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

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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
• 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 ≥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

**B6C3F1/N Mice**
- Mice ~5 weeks of age at study start
- N=50/group

**Hsd:SD Rats**
- N=45 dams/group

**Biological Sampling:**
- Dams and fetuses (N=5/group)
- Dams and pups (N=5 dams/group; 4 pups/group)
- Full necropsy + histopathology (2/sex/litter; N=50/sex/group)

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Study Design

**0, 300, 1000, 3000, 10000 ppm via feed**

**GD**
- 6
- 18

**PND**
- 4
- 21

**Wean**

**2 years**

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**Mice ~5 weeks of age at study start**
- N=50/group

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**Full necropsy + histopathology (N=50/sex/group)**
### DBP consumption (mg/kg/day; mean)

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

- 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
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

Results: Rats

• Non-linear increases in MBP internal concentrations
• Moderate gestational transfer was observed
• Low lactational transfer was observed
• 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; PPARα activation

<table>
<thead>
<tr>
<th></th>
<th>0 ppm</th>
<th>300 ppm</th>
<th>1000 ppm</th>
<th>3000 ppm</th>
<th>10000 ppm</th>
<th>Historical Control (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (males)</td>
<td>49</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>49</td>
<td>NA</td>
</tr>
<tr>
<td>Pancreas, acinus, carcinoma or adenoma</td>
<td>6# (12%)</td>
<td>4 (8%)</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td>10 (20%)</td>
<td>0-28%</td>
</tr>
</tbody>
</table>

*p≤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® 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

<table>
<thead>
<tr>
<th></th>
<th>0 ppm</th>
<th>300 ppm</th>
<th>1000 ppm</th>
<th>3000 ppm</th>
<th>10000 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (males)</td>
<td>48</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>15*</td>
<td>13</td>
<td>13</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>(31%)</td>
<td>(26%)</td>
<td>(26%)</td>
<td>(36%)</td>
<td>(44%)</td>
</tr>
<tr>
<td>Adenoma (unspecified site)</td>
<td>15</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(31%)</td>
<td>(20%)</td>
<td>(24%)</td>
<td>(28%)</td>
<td>(12%)</td>
</tr>
</tbody>
</table>

*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