THE HAMNER INSTITUTES FOR HEALTH SCIENCES

DiNP Dose-Response Studies:

Gestation PK and Developmental Effects
Postnatal Effects

Rebecca Clewell, PhD
Associate Director
Institute for Chemical Safety Sciences

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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

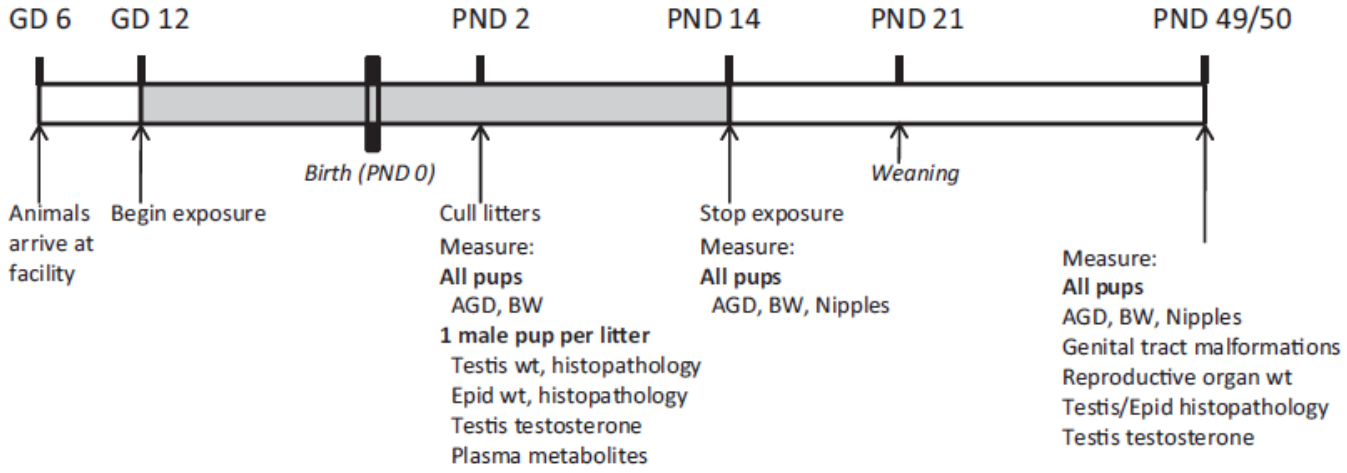
- Similar kinetics to DEHP, indicates reduced potency of DiNP is due to **pharmacodynamic** differences

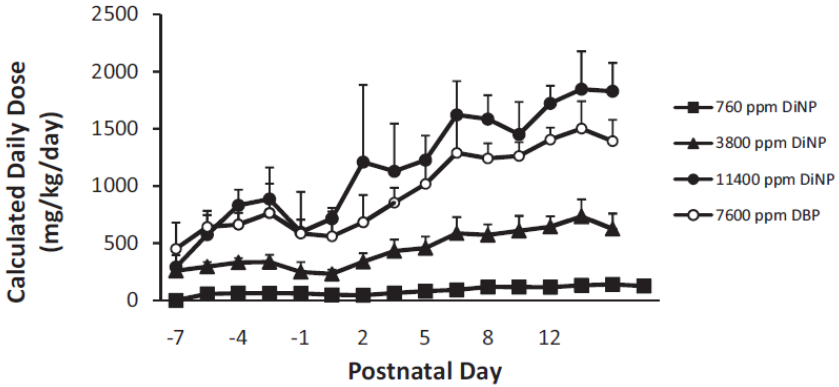
Clewell et al., 2013a

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

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

Conclusions from DiNP perinatal studies

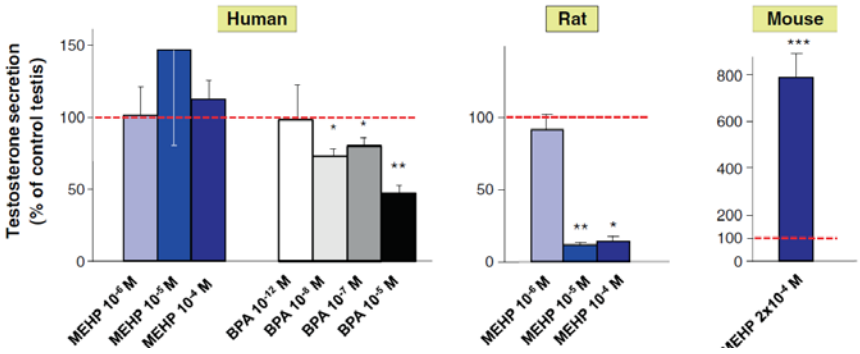
- The current studies on DiNP are the most well designed, comprehensive studies available for testing the effects of DiNP on the male reproductive tract
- A clear NOEL for effects on the developing male rat reproductive tract was established for DiNP of 760 ppm (50 mg/kg/day)
- A LOEL of 3800 ppm DiNP (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

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