SILENT SPRING INSTITUTE

RESEARCHING THE ENVIRONMENT AND WOMEN'S HEALTH

Integration of Mammary Gland Endpoints in Risk Assessment: Key Issues and Research Needs

Mammary Gland Evaluation and Risk Assessment Workshop

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Outline

- MG in context of other toxicology data
  - Risk assessment terminology
  - Case studies
- What is an adverse effect? A carcinogen?
- Key issues and research directions
  - Methodology for MG evaluation
  - Lactation
  - Cancer

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Important Toxicology Terms

- Non-cancer effects
  - No Observed *Adverse* Effect Level (NOAEL)
  - Lowest Observed *Adverse* Effect Level (LOAEL)
- Is it a carcinogen?
  - Weight of evidence, hazard identification
- EPA Integrated Risk Information System (IRIS)
- US National Toxicology Program (NTP)

  . . .where chemicals go to be tested
- Testing also done by industry using guideline studies e.g. pesticide regulation
- Registration, Evaluation and Authorization of Chemicals (REACH)

Key Toxicology Study Designs

- Two-year chronic cancer bioassay
  - Typically no in utero/early life exposure
  - Large number of animals per dose (50)
- Reproductive Toxicity
- Developmental Toxicity
- Multigenerational
  - Two generation
  - Reproductive Assessment by Continuous Breeding (RACB)
- EPA Endocrine Disruptor Screening Prog. (EDSP) Tier 1
  - Male and female pubertal
- OECD extended one generational reproductive (draft)

Case Studies – Genistein and EE2

- Estrogenic
- In utero exposure
  - accelerated MG development in pups, persistent changes
  - accelerated puberty markers
- New study design – NTP/NCTR - RACB with chronic cancer bioassay
  - MG whole mount data not reported
  - MG histopathology (Latendresse et al., 2009)
    - MG hyperplasia in males and not females
    - Did not observe feminization of male MG
    - EE2 more potent (2 ppb) but Gen shows similar effects (500 ppm)
    - MG hyperplasia more prominent at 140 days vs. 2 years
    - Hyperplasia appears reversible, does not lead to neoplasia
    - Hyperplasia also observed with adult only exposure, but at higher dose (EE2 50 ppb vs 2 ppb)

Dioxin

- Ah receptor agonist
- In utero/lactational exposure
  - Impaired male repro tract develop (0.05-0.075 microgram/kg)
  - Delays/stunts MG differentiation/development in dams and offspring. Persistent change. ~1 microgram/kg
  - Increase MG tumors in offspring after DMBA
  - Delays puberty in offspring (VO)
  - ↑ Ov tumors
- Postnatal or adult exposure
  - Impaired fertility
  - Decreased MG tumors
  - Diminished MG tumors following DMBA

In Utero Dioxin + DMBA

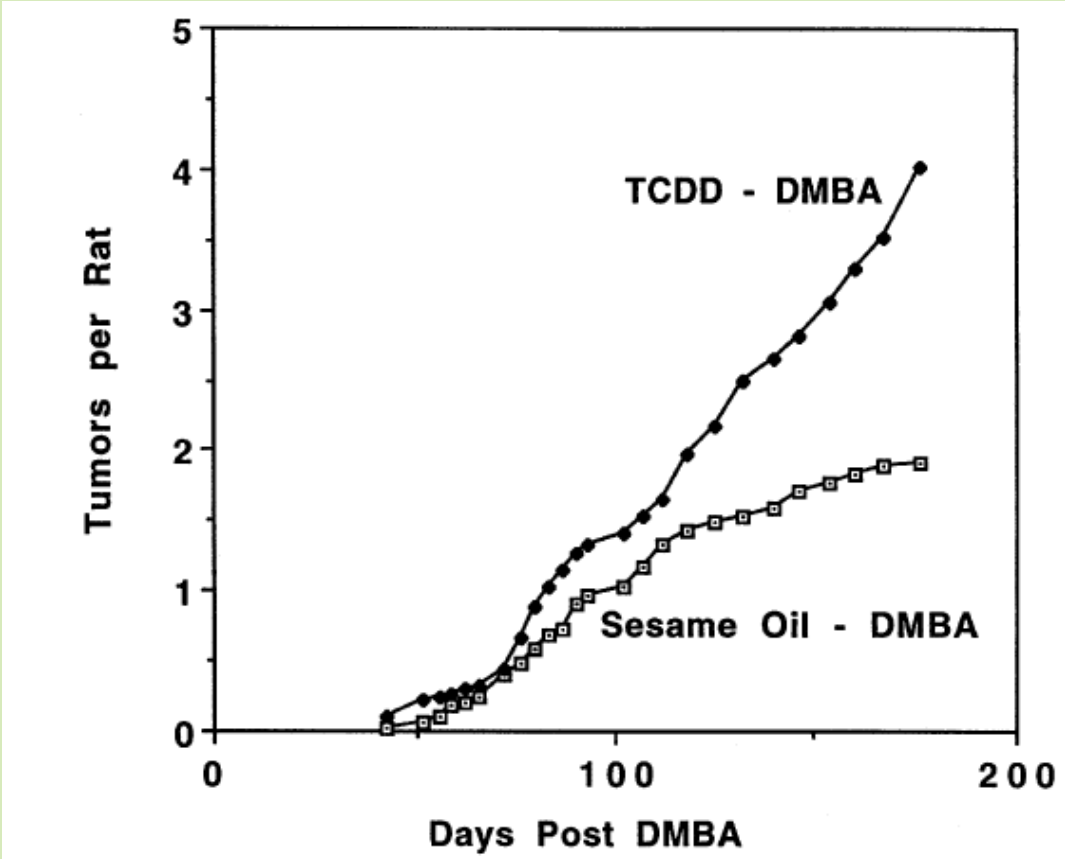


Fig. 2. Ontogeny of palpable mammary tumors in female rats exposed prenatally to TCDD and treated with DMBA on day 50 post-partum.

Brown and Lamartiniere, 1998

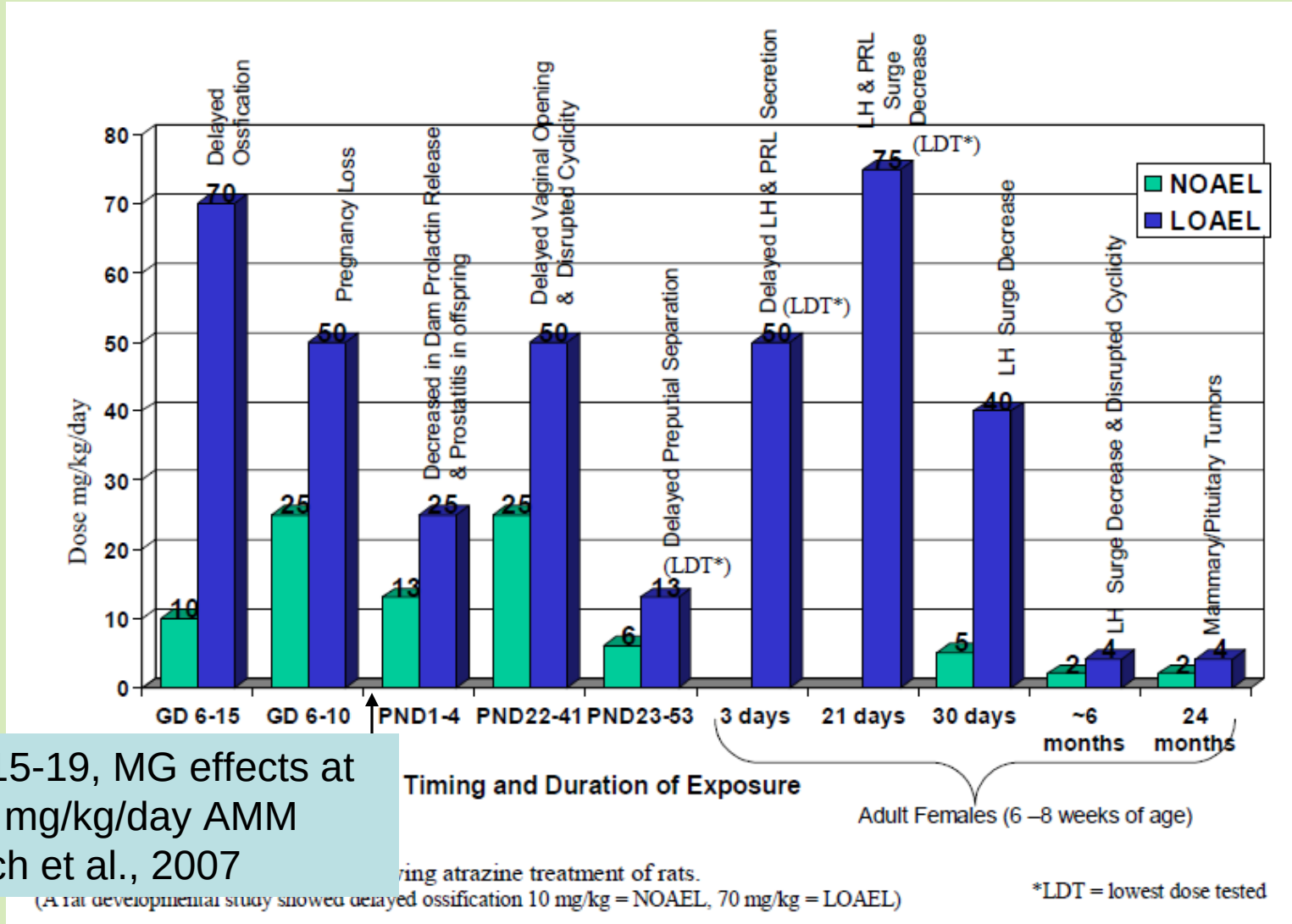
Bisphenol A

- Estrogenic (and also . . .)
- Exposure route affects dose required for effect
- In utero/early life exposure in mice (subcut)
  - Increased branching and alveolar buds
  - Hyperplasia, carcinoma in situ
  - ↑ sensitivity to estradiol
  - ↑ PR in epith cells at puberty
  - ↓ apoptosis
  - Incidental finding of mammary adenocarcinoma
- MG effects at lower dose than puberty markers
- July 2009 meeting of CA Prop 65 DARTIC
  - Not enough evidence that BPA causes reproductive toxicity
  - Ask OEHHA to look further at evidence that bisphenol A exposures in utero or pre-conception may lead to precancerous lesions and eventually cancers (e.g., breast and prostate)

Atrazine

- Neuroendocrine MOA (rat)
  - Suppresses LH surge from pituitary
  - Cumulative MOA for triazines and their metabolites
- 6-mo rat study 1.8/3.8 mg/kg-day = NOAEL/LOAEL for LH suppression
- Affects fertility, cyclicity, pubertal timing

Atrazine



GD 15-19, MG effects at 0.09 mg/kg/day AMM
Enoch et al., 2007

EPA 2006 Triazine Cumulative Risk Assessment

Atrazine Metabolite Mixture MG Whole Mounts

- In utero exposure, LE rats
  - persistent MG changes in offspring at 0.09 mg/kg-d and higher – delayed/stunted dev.
  - ↑ pituitary weight
  - ↑ pituitary prolactin, but no change LH
  - no change in VO at these doses
- Impaired lactation in F2 at higher doses?
- EPA risk assessment permits 0.018-0.1 mg/kg-day

Perfluorooctanoic Acid (PFOA)

- PPAR-alpha agonist; thyroid, other MOA possible
- Mouse metabolism better mimics human
- In utero exposure - mouse
  - decreased pup wt and survival
  - diminished differentation/growth of dam MG
  - Milk protein gene expression findings difficult to interpret
  - Stunted MG branching and growth in pups, mult. strains
  - No effect on MG in non-pregnant adult mouse
- Chronic cancer bioassay (rat) – mammary fibroadenomas**, Leydig adenomas

Dibutyl phthalate

- Anti-androgen/impairs T synthesis
- Effects on developing male reproductive system – AGD, T, retained nipples, etc.
  - US EPA IRIS NOAEL 30 mg/kg-day for fetal T
- Cal EPA LOAEL 1.5-3 mg/kg-day
  - Prenatal exposure study
  - Histopathol. effects on male and female MG

Hmm, so what?

- They are not all estrogenic
- May be most sensitive effect in some cases – more data needed
- Sometimes it affects dam, sometimes offspring, sometimes both
- Still don’t know much about relationship with impaired lactation, pubertal timing, cancer

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What is adverse?

- “A biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces the organism’s ability to respond to an additional environmental challenge.” (US EPA IRIS)
- OECD Guidelines
  - Altered pubertal timing (preputial separation, VO)
  - Anogenital distance

What is a carcinogen?

- **Carcinogenesis:** The origin or production of a benign or malignant tumor. The carcinogenic event modifies the genome and/or other molecular control mechanisms of the target cells, giving rise to a population of altered cells (EPA IRIS).
- Is something that alters susceptibility to carcinogenic insult a carcinogen?
- How to evaluate/demonstrate relationship of the MG changes to carcinogenic susceptibility

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Future Work: Methods for MG Evaluation

- Standardized protocol
  - Define procedures
  - Clear definitions of structures
  - Clear description of scoring/evaluation methods
  - Reliability, consistency
- Greater clarity on mechanisms that give rise to MG changes and the potential significance of the changes
- Define endpoints/procedures:
  - Whole mounts
  - Histology, immunohistochemistry
  - Early biomarkers of effect (e.g. gene expression)

Future Work: Relationship of MG changes and pubertal timing

- Adding MG evaluation to developmental repro tox studies will elucidate relationships between MG changes and
  - Timing (VO, preputial separation, first estrus)
  - Cyclicity

. . . and may help elucidate strain/species differences

Future Work: Relationship of MG Changes and Lactation Effects

- What are options for assessing effects on lactation?
  - Dams and F1s can be affected
  - Pup weight and survival
  - Milk spots in pups
  - Milk proteins, milk protein gene expression

Future Work: Relationship of MG Changes and Cancer

- Possible intermediate markers of cancer
  - Hyperplasia
  - Other immunohistochemical markers
  - Gene expression changes
  - Presence of nodules and bridging
  - Laser capture microdissection to select single cell types
  - Changes in hormone levels, receptor levels, receptor sensitivity
  - Effects on stromal-epithelial interactions
  - Other?

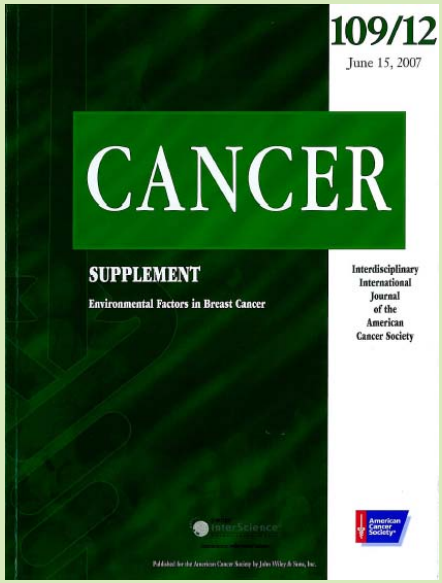
Future Work: Relationship of MG Changes and Cancer (cont.)

- Approaches
  - Chronic cancer bioassay with in utero exposure, take tissues longitudinally
  - Look at MG from F1 in multigen studies. Keep F1s alive and examine MG tissues. Can look for tumors but small n for that.
  - Carcinogen challenge experiments (e.g. DMBA)
  - Examine MG tissue blocks held from chronic studies of chemicals that did cause MG tumors – may indicate whether we can see tissue changes from these chemicals early, and what sorts of changes we see

What have we learned from the two-year chronic bioassay about chemicals that cause MG tumors?

Useful to review these, since the same issues will emerge as work progresses to tie MG changes to cancer

Animal Mammary Gland Carcinogens



216 chemicals

- 29 High Production Vol. (>1 million lbs/yr)
- 35 air pollutants
- 73 in consumer products
- 25 had > 5,000 women workers exposed
- 10 were food additives
- 47 pharmaceuticals (17 hormones)

Rudel et al., 2007 Cancer

methylene chloride
CAS RN 75-09-2

Originating list
Carcinogenicity Potency Database, National Toxicology Program studies, IARC Monographs, Chemical Carcinogenesis Research Information System

Associated chemicals
none

Major use
Chlorinated solvent

Widespread exposure
More Likely

Human exposure summary
Widespread exposure occurs during the production and industrial use of methylene chloride and during the use of a variety of consumer products containing it. Consumer products that may contain the chemical include: fabric cleaners, furniture polish, paint strippers, wood sealant and stains, spray paints, adhesives, shoe polish and art supplies (SRD). Used until 1989 as a propellent for hair spray. Substantial losses to the environment lead to ubiquitous low-level exposures from ambient air and groundwater (IARC 1999 vol.:71 p.25, NTP 11th ROC).

Image from the National Library of Medicine

methylene chloride
CAS RN 75-09-2

Chemical Summary | Exposure and Risk Assessment | Cancer Studies

Cancer studies: Experimental details

National Toxicology Program Technical Report 306, 1986

Link
http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr306.pdf

Notes
"Clear evidence" in rats is based on the mammary tumors. Rats: 0, 1000, 2000, 4000 ppm:benign mammary tumors in female rats: 5/50, 11/50, 13/50, 22/50. In male rats, they looked at the combined benign mammary tumors and the integumentary ones, for significant increase. In Discussion, for mammary tumors they note Burek 1980 and 1984 for mammary tumors, Nitschke 1982. For"negative" they note National Coffee Association studies of 1982 and 1983 in which much lower levels were used (highest was 250 mg/kg).

Route	Species	Sexes	Strain
inhalation	Rat, mouse	M,F	F344/N rats, B6C3F mice

Doses
Rats: 0, 1000, 2000, 4000ppm, Mice: 0, 2000, 4000ppm; for 6 hrs/day, 5 days/wk for 102 weeks. 50 animals in each group. Age exposure started 8-9 weeks for rats, 7-8 weeks for mice.

Time after cessation of dosing
1 week

Likely human relevance indicated by mutagenicity, multi-species tumors

- 93 of 132 IARC chemicals - “sufficient” evidence in animals
- 84% with some evidence of mutagenicity
- CPDB analysis – multi-species carcinogens
  - 91% MG carcinogens in rats also caused tumors at some site in mice
  - 89% MG carcinogens in mice also caused tumors at some site in rats

Risk assessments ignore MG tumors

- 20 of 216 chemicals have EPA slope factor
  - ➢3 of 20 are based on MG tumors
- 31 of 216 are in NIOSH list of potential carcinogens
  - ➢9 of 31 list MG as a tumor site in animals
- 11 of 221 have OSHA-required medical surveillance
  - ➢0 of 11 require mammography
- High-profile risk assessments don’t mention breast cancer or mammary gland tumors

  e.g., diesel exhaust, disinfection byproducts

Issues related to interpretation of mammary gland tumors

- Inconsistent treatment of fibroadenomas
- High background fibroadenomas in rats
- Rodent/human diffs. in hormonal regulation
- Treatment-related weight loss

Summary of Priority Research Areas

- Standardize MG evaluation methods
- Identify early effect markers for impaired lactation, cancer
- Explore relationships between altered MG development and effects on pubertal timing, impaired lactation, cancer
- Risk assessment
  - Use of upstream indicators of adverse effects
  - Exposures as risk factors for cancer, rather than carcinogens

Advocate!
Add MG Evaluation to Test Protocols

- OECD Extended One Generation Reproductive Toxicity
  - Comments on draft guideline due Dec 9, 2009
    - Don Bergfelt, US EPA, Bergfelt.Don@epa.gov
    - Christina Augustyniak, US EPA, Augustyniak.Christine@epamail.epa.gov
- EDSP Pubertal
- Other?

"…Certainly for the generations yet unborn, prevention is the imperative need."

--Rachel Carson, 1962