Weighing Evidence from National Toxicology Program Cancer Bioassays

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Breast Cancer and the Environment
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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

Substance profiles are intended to describe the following: 1) carcinogenicity, 2) biological pathways, and 3) potential for human exposure. The information presented is based on the most recent data available.

Carcinogenicity

PCBs are suspected carcinogens in humans. The International Agency for Research on Cancer (IARC) has classified PCBs as Group 2B, which means that they are probably carcinogenic to humans.

Biochemical pathways

PCBs are known to interfere with the production of certain enzymes and disrupt the normal functioning of cells. They can cause liver damage, immune system suppression, and reproductive problems.

Potential for human exposure

PCBs are known to be bioaccumulative, which means that they can build up in the food chain. They can be found in fish, dairy products, and other foods that humans consume. Monitoring programs have been set up to monitor PCB levels in the environment.

Risk assessment

PCBs are known to be toxic to humans. They are suspected carcinogens and can cause liver damage, immune system suppression, and reproductive problems. They are bioaccumulative and can build up in the food chain.

Regulations

PCBs are regulated by various federal and state agencies. The United States Environmental Protection Agency (EPA) has set limits on PCB concentrations in waste and in the environment.

References


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.
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, sulflinate
Biology of mammary cancer in NTP studies

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

- Estrogen
- LH
- Prolactin (Prl)

Lights on: D D P E D D P E D D
Lights off: D D P E E E E E E E E E

Ovulatory LH Threshold

Normal Cycling

Failed Ovulation (CE)
Identified critical periods in mammary gland development

**Exposure Periods**

- **Gestational/Neonatal**
  - Breast bud outgrowth
  - Potential Health Impacts:
    - Altered developmental programming (+/-)
    - Altered pubertal development (+/-)
    - Inappropriate gender-specific characteristics

- **Peripubertal**
  - Ductal outgrowth & TEB differentiation
  - Potential Health Impacts:
    - Precocious development
    - Elongated TEB presence (delayed development)
    - Altered sensitivity to carcinogens/xenobiotics

- **Pregnancy**
  - LA development & milk formation
  - Potential Health Impacts:
    - Affects lactation (milk content, ability, length)
    - Offspring mortality
    - Altered protective effects of pregnancy to breast cancer risk

**Age**

- Birth

**Potential Health Impacts**

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

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

*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

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 → 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) ‡ lobular carcinoma, DCIS
    ß Over expressed in 30% of human breast cancer
  – PyV-MT, c-src, c-myc ‡ scirrhouis carcinoma
  – PyV-MT ‡ papillary adenocarcinoma
  – Wnt-1 (int-2) ‡ acinar adenocarcinoma
  – BRCA1, SV40-Tag ‡ 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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