exposure may possibly be exposed to releases of polychlorinated biphenyls in air. In 1977, NIOSH estimated that 22,000 workers and potential occupational exposure as a result of overall annual releases of 100,000 lb of polychlorinated biphenyls (IARC 1979). Additional exposure information may be found in the ATSDR Toxicological Profile for Polychlorinated Biphenyls (ATSDR 2002).

**Regulations**

**DOT**

PCBs are listed as hazardous materials and marine pollutants and also as hazardous waste for air transport by DOT and for marine pollutants (IARC 1979).

**EPA**

**CERCLA**

PCBs are listed as hazardous materials (IARC 1979).

**RCRA**

PCBs are listed as hazardous materials (IARC 1979).

**SDWA**

PCBs are listed as hazardous materials (IARC 1979).

**State Water Act**

PCBs are listed as hazardous materials (IARC 1979).

**Water Quality Criteria**

Based on the water quality criteria for drinking water (IARC 1979), the maximum contaminant level (MCL) for PCBs is 0.005 µg/L. Concentration of PCBs in drinking water should not exceed 0.005 µg/L. Based on the water quality criteria for drinking water (IARC 1979), the maximum contaminant level (MCL) for PCBs is 0.005 µg/L.

- 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.



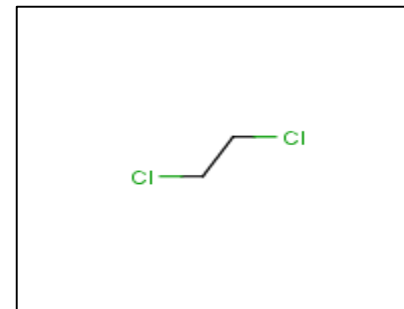
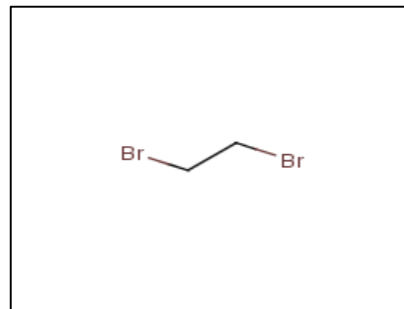
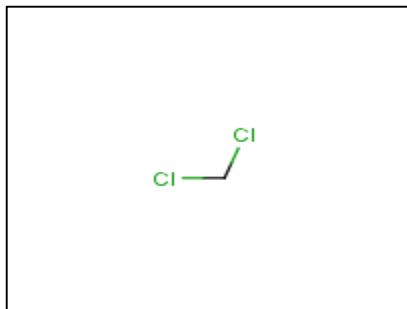
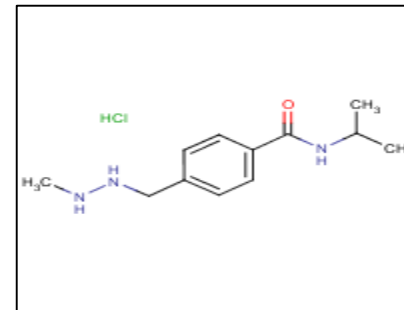
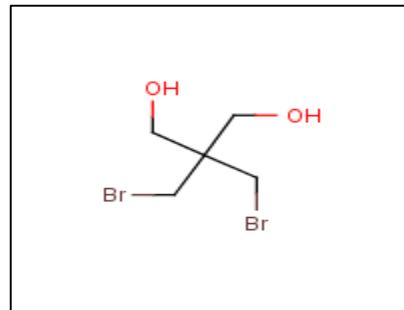
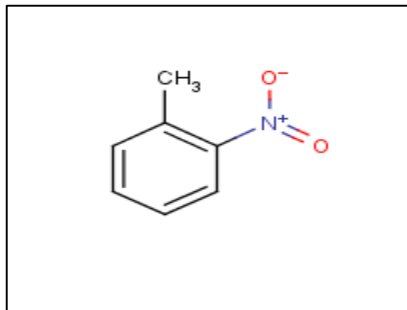
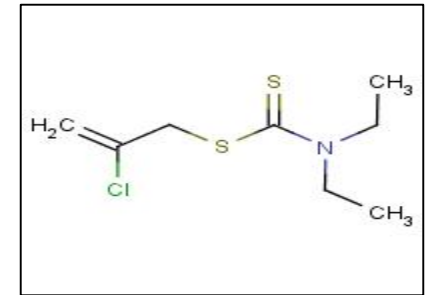
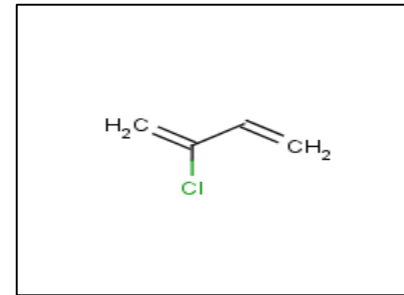
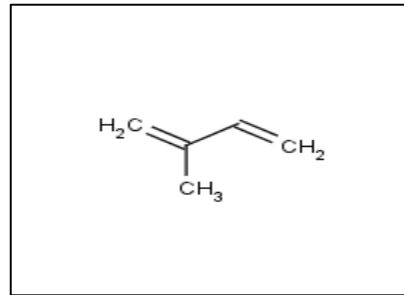
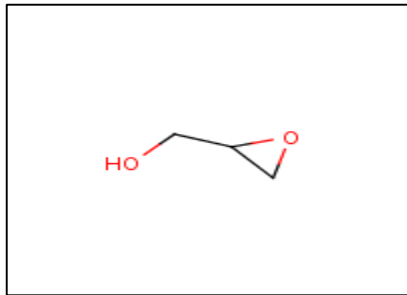
## **Listing criteria for the RoC (continued)**

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, sulfalate





## 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)

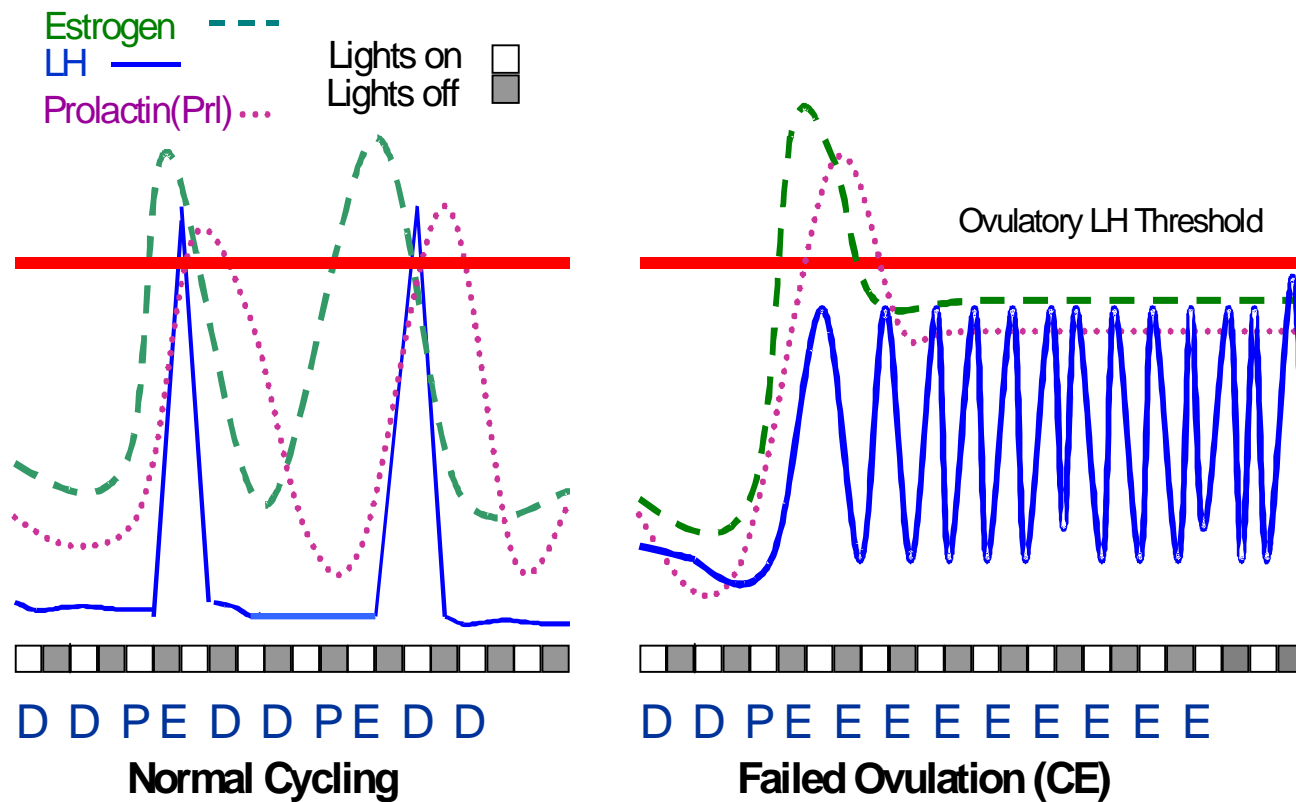


## “Strain specific” sensitivities for hormonal mammary carcinogens

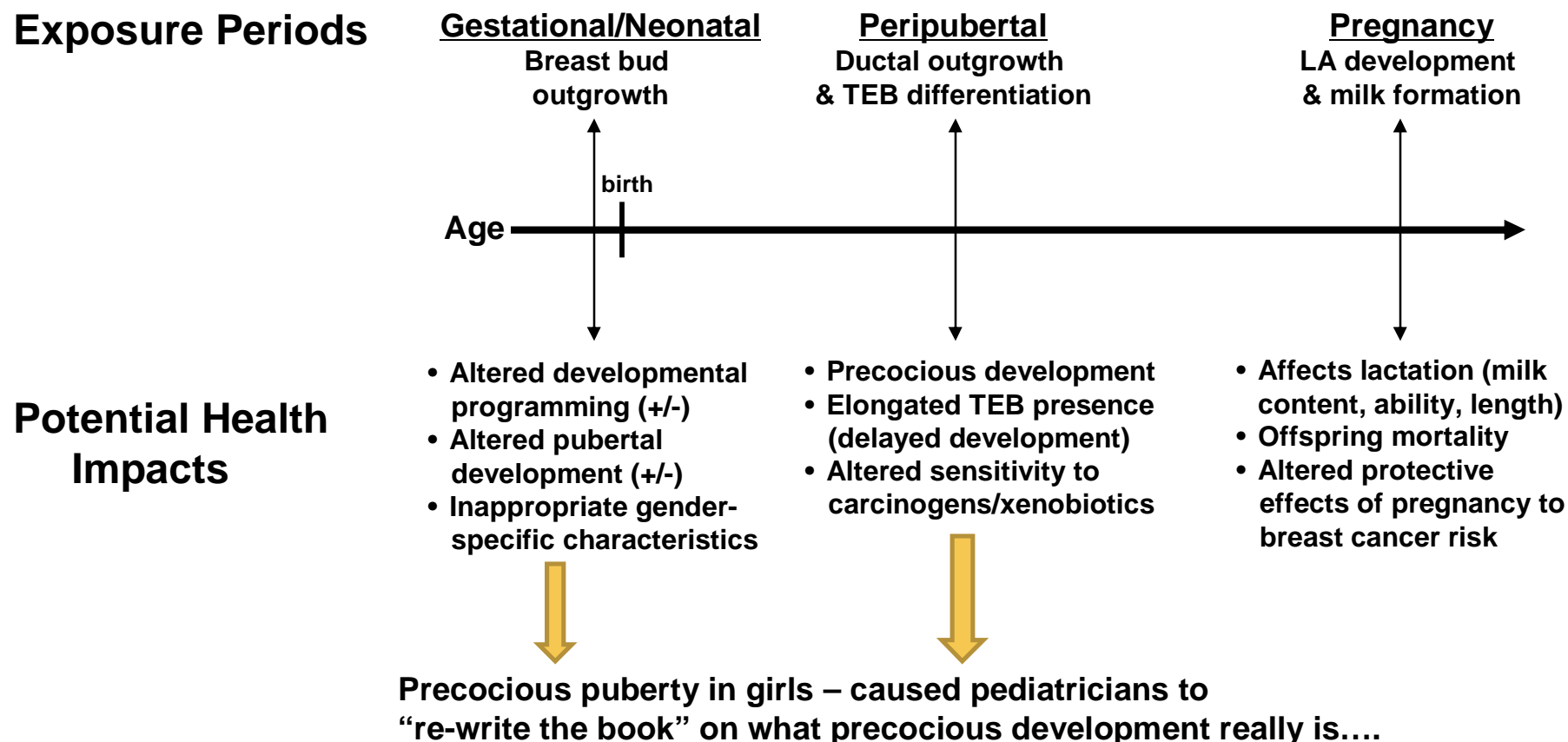
- Sprague Dawley (“susceptible”)
  - Estrogenic agents
  - Agents that accelerate reproductive senescence
  - Agents that increase prolactin
- Fischer 344 (“susceptible”)
  - Agents that increase prolactin
- Wistar Furth (“susceptible”) --- prolactin
- Wistar Han (“susceptible”)
- Wistar Kyoto (“resistant”)
- 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



from Fenton, 2006 *Endocrinology*

## Developmental events in human and rodent mammary tissue

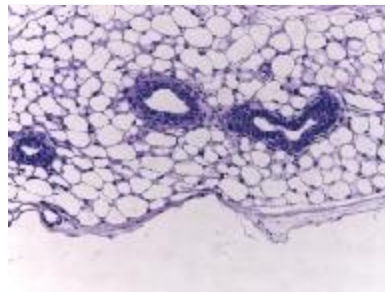
Developmental Event	Human	Rodent
milk streak evident	EW4-6	GD10-11 (mice)
mammary epithelial bud forms	EW10-13	GD12-14 (mice), GD 14-16 (rat)
female nipple and areola form	EW12-16	GD18 (mice)/GD20 (rat)
branching and canalization of epithelium	EW20-32	GD16 to birth (mice), GD 18 to birth (rat)
secretion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

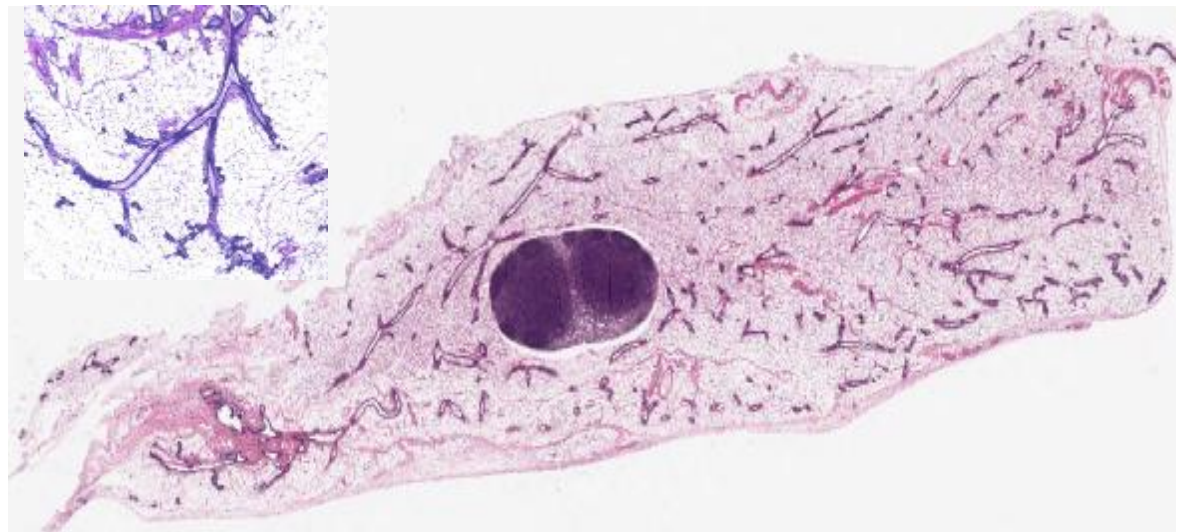
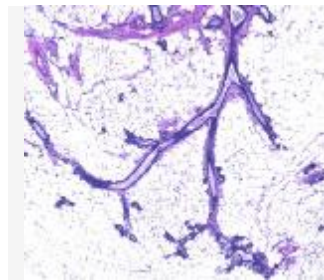


## Inguinal mammary gland sampling

Cross Section



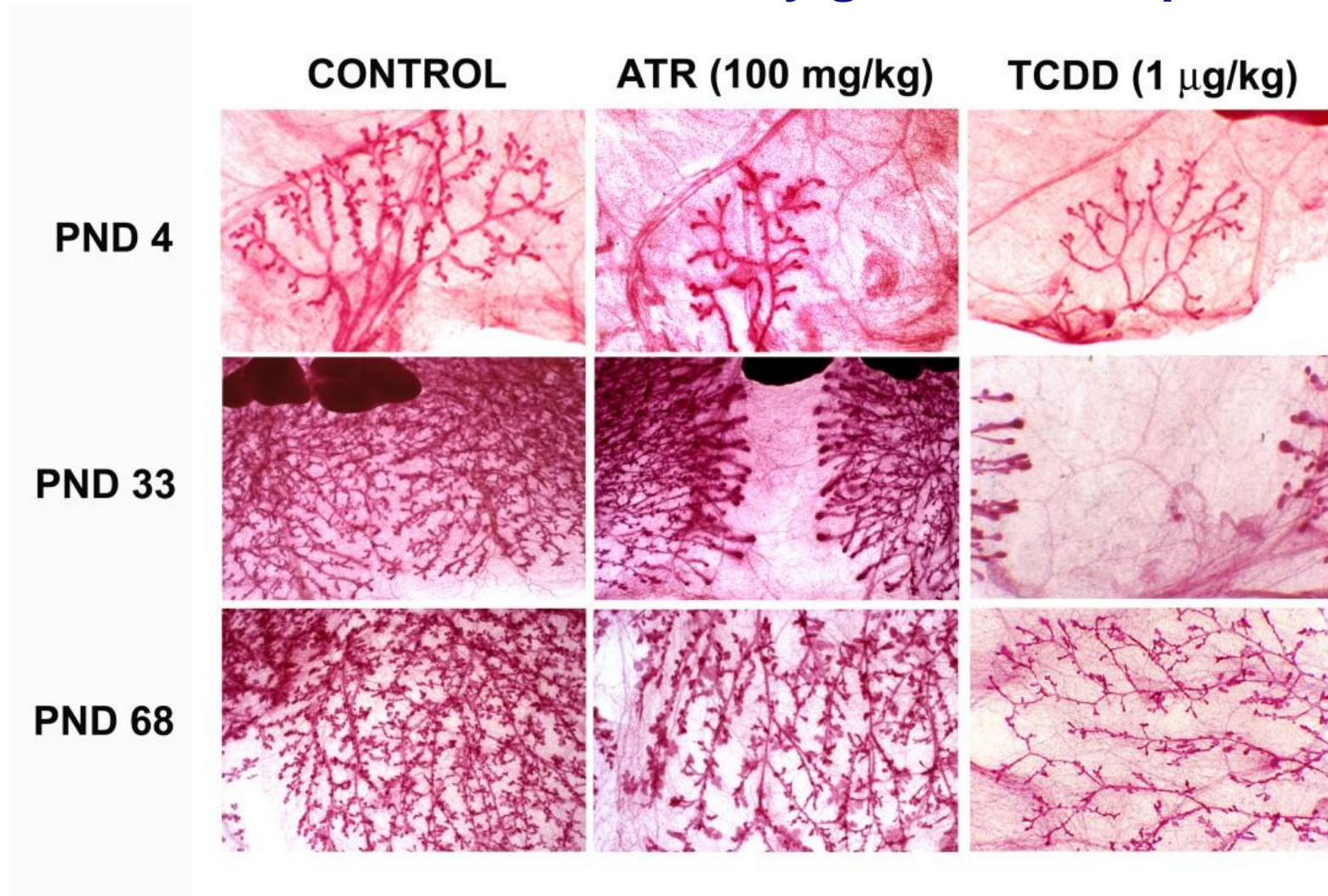
Horizontal Section







## 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- $\alpha$ ,  $\beta$
- Growth factor receptors
  - TGF- $\beta$ , 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 → 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”



## 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



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