Mammary Gland

Mammary Gland – "Low" Dose Effects

- 2 reports of "pre-neoplastic" lesions in mammary gland (Durando *et al*. 2007, Murray *et al*. 2007)
- Mammary gland identified as BPA target tissue in other other studies
  - Increase in undifferentiated structures, altered rate of maturation
    - sc route of administration to pregnant rodents
  - New oral dosing study reported effects on undifferentiated structures mammary gland (Moral *et al*. 2008)
- Human correlate lesions have been described as risk factors for invasive breast cancer in women

Mammary Gland Lesions (Durando *et al*. 2007)

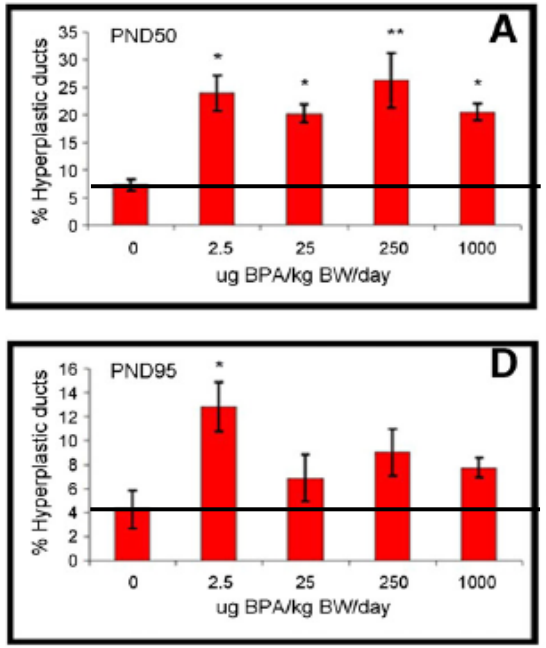
- Wistar-derived rats
- 25 μg/kg/day BPA (sc mini-pump in dam GD8-23; 11-14 litters/group)
- Ductal hyperplasia and carcinoma *in situ*

Treatment			Results	
In utero	**NMU (μg/kg)**	**Day of sacrifice**	**Hyperplastic ducts (%)**	**Tumor incidence**
control	----	PND 110	8.2 ±1.5[d]	0/6
25 BPA	----	PND 110	18.0 ± 3.2[e]	0/5
			↑ 2.2-fold	
control	---	PND180	3.2 ± 1.3[f]	0/6
25 BPA	---	PND180	16.2 ± 2.3[e]	0/6
			↑ 5.1-fold	
control	25	PND180	15.7 ± 1.2[e]	0/10
25 BPA	25	PND180	35.5 ± 3.7[g]	2/15
			↑ 2.3-fold	carcinoma *in situ*

Modified Table 3 from: Durando M *et al.* (2007) Environ Health Perspect. Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. 11580-86.

Mammary Gland Lesions (Murray *et al*. 2007)

- Wistar-Furth rats
- 2.5 – 1,000 μg/kg/day BPA (sc mini-pump in dam GD9 – PND 1; 4 - 6 litters/group)
- Ductal hyperplasia and carcinoma *in situ*

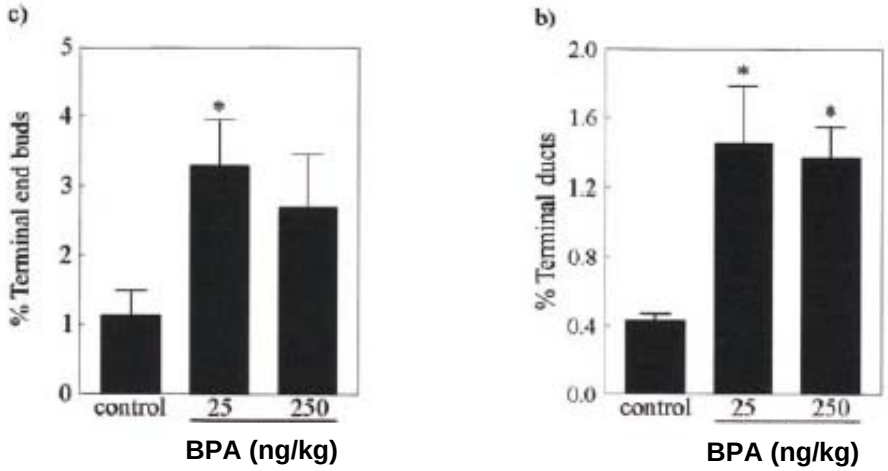


Dose (ug/kg/day)	Carcinoma *in situ*	
	PND50	PND95
0	0	0
250	1/4	1/4
1000	2/6	2/6

Modified Figure 2 from: Murray TJ *et al*. (2007) Reprod Toxicol. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. 23(3): 383-390.

Mammary Gland - Supporting Information (Markey et al. 2001)

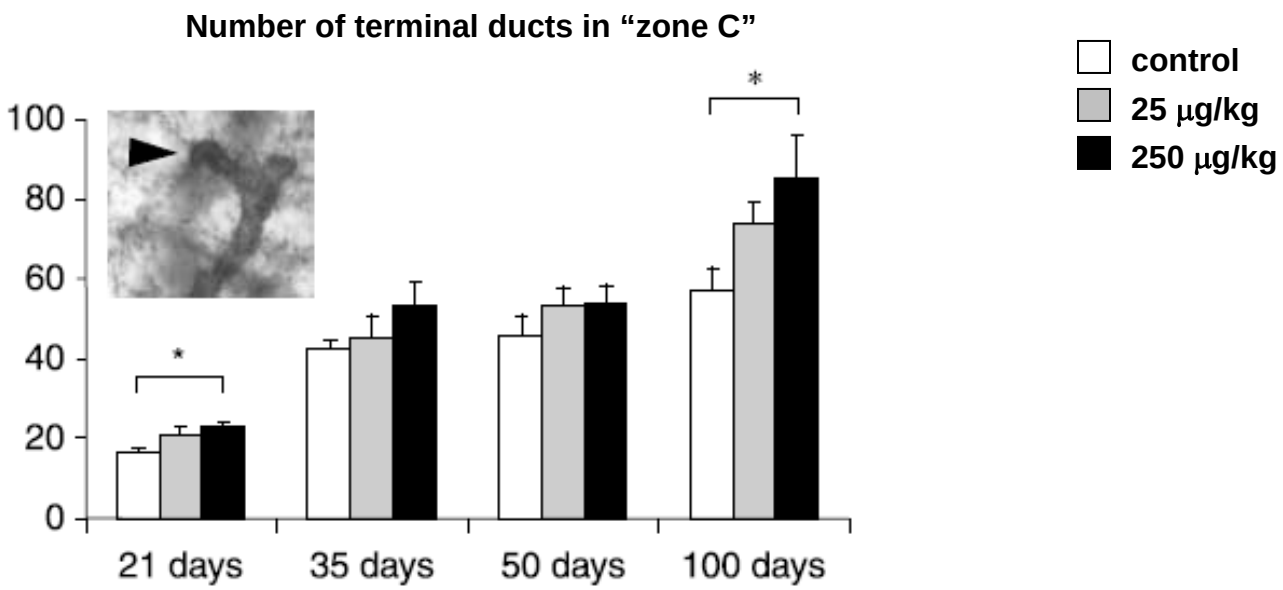
- CD-1 mice
- 25 and 250 ng/kg/day BPA (sc mini-pump in dam GD9-PND1; 6-10 litters/group)
- Increased terminal end buds and terminal ducts



Modified Figure 3 from: Markey CM *et al*. (2001) *Biol Reprod.* In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. 65(4): 1215-1223 [Erratum: Biol Reprod 2004;1271:1753].

Mammary Gland - New Supporting Information (Moral *et al*. 2008)

- Sprague-Dawley rats
- 25 and 250 μg/kg/day BPA (oral to dam GD10 – PND21; 10 litters/group)
- Increased number of terminal ducts at 250 μg/kg/day and modified gene signature at both doses



Modified Figure 1 from: Moral R *et al*. 2008. Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. J Endocrinol 196(1):101-112.

Mammary Gland - Reproducibility

- These effects may not have been detected in guideline compliant studies
  - Not assessed, including in rat multigenerational study by Tyl *et al*. 2002
  - No whole mount preparation in mouse (Tyl *et al*. 2008) or rat multigenerational (Ema *et al*. 2001)
- NTP 2-year bioassay did not report effects mammary gland tumors in BPA-treated female rats or mice
  - Did not include perinatal exposure or whole mounts

Mammary Gland - Data Limitations

- Unknown if lesions would progress to cancer
- Underpowered statistically to detect differences in incidence of carcinoma *in situ,* i.e., Murray *et al.* (2007)
- Murray *et al.* (2007) tested a wide dose range but did not see a clear relationship between dose and percent hyperplasic ducts
- sc route of administration to adult animals only useful for hazard identification

Mammary Gland – CERHR Expert Panel Criticisms of Study Design

- The CERHR Expert Panel considered the key studies "inadequate" [Durando *et al.* 2007, Murray *et al.* 2007]
  - Not based solely on route of administration

Mammary Gland – CERHR Expert Panel Criticisms of Study Design

- 100% DMSO as vehicle in mini-pump (Durando *et al*. 2007)
  - > 50% DMSO not recommended by manufacturer
    - NTP not convinced that 100% DMSO can account reported effects
    - Authors report no indication of DMSO toxicity
    - Does raise concerns on accuracy of administered dose
- Small sample size (Murray *et al*. 2007)
  - Durando *et al*. 2007 was larger
- Control for litter effects (Murray *et al*. 2007)
  - Author clarified use of 1 animal/litter in histological examination
- Route of administration for both studies

Weight of Evidence for Mammary Gland

- **Several studies identified mammary gland as target**
- **Absent or limited assessment in guideline studies**
- **New data using oral route**
- **Additional technical information from author**

- **"Preneoplastic" progression?**
- **Small sample size to detect carcinoma *in situ***
- **Use of sc mini-pump**
- **Use of >50% DMSO in sc mini-pump**

Clear evidence of adverse effects

Some evidence of adverse effects

Limited evidence of adverse effects

Insufficient evidence for a conclusion

Limited evidence of no adverse effects

Some evidence of no adverse effects

Clear evidence of no adverse effects

CERHR Expert Panel

- The CERHR Expert Panel did not have a conclusion related to the mammary gland
  - Considered a research need

Questions and Discussion