DiNP Dose-Response Studies: Gestation PK and Developmental Effects Postnatal Effects

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Summary – DiNP Gestation Study

• NOEL for DiNP effects
  – 50 mg/kg/day: T inhibition, MNG, increased liver weight
  – >750 mg/kg/day: AGD, ST diameter

• DiNP metabolites are present in the fetal testes

• Apparent saturation of oral absorption at highest dose (750 mg/kg/day)
  – Evidenced by tissue metabolite data
  – Causes plateau in liver wt and T inhibition
  – Likely result of oral gavage administration

• DiNP is consistently less potent than DBP and DEHP where there is equivalent D-R data

• Similar kinetics to DEHP, indicates reduced potency of DiNP is due to pharmacodynamic differences

Clewell et al., 2013a
Postnatal Effects Study

- **Objective**: determine a NOEL for effects on the developing male rat reproductive tract for di-isononyl phthalate (DiNP).

- **Study Design**
  - 20 – 25 litters per treatment group
  - All necropsies and observations completely **BLINDED**

Clewell et al., 2013b
## Comparison of effects - DiNP Postnatal Study

<table>
<thead>
<tr>
<th>500 mg/kg/day DBP</th>
<th>&gt; 250 mg/kg/day DiNP</th>
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<tbody>
<tr>
<td>• No body weight effects</td>
<td>• PND 2 body weight (750 mg/kg)</td>
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<tr>
<td>• Nipple retention</td>
<td>• PND 14 body weight (≥250 mg/kg)</td>
</tr>
<tr>
<td>• AGD (absolute and scaled) PND 2 + 14</td>
<td>• PND 14 reduced AGD (750 mg/kg)</td>
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<tr>
<td>• Phallus development</td>
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<td>• Epididymal development</td>
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<td>• Preputial separation</td>
<td></td>
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<tr>
<td>• Weight of 4 reproductive organs</td>
<td></td>
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<tr>
<td>• PND 2 ST – some enlarged tubules</td>
<td>• No change in ST diameter</td>
</tr>
<tr>
<td>• PND 2 #MNG/section, large LC aggregates</td>
<td>• PND 2 # MNG/section (≥ 250 mg/kg), large LC aggregates (750 mg/kg)</td>
</tr>
<tr>
<td></td>
<td>• Effects were seen to be transient (not observed at PND 49)</td>
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Effects were seen to be transient (not observed at PND 49)

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*DBP*: Di(2ethylhexyl)phthalate

*AGD*: Absolute and scaled

*PND*: Postnatal Day

*MNG*: Mesenchymal nephrogenic zone

*LC*: Leydig cell
Conclusions from DiNP perinatal studies

• The current studies on DiNP are the most well designed, comprehensive studies available for testing the effects of DiNP on the male reproductive tract

• A clear NOEL for effects on the developing male rat reproductive tract was established for DiNP of 760 ppm (50 mg/kg/day)

• A LOEL of 3800 ppm DiNP (250 mg/kg/day) based on the significant increase in MNGs on GD 20/PND 2, testosterone reduction on GD 19, and decreased pup body weight on PND 14
  – All effects were recoverable at later time points

• No evidence for DiNP-induced effects attributed to the rat phthalate syndrome at doses up to 750 mg/kg/day using global statistical analysis

• The role of testosterone as part of the mechanism leading to each of the male reproductive effects is unclear
  – Data indicate that decreased testosterone may be necessary for the induction of some effects, but is clearly not sufficient at doses up to 750 mg DiNP/kg/day

• Although the kinetics of DiNP are similar to DEHP, the mechanism and/or potency of DiNP is clearly different
Possible reasons for lack of malformations with DiNP

- Testosterone reduction is not sufficient to produce malformations (i.e., permanent effects).
  - Doses of lower molecular weight phthalates causing malformations such as hypospadias and cryptorchidism, correspond to ~ 90% inhibition of testosterone. DiNP causes ~ 70% inhibition at very high doses (750 – 1500 mg/kg/day).

- Testosterone inhibition **alone** is not sufficient to induce downstream malformations.
  - Cryptorchidism, for example is a combination of testosterone and INSL3 reduction.
  - (Wilson et al., 2004)

- More importantly...
  - Species differences in phthalate effects.
  - Susceptibility appears to be rat specific
    - Not applicable to human
  - (Johnson et al., 2012; Habert et al., 2014; Heger et al., 2012; Mitchell et al., 2012)
• NOTE – error in doses for Clewell reference on pg 3-47 of IRIS document

References


