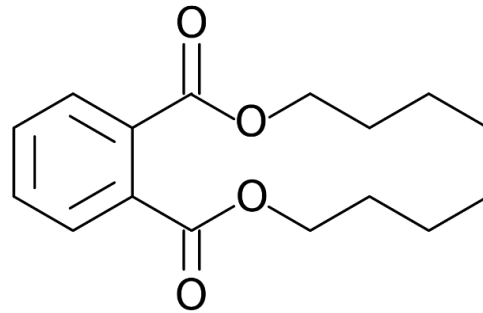


**Draft NTP Technical Report on the  
Toxicology and Carcinogenesis Studies of  
Di-n-butyl Phthalate (CASRN 84-74-2)  
Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley<sup>®</sup> SD<sup>®</sup>) Rats  
and B6C3F1/N Mice**



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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  $\mu\text{g}/\text{kg}/\text{day}$ 
  - Primarily through food; some inhalation and dermal
- Rapid metabolism to monobutyl phthalate (MBP) in the gut
  - Broad distribution, minimal accumulation, urinary excretion

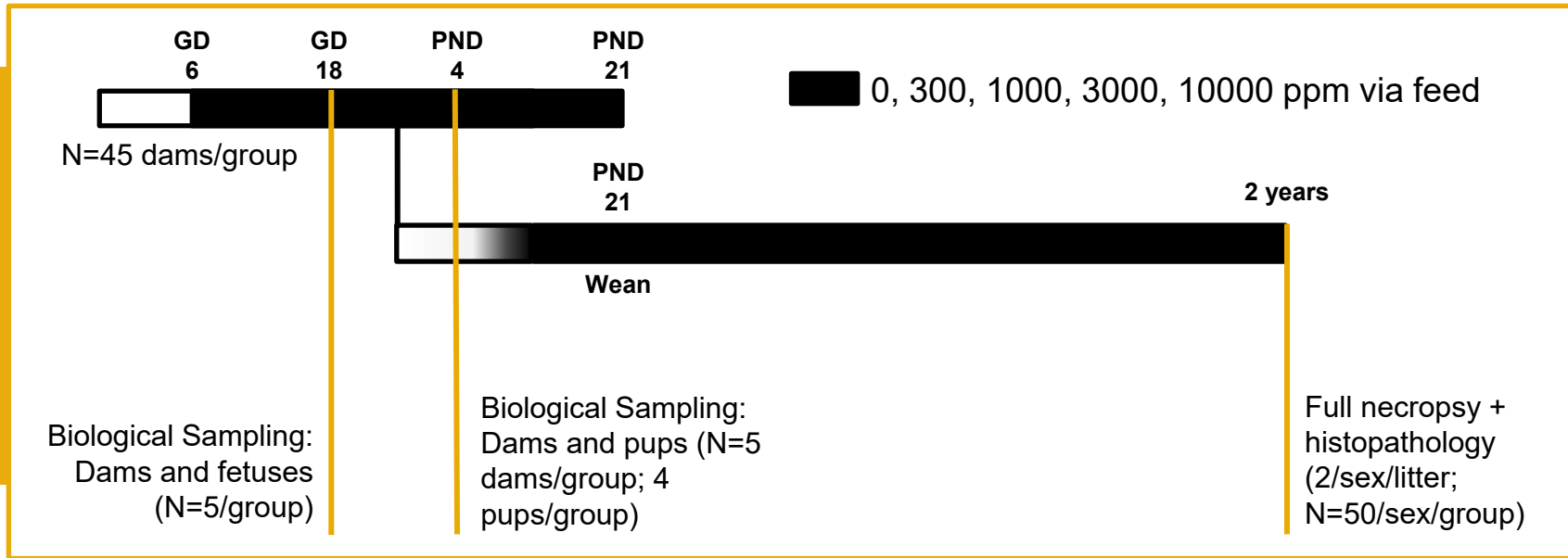
- Previous hazard assessments and carcinogenicity assessments in animals of phthalates have not evaluated the effects of combined early life and postnatal exposures
  - Smith et al. 1953: Male rats, 1 year exposure, adult exposure only
  - Barlow et al. 2004: Male SD rats, 6-18 months exposure, prenatal 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

## Findings from subchronic feed studies (TOX 30)

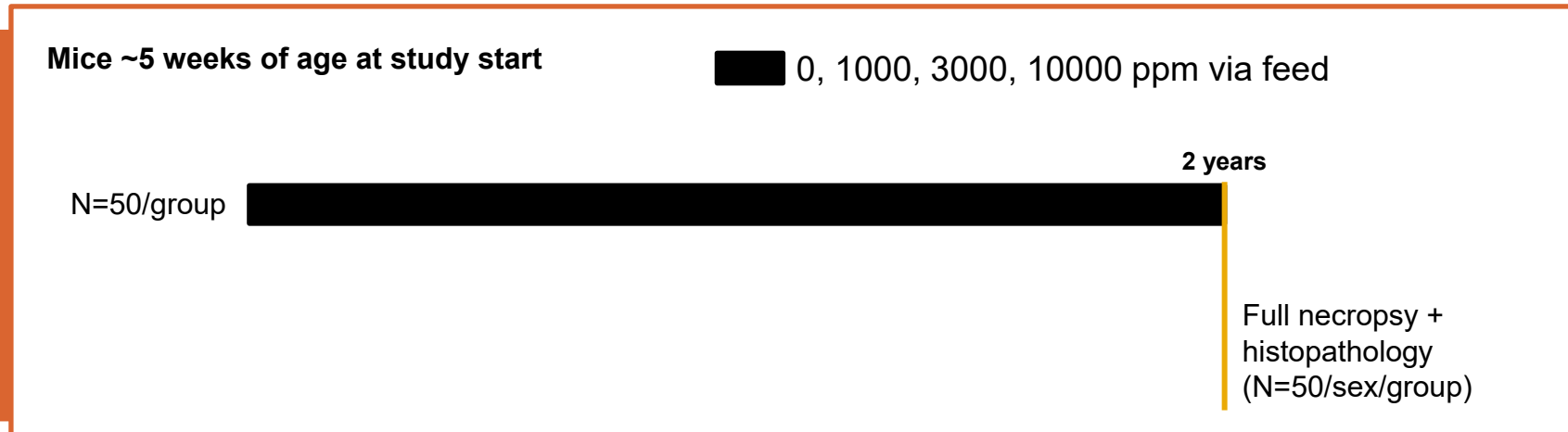
- Rat (F344/N) Perinatal dose-range finding study
  - 0, 1250, 2500, 5000, 7500, 10000, 20000 ppm *in utero* exposure
  - High mortality of pups at 20,000 ppm, slight decreases in number of live pups/litter and pup body weight at 10,000 ppm
- Mice (B6C3F1/N) 13-week study
  - 0, 1250, 2500, 5000, 10000,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

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

**Hsd:SD Rats**



**B6C3F1/N Mice**



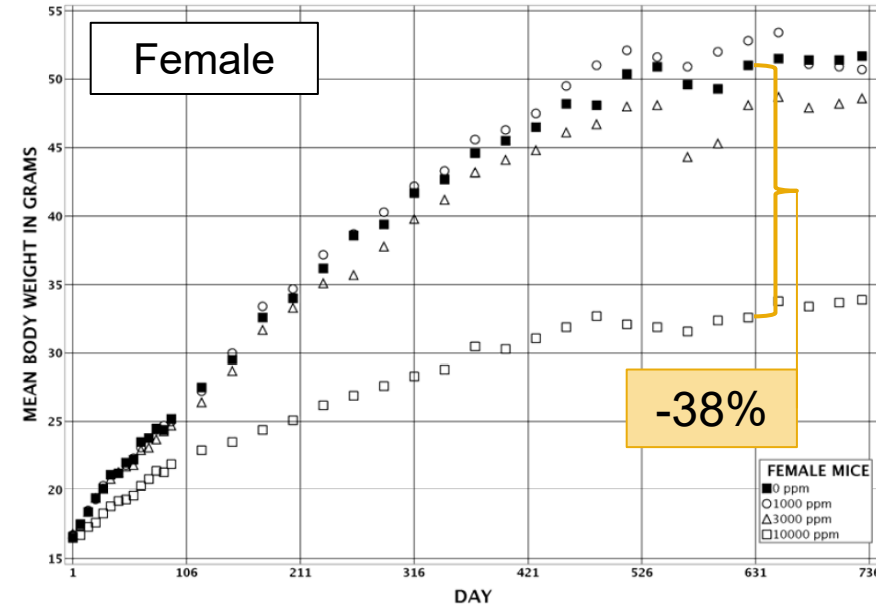
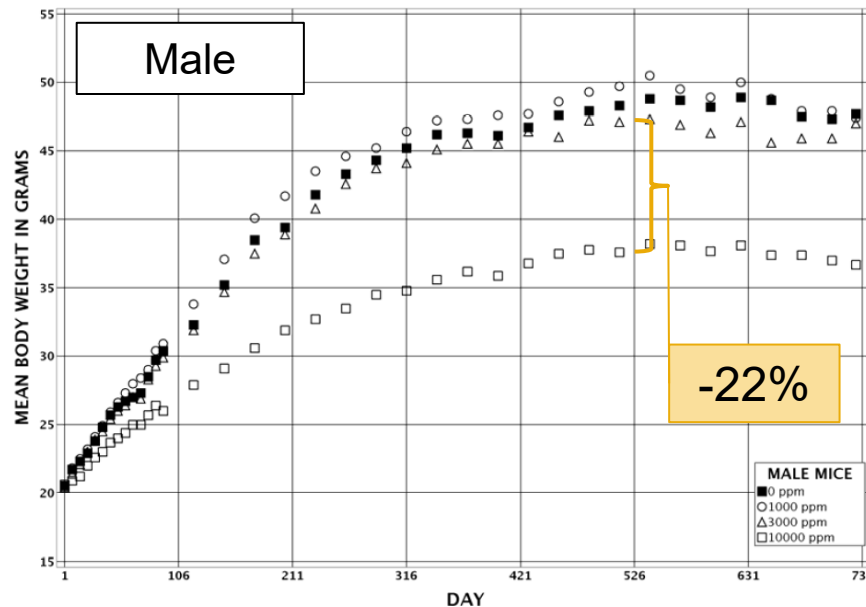
## DBP consumption (mg/kg/day; mean)

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

## 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



## 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



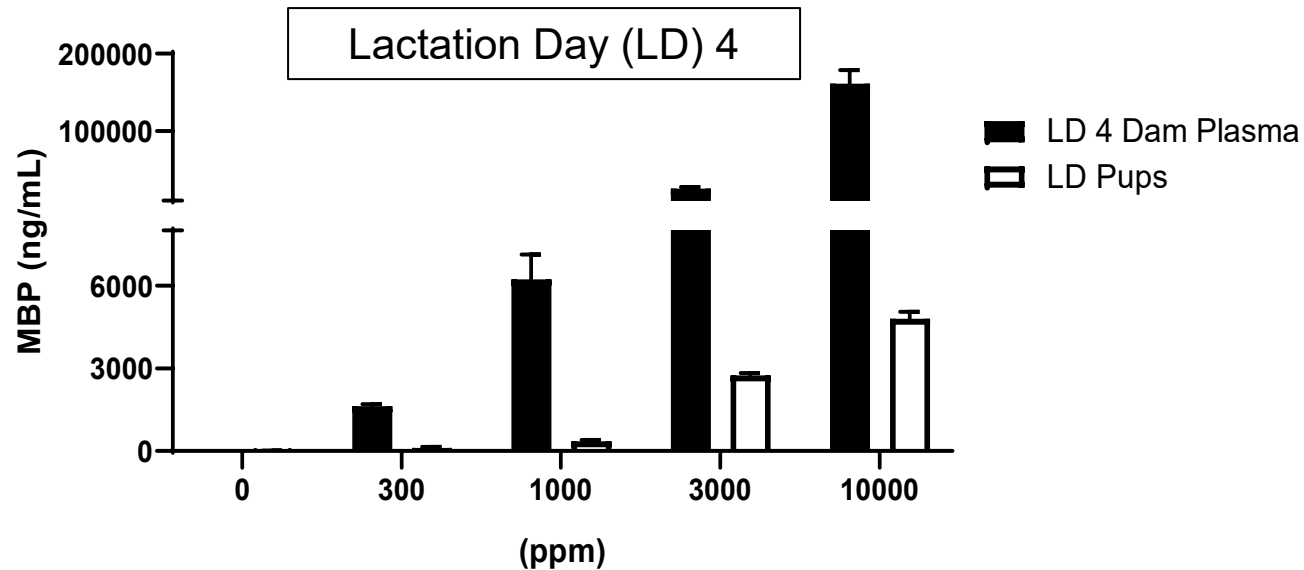
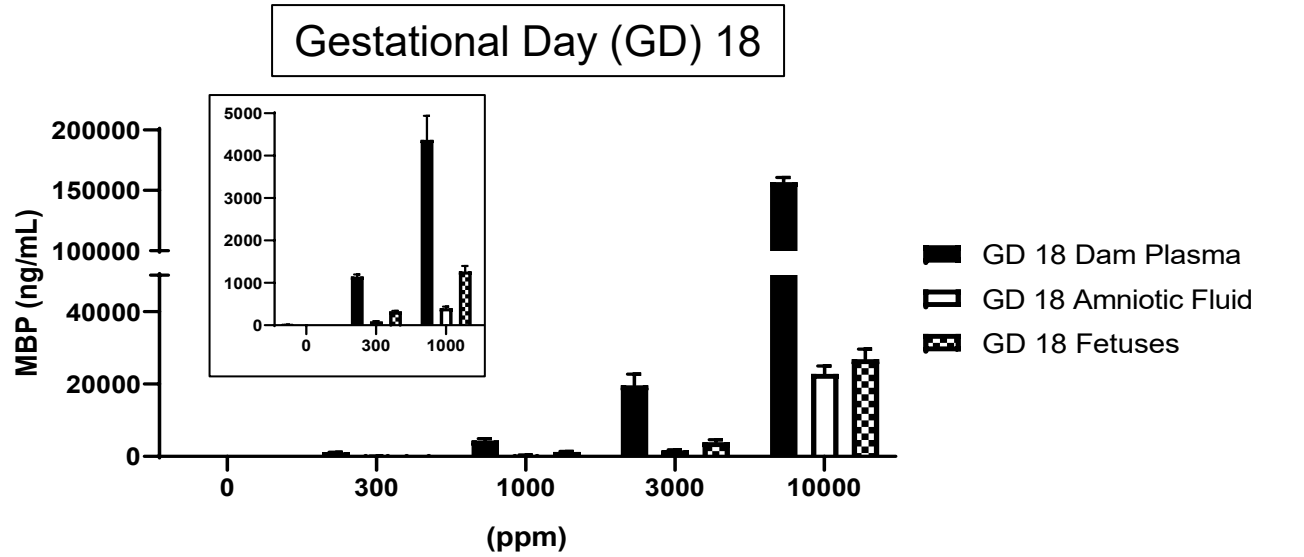
## Neoplasms

- No exposure-related increases in incidences of neoplasms

## 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

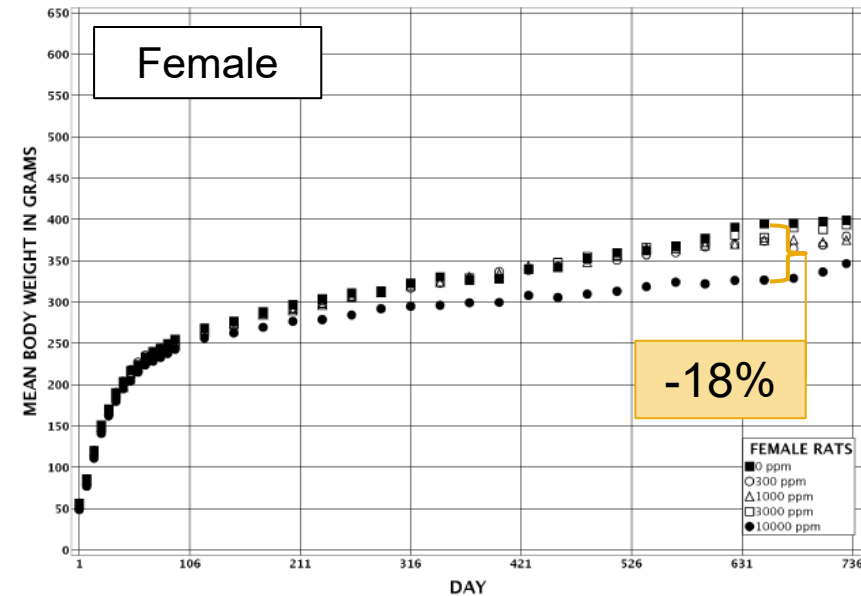
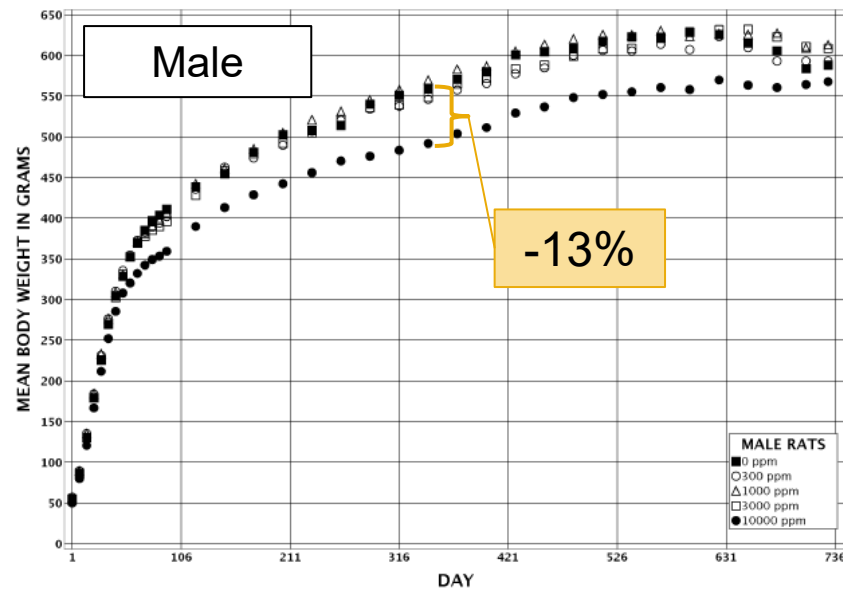
## Internal concentrations of DBP metabolite, MBP



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

## Chronic Phase

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



## 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

## 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

## 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; PPAR $\alpha$  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 <sup>#</sup> (12%)	4 (8%)	3 (6%)	1 (2%)	10 (20%)	0-28%

<sup>#</sup>p $\leq$ 0.05 indicates a significant trend; Numbers in brackets indicate severity; NA- not applicable

- Rats

- *Equivocal evidence of carcinogenic activity* in male Hsd:Sprague Dawley<sup>®</sup> SD<sup>®</sup> rats based on marginal increases in the incidence of pancreatic acinus adenomas and carcinomas
- *No evidence of carcinogenic activity* in female Hsd:Sprague Dawley<sup>®</sup> SD<sup>®</sup> 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?**

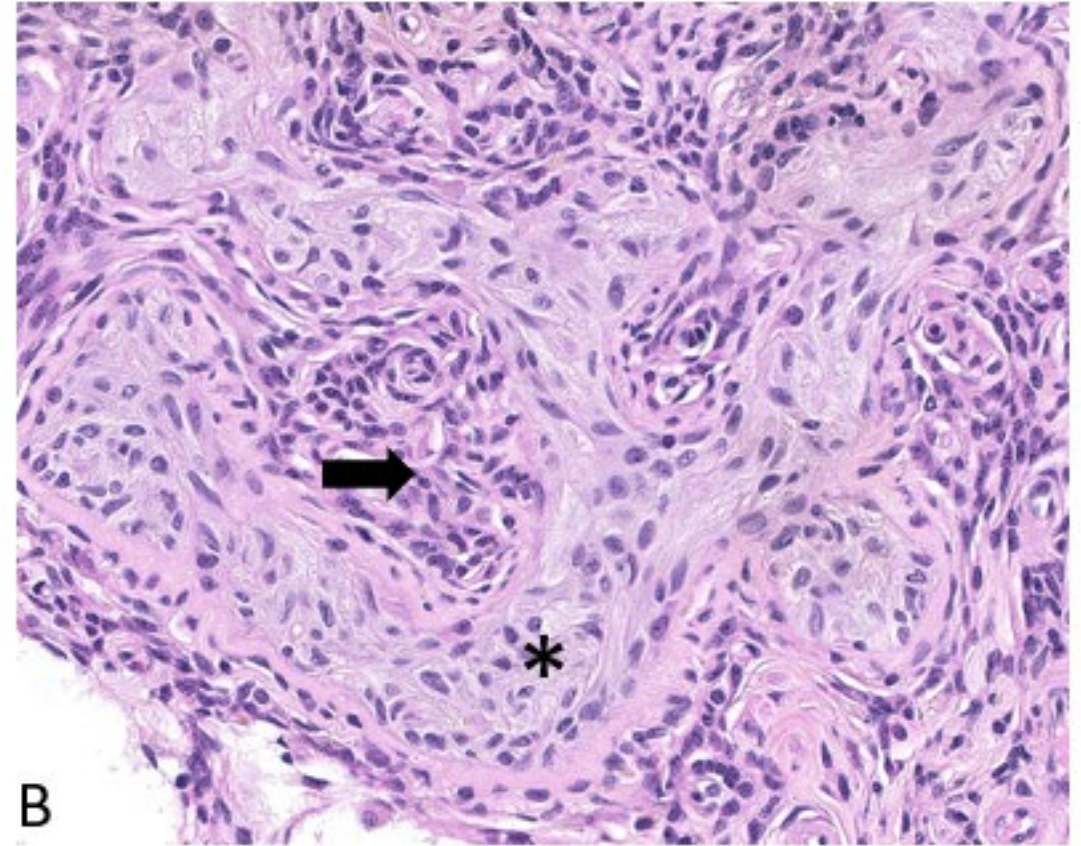
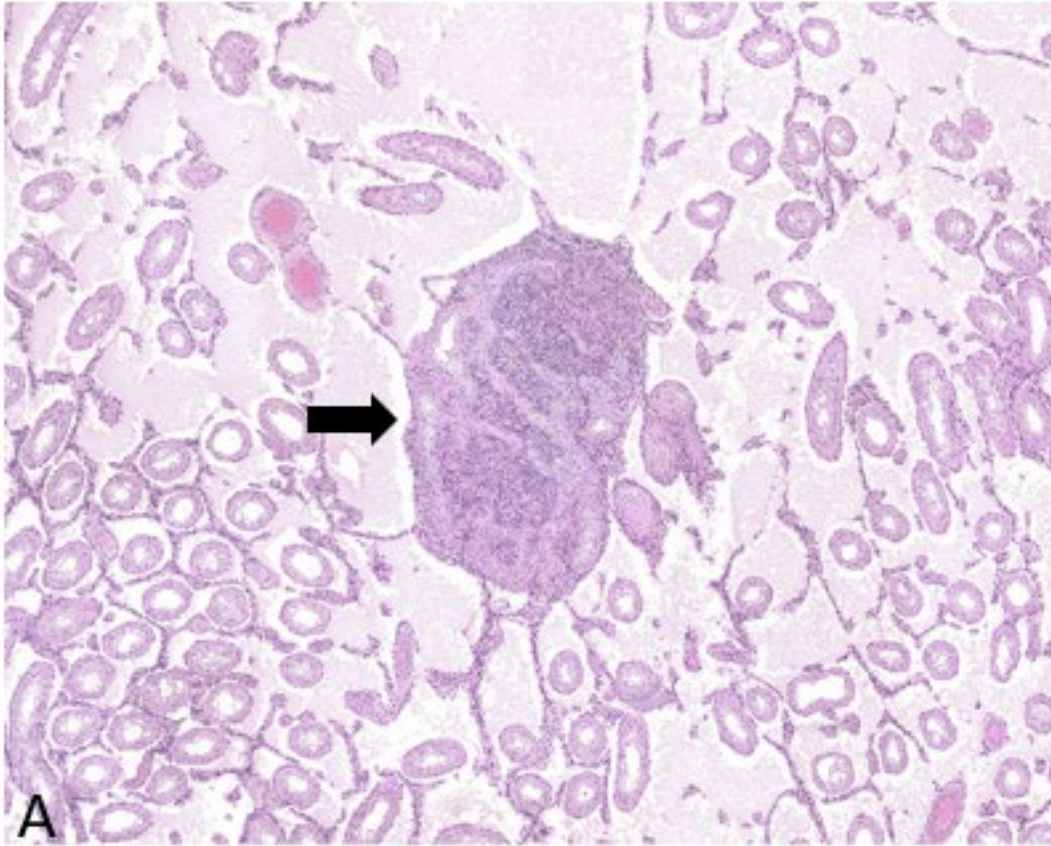
# Extra slides

## Pituitary Gland, Pars Distalis

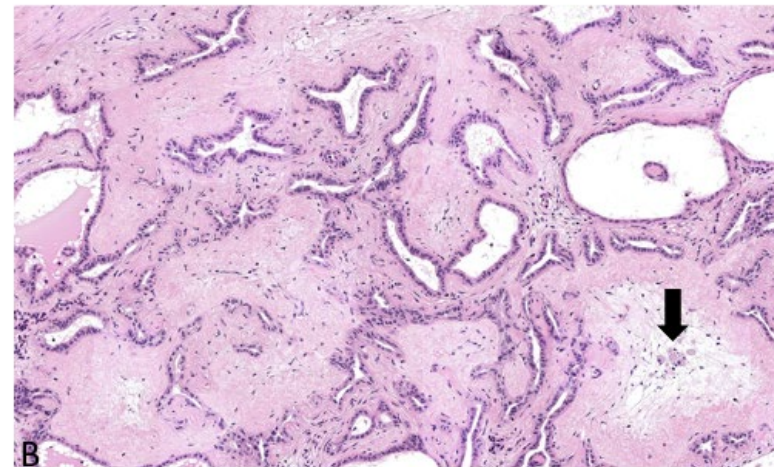
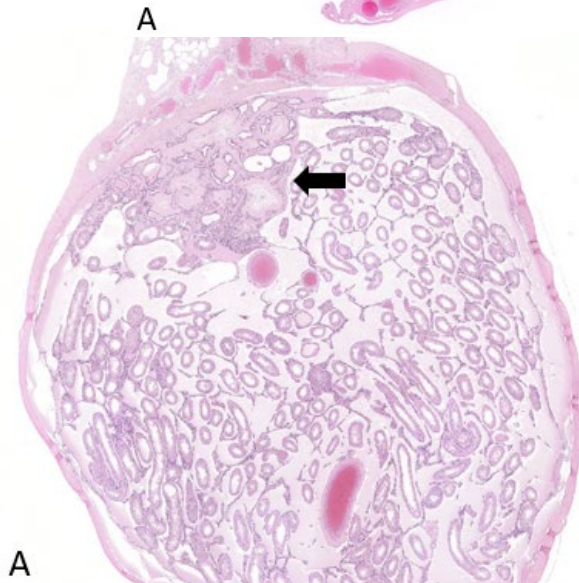
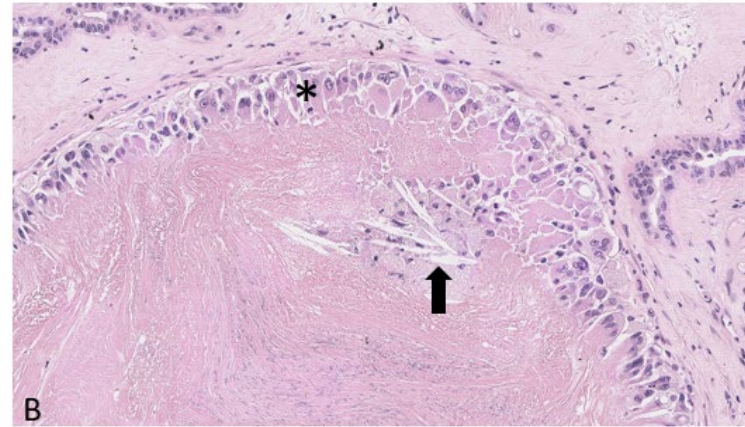
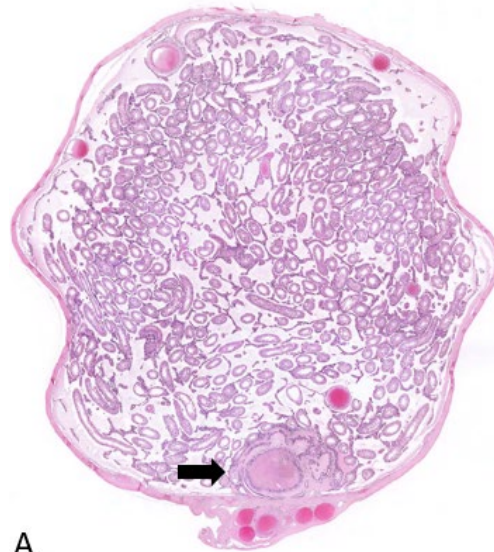
	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
N (males)	48	50	50	50	50
Hypertrophy	0	0	0	0	29
Hyperplasia	15* (31%)	13 (26%)	13 (26%)	18 (36%)	22 (44%)
Adenoma (unspecified site)	15 (31%)	10 (20%)	12 (24%)	14 (28%)	6 (12%)

#p≤0.05 indicates a significant increasing trend

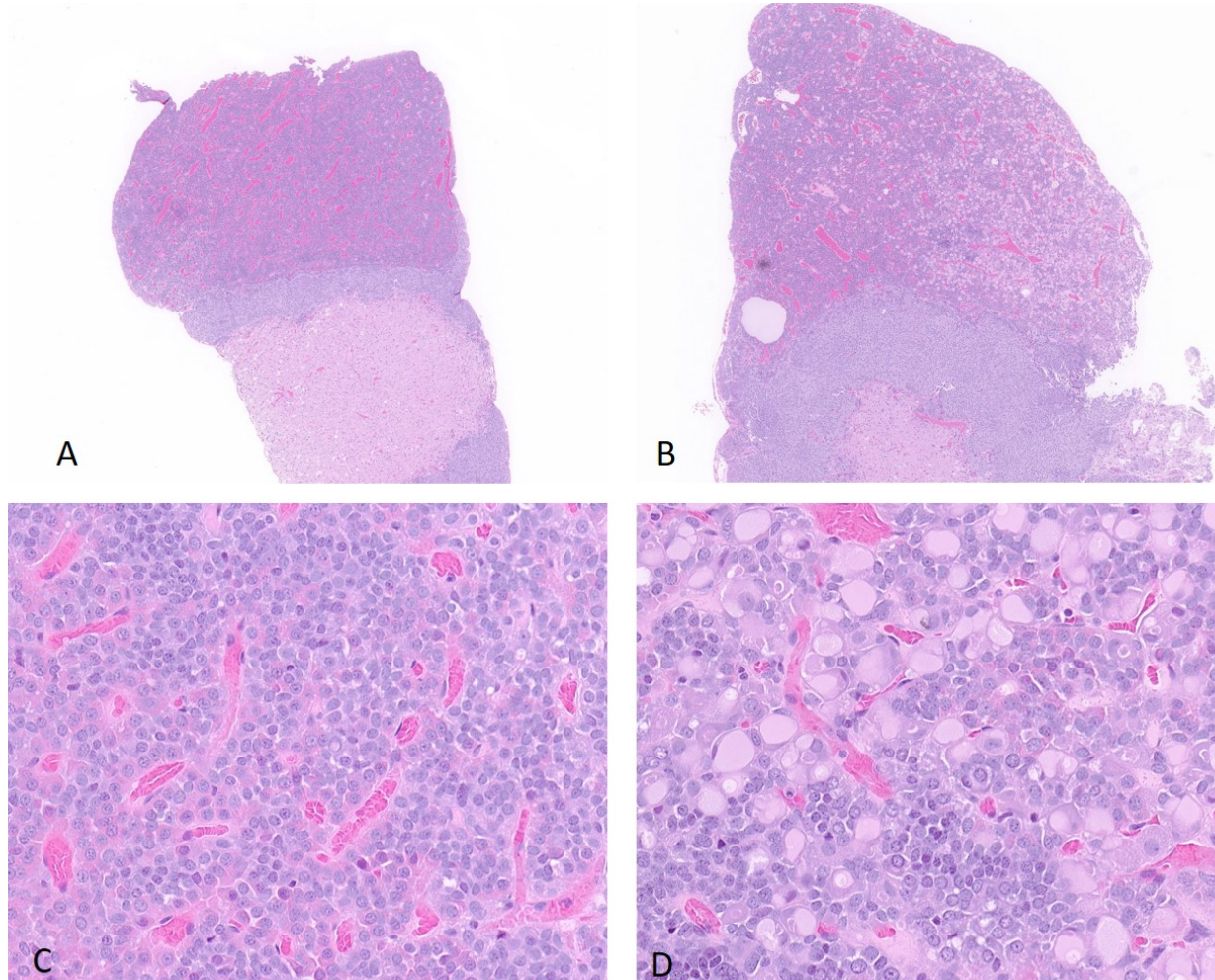
## Testis: Seminiferous tubule dysgenesis, atrophy, and edema



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



## Pituitary gland: pars distalis hypertrophy (“gonadectomy” cells)



## Kidney: Renal tubule hyperplasia

