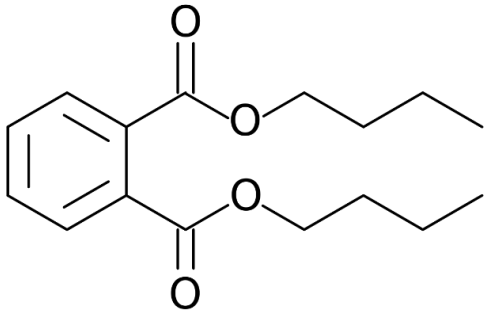
NTP
National Toxicology Program

Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Di-n-butyl Phthalate (CASRN 84-74-2) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice



Mimi Huang, PhD, Study Scientist
Mark Cesta, DVM, PhD, DACVP, Study Pathologist
Division of the National Toxicology Program, National Institute of Environmental Health Sciences

Cynthia Willson, PhD, DVM, DACVP, Study Pathologist
Integrated Laboratory Systems, LLC

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Dibutyl phthalate (DBP) is a common environmental contaminant





- Use: Plasticizer, manufacture of latex adhesives, varnishes and solvents, vinyl fabrics and flooring, personal care products, pharmaceuticals, food packaging
- Human exposure: 1-10 µg/kg/day
  - Primarily through food; some inhalation and dermal
- Rapid metabolism to monobutyl phthalate (MBP) in the gut
  - Broad distribution, minimal accumulation, urinary excretion

Study Rationale

- Previous hazard assessments and carcinogenicity assessments in animals of phthalates have not evaluated the effects of combined early life and postnatal exposures
  - Smith et al. 1953: Male rats, 1 year exposure, adult exposure only
  - Barlow et al. 2004: Male SD rats, 6-18 months exposure, prenatal exposure only
- Dibutyl phthalate (DBP) was evaluated in the NTP 2-year rodent carcinogenicity assay
  - Given that developmental toxicities are associated with prenatal DBP exposure in rodents and given widespread exposure in women of child-bearing age, perinatal exposure was included in the rat study

Dose Selection Rationale

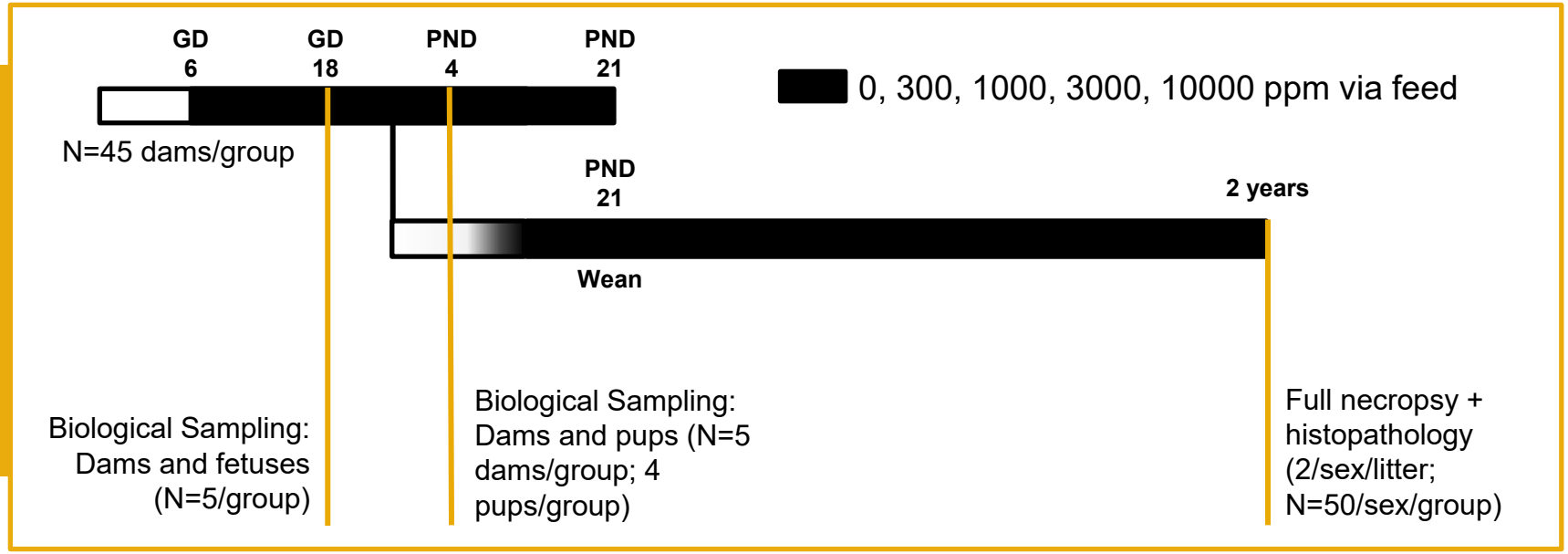
Findings from subchronic feed studies (TOX 30)

- Rat (F344/N) Perinatal dose-range finding study
  - 0, 1250, 2500, 5000, 7500, 10000, 20000 ppm *in utero* exposure
  - High mortality of pups at 20,000 ppm, slight decreases in number of live pups/litter and pup body weight at 10,000 ppm
- Mice (B6C3F1/N) 13-week study
  - 0, 1250, 2500, 5000, 10000, 20000 ppm
  - Survival unaffected up to 20000 ppm
  - Significant decreases (6-15% of control) in BW at ≥5000 ppm

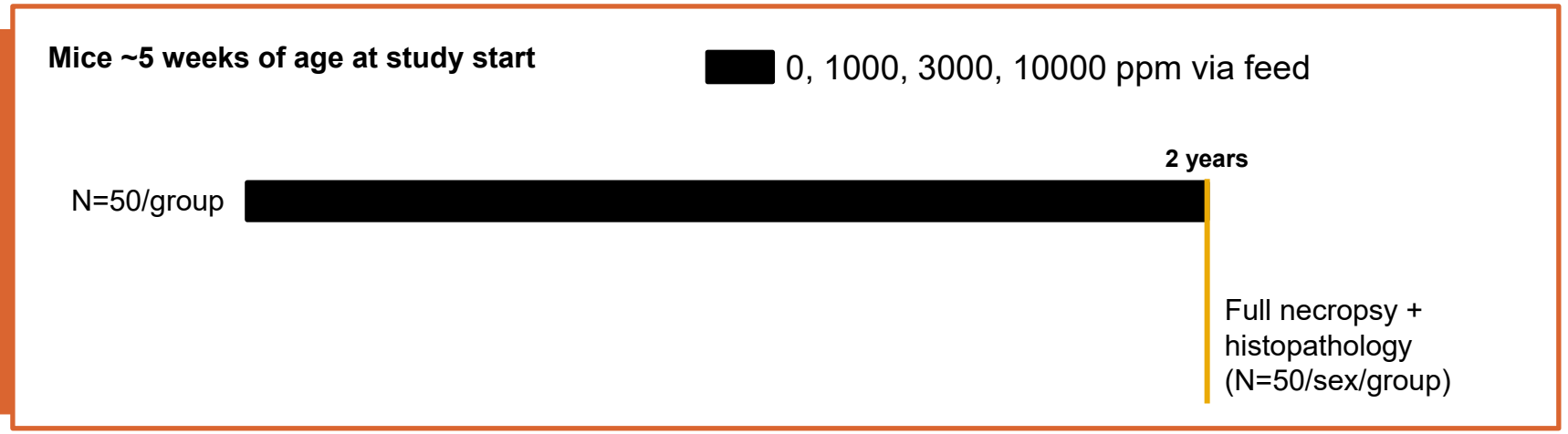
Top dose of 10000 ppm for both mice and rats
Low dose of 300 ppm included in rat study for reproductive effects

Study Design

Hsd:SD Rats



B6C3F1/N Mice



Results: Chemical Consumption

DBP consumption (mg/kg/day; mean)

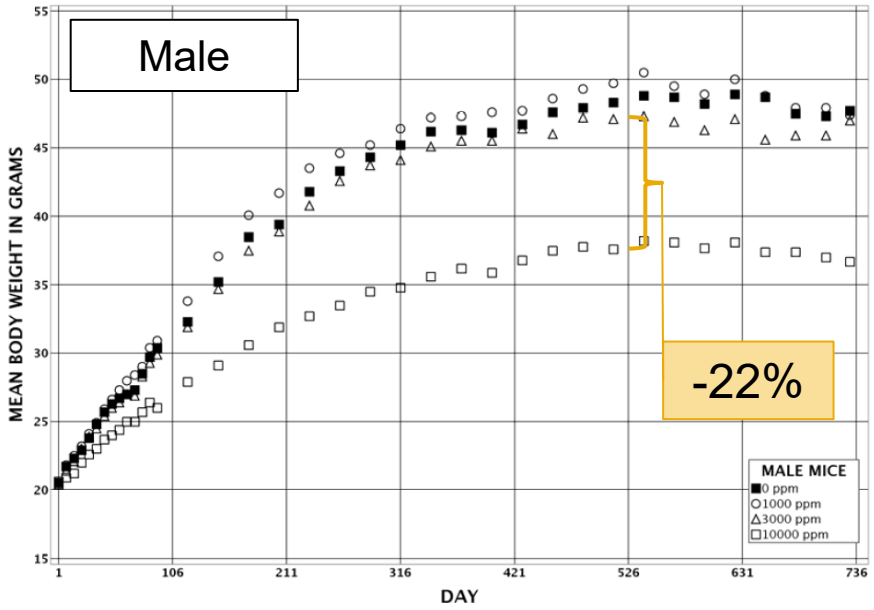
Study Phase	300 ppm	1000 ppm	3000 ppm	10000 ppm
Dams: Gestation	20	105	205	713
Dams: Lactation	52	174	522	1705
Male Rats	16	53	152	510
Female Rats	17	57	169	600
Male Mice	NA	112	347	1306
Female Mice	NA	105	329	1393

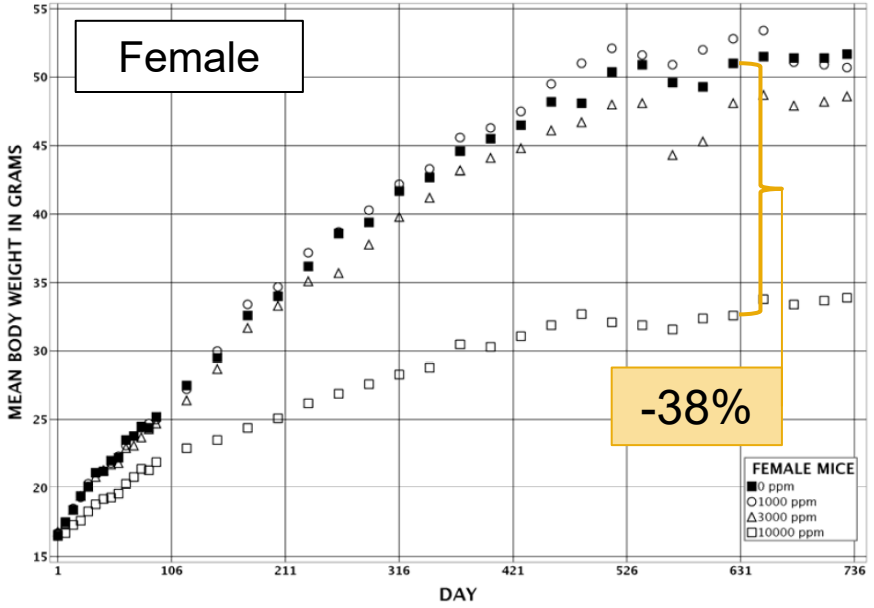
- Similar across sexes
- Approximately twice as high in mice
- DBP measurements in control feed were below limit of quantitation

Results: Mice

Survival and in-life

- No exposure-related differences in survival
- Body weight of most treated groups was within ~20% of controls
  - Female mice 10000 ppm terminal body weight was 35% lower than controls
- No exposure-related clinical observations





Results: Mice

Non-neoplastic findings

- Increased incidences of microscopic lesions in the male reproductive system at the top dose only (10000 ppm; ~1300 mg/kg/day)
  - Testicular degeneration, epididymal exfoliated germ cells
- Increased incidences in the liver:
  - Cytoplasmic alteration in male and female mice at the top dose
  - Multinucleated hepatocytes in male mice
- Renal tubular hyperplasia in female mice only
  - Observed in previous studies of peroxisome proliferators

Results: Mice

Neoplasms

- No exposure-related increases in incidences of neoplasms

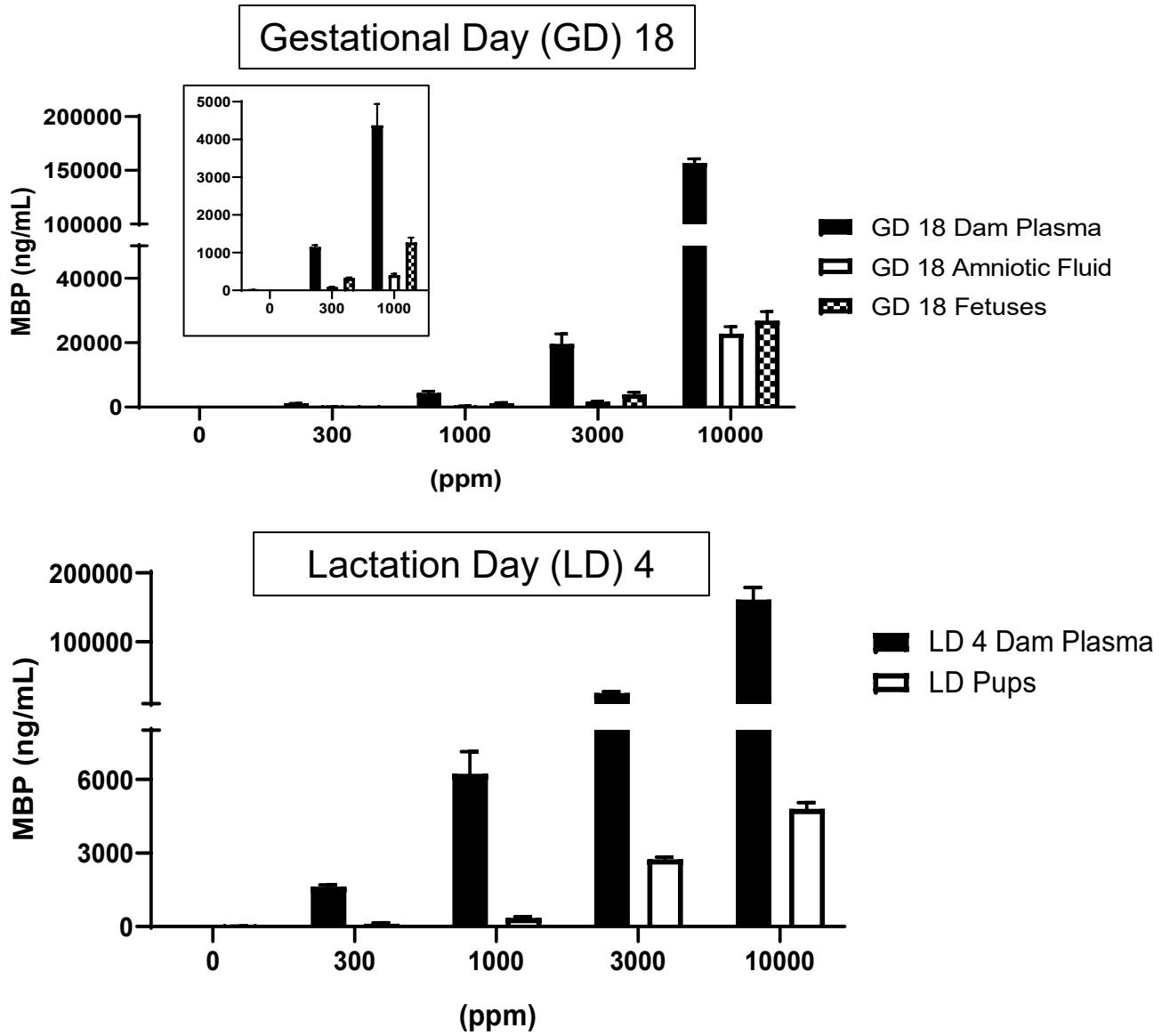
Results: Rats

Perinatal phase

- No significant differences in mortality or dam body weight during gestation or lactation
- No significant differences in gestation length, litter size, sex ratio
- Male and female pup body weight in top dose group (10000 ppm) was 12-13% lower than controls

Results: Rats

Internal concentrations of DBP metabolite, MBP

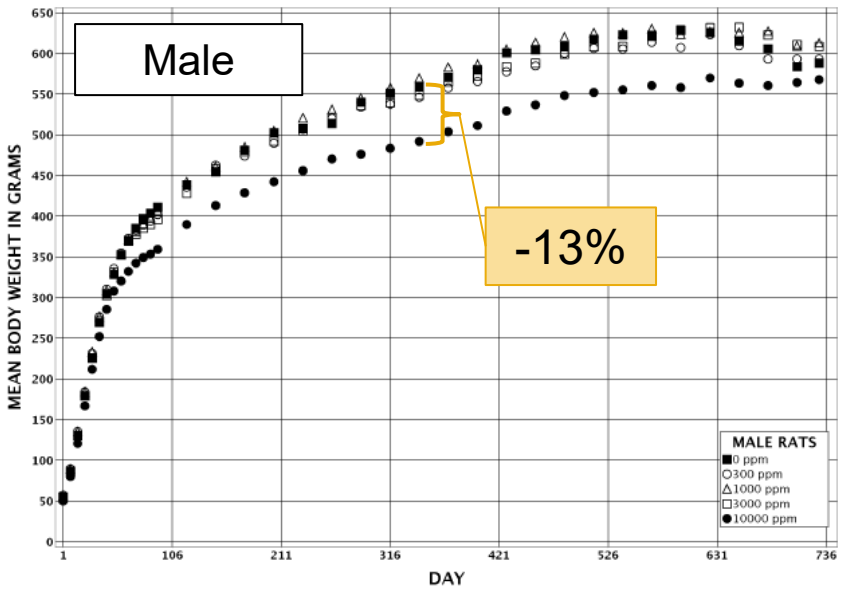


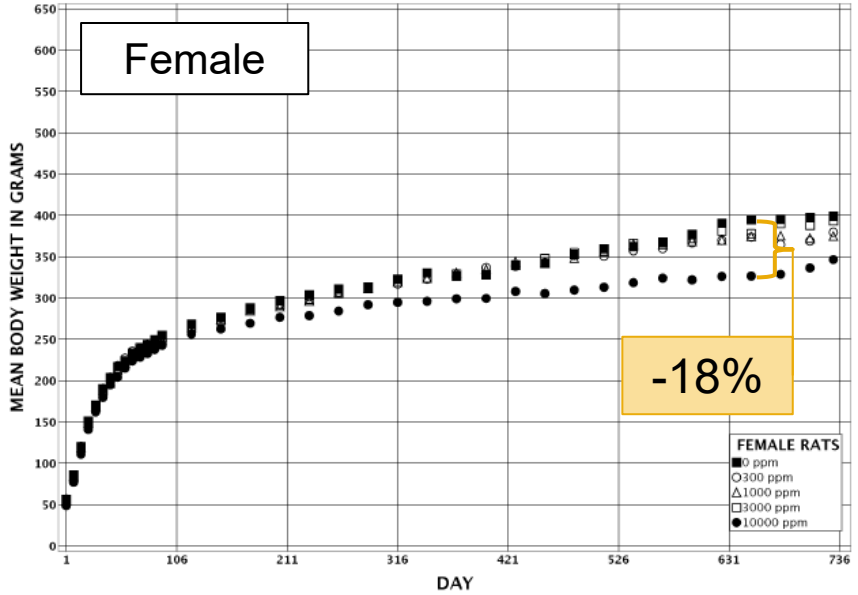
- Non-linear increases in MBP internal concentrations
- Moderate gestational transfer was observed
- Low lactational transfer was observed

Results: Rats

Chronic Phase

- No exposure-related differences in survival
- Body weight of all treated groups was within ~20% of controls
- No exposure-related clinical observations





Results: Rats

Non-neoplastic findings: Male reproductive system

- Increased incidences of gross and microscopic lesions in the *male reproductive system* at the top dose only (10000 ppm; ~500 mg/kg/day)
  - Gross
    - Cryptorchidism (undescended testis)
    - Agenesis (testis, epididymis, prostate, vas deferens)
    - Small (testis, epididymis, prostate, seminal vesicle)
  - Microscopic
    - Testis: Atrophy, Edema, Seminiferous tubule dysgenesis, Rete testis fibrosis, Leydig cell hyperplasia
    - Epididymis: Hypospermia
    - Accessory sex organs: Decreased secretory fluid in prostate & seminal vesicles

Results: Rats

Non-neoplastic findings: Liver and Pituitary Gland

- Increased incidence in *hepatocyte cytoplasmic alteration* in male and female rats at the top dose

- Increased incidence in *pars distalis hypertrophy* in the pituitary gland in male rats at the top dose
  - Consistent with “gonadectomy” or “castration” cells

Results: Rats

Neoplasms

- Exposure-related trend in neoplasms observed in pancreas of male rats only
  - Top dose incidence falls within historical control ranges
  - There is a potential mechanism of action; PPARα activation

	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm	Historical Control (range)
N (males)	49	50	50	50	49	
Pancreas, acinus, hyperplasia	19 [2.3]	21 [2.1]	18 [2.1]	23 [2.0]	18 [2.1]	NA
Pancreas, acinus, carcinoma or adenoma	6# (12%)	4 (8%)	3 (6%)	1 (2%)	10 (20%)	0-28%

#p≤0.05 indicates a significant trend; Numbers in brackets indicate severity; NA- not applicable

Draft Conclusions

- Rats
  - *Equivocal evidence of carcinogenic activity* in male Hsd:Sprague Dawley® SD® rats based on marginal increases in the incidence of pancreatic acinus adenomas and carcinomas
  - *No evidence of carcinogenic activity* in female Hsd:Sprague Dawley® SD® rats at 300, 1000, 3000, or 10000 ppm
  - Exposure to DBP increased incidences of gross and non-neoplastic lesions in the male reproductive system, liver, and pituitary gland pars distalis (male rats)
- Mice
  - *No evidence of carcinogenic activity* in male and female B6C3F1/N mice at 1000, 3000, or 10000 ppm
  - Exposure to DBP increased incidences of nonneoplastic lesions in the male reproductive system, liver, and kidney (female mice)

Thank you!

Questions?

Extra slides

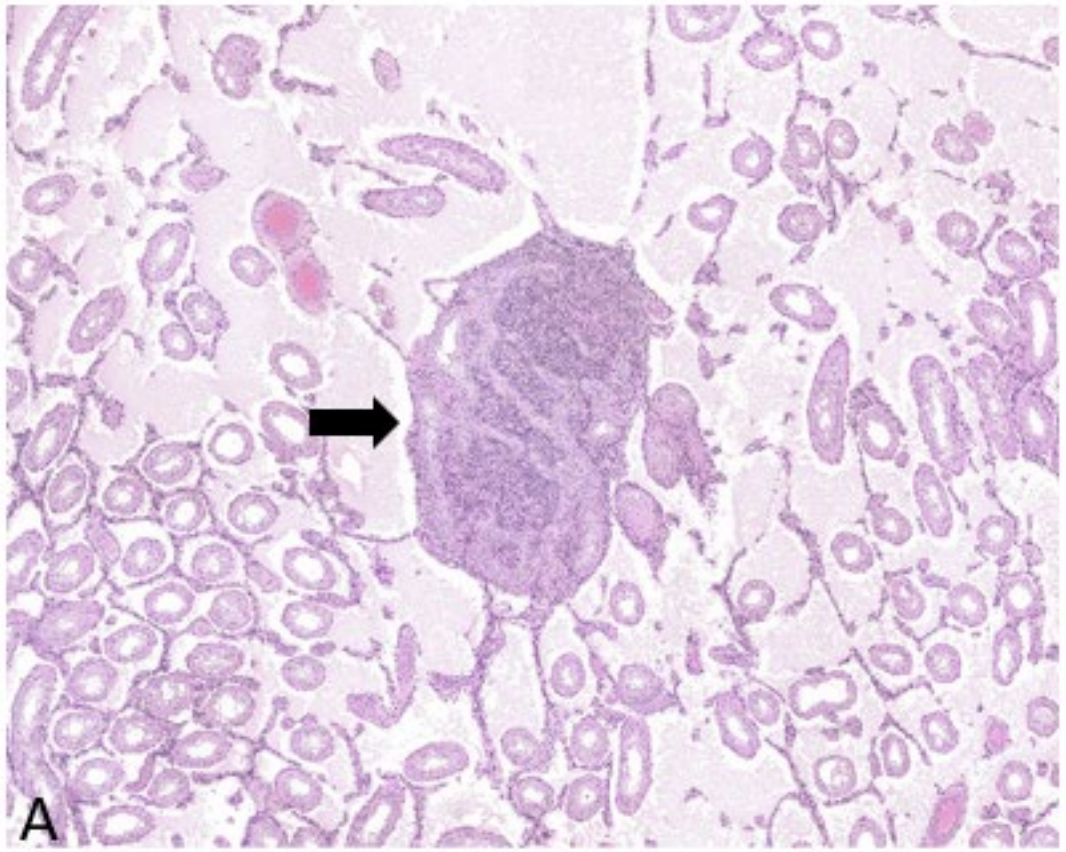
Pituitary Gland, Pars Distalis

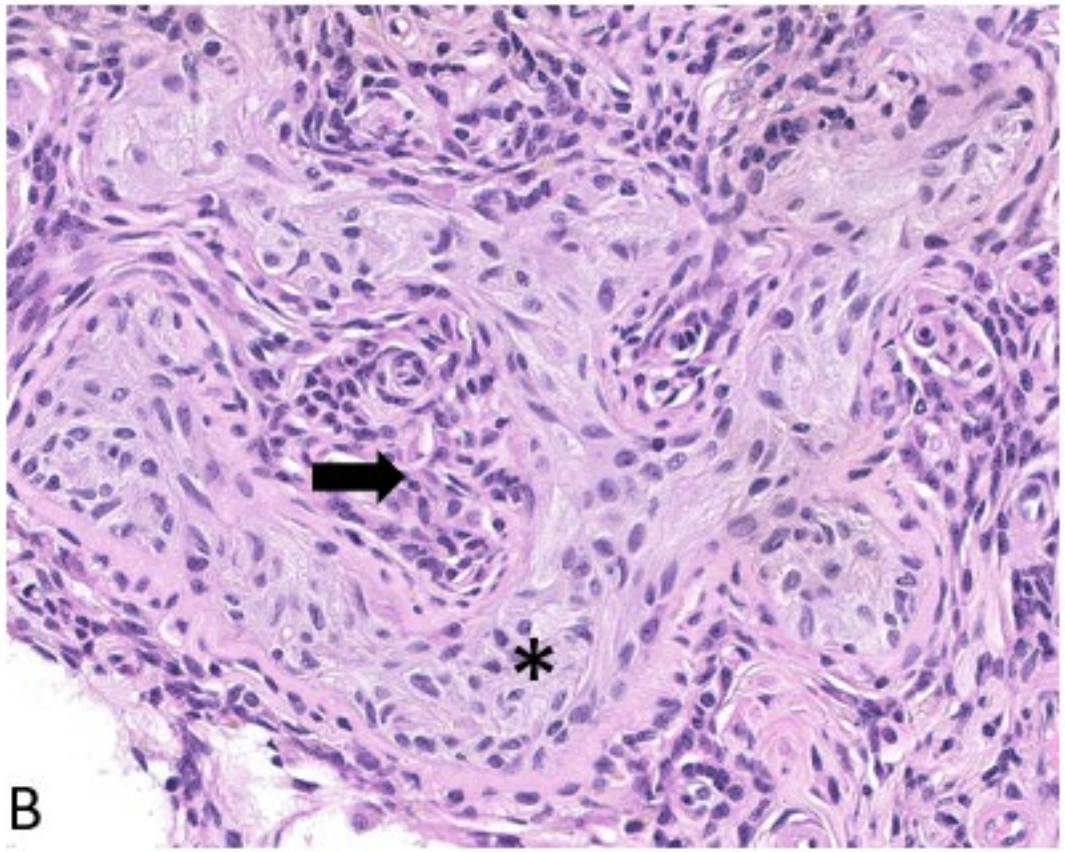
	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
N (males)	48	50	50	50	50
Hypertrophy	0	0	0	0	29
Hyperplasia	15* (31%)	13 (26%)	13 (26%)	18 (36%)	22 (44%)
Adenoma (unspecified site)	15 (31%)	10 (20%)	12 (24%)	14 (28%)	6 (12%)

#p≤0.05 indicates a significant increasing trend

Histopathology: MALE RATS

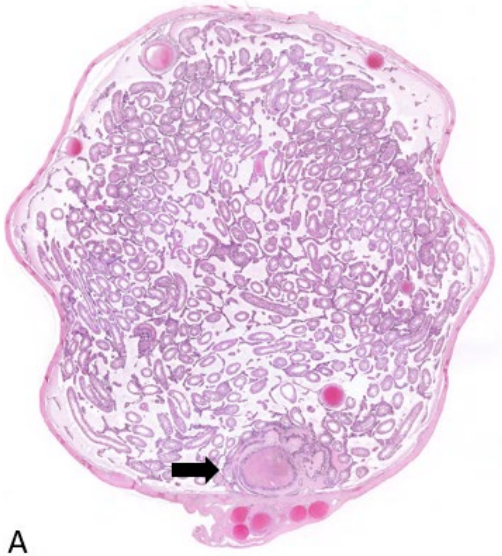
Testis: Seminiferous tubule dysgenesis, atrophy, and edema

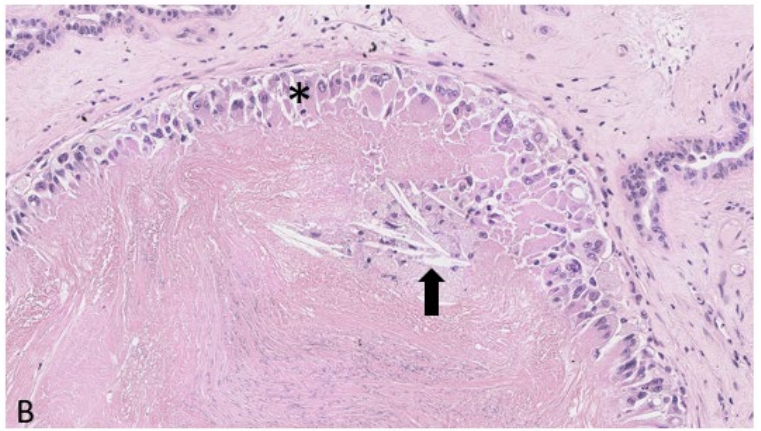


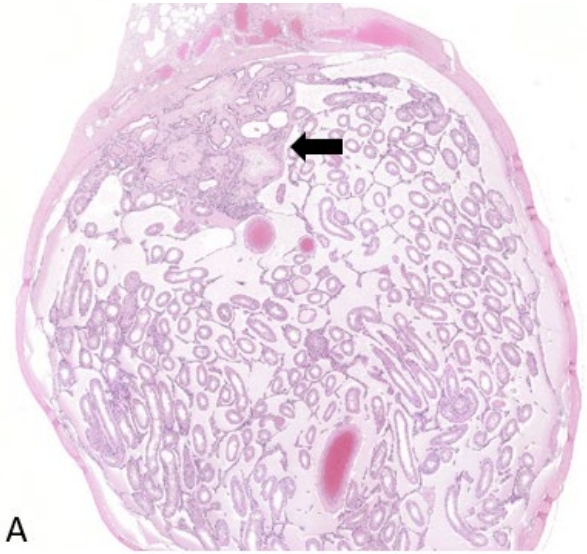


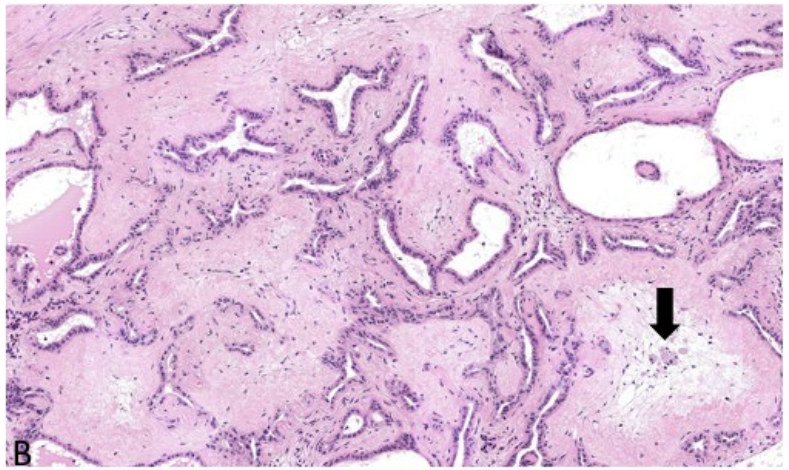
Histopathology: MALE RATS

Testis: Rete testis sperm granuloma (top) and fibrosis (bottom)



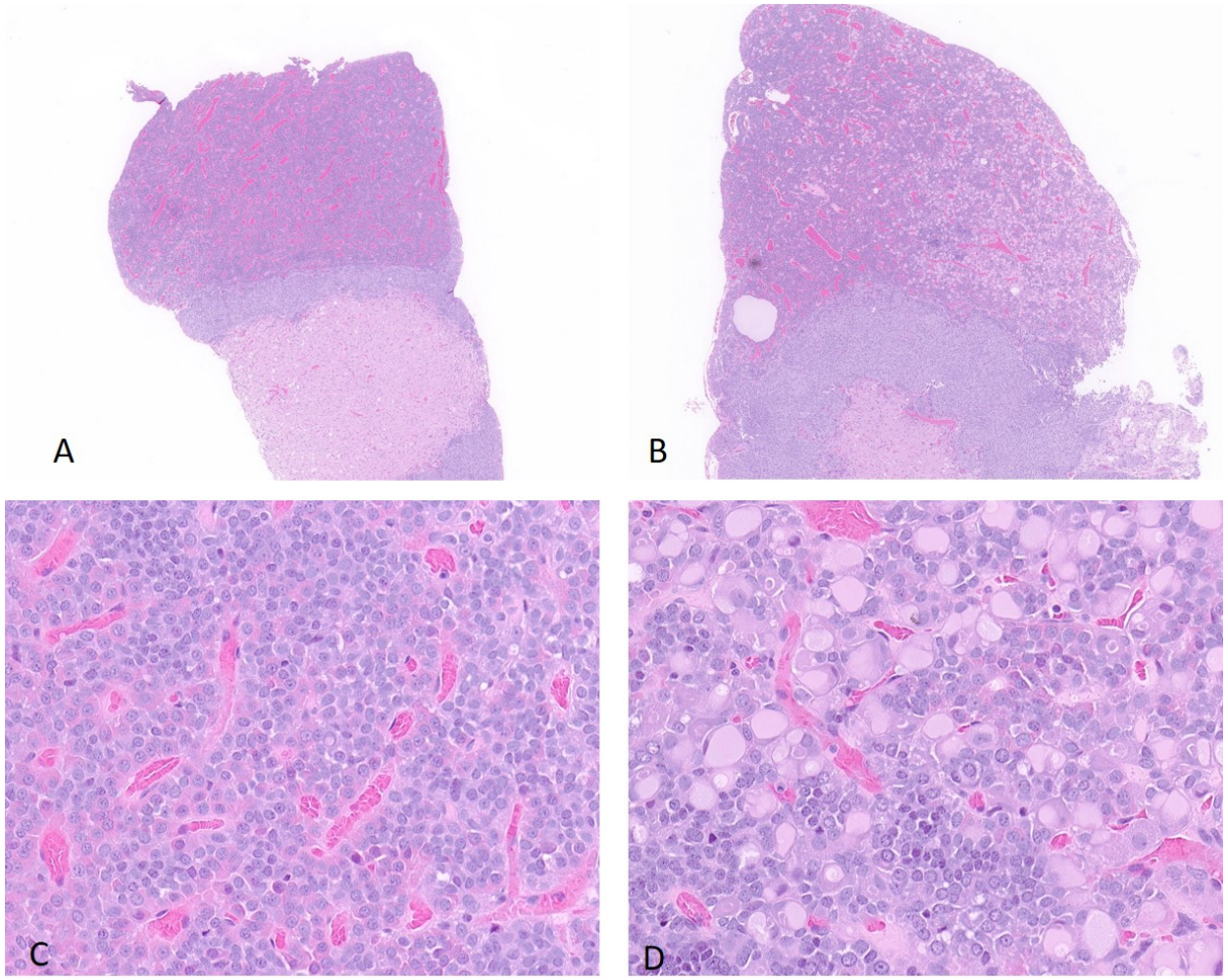






Histopathology: MALE RATS

Pituitary gland: pars distalis hypertrophy ("gonadectomy" cells)



Histopathology: FEMALE MICE

Kidney: Renal tubule hyperplasia