Endocrine Activity of Dibutyl, Diethyl and Dimethyl Phthalate

Presentation to CIR

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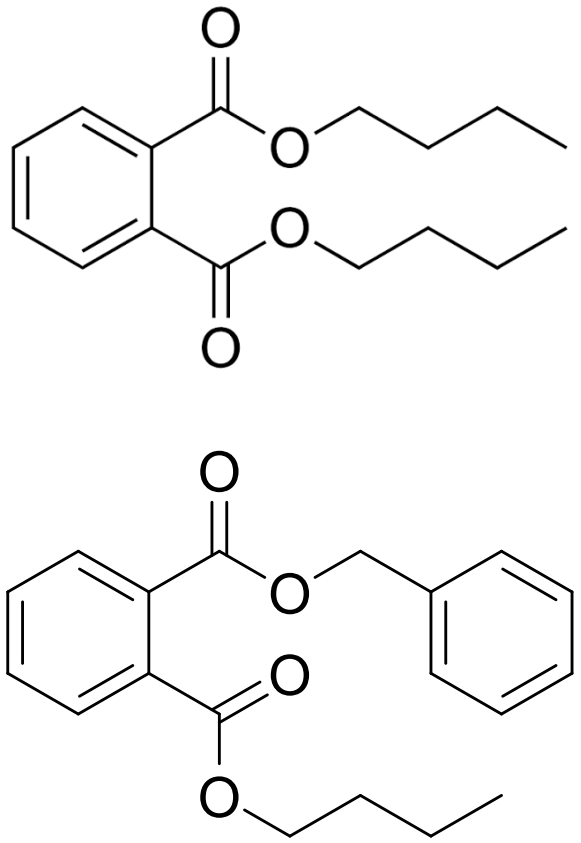
Disclaimer

- I am a full-time employee of Procter & Gamble
- P&G does not formulate with any phthalate ester

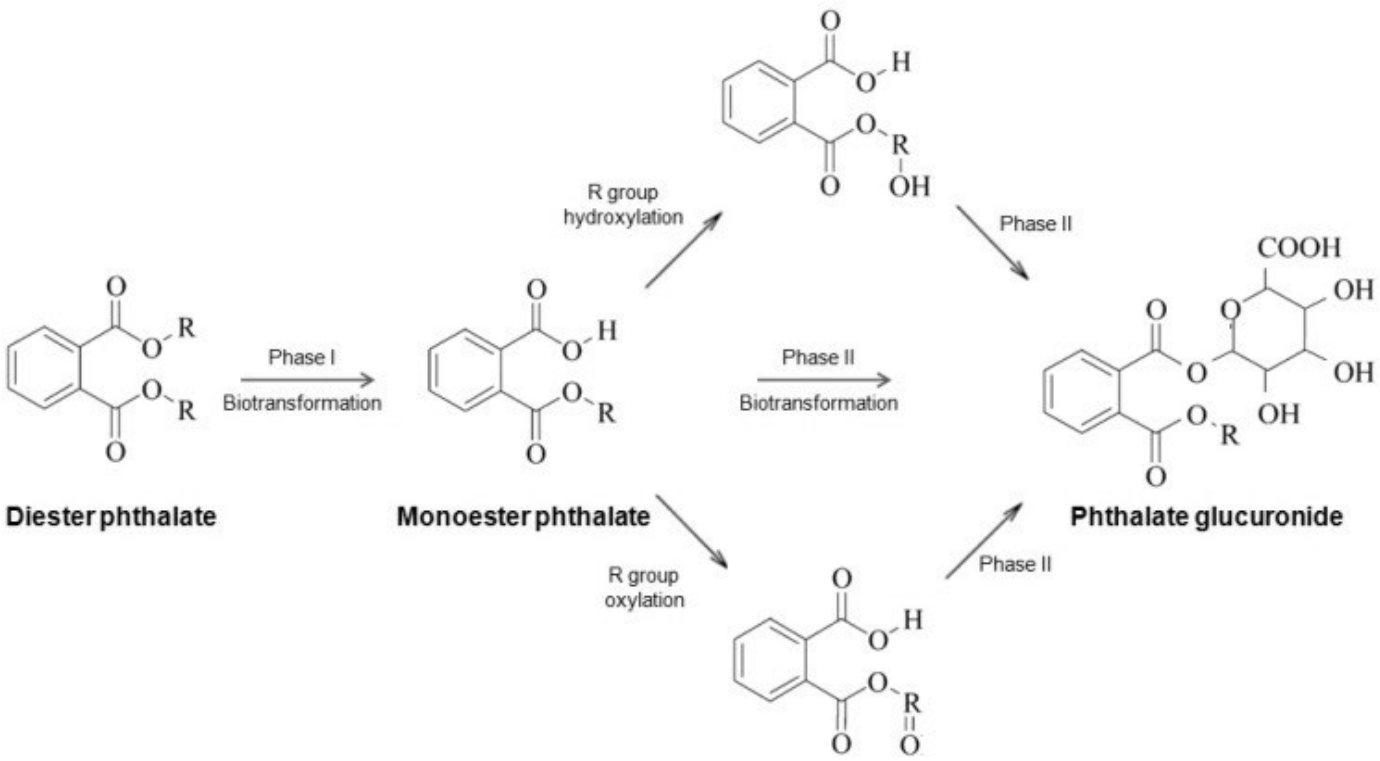
Phthalate Esters: Chemistry and Uses



Uses:

- Plasticizers: they make plastic polymers more flexible, less brittle
- Fragrance additives (smaller side chains, like methyl and ethyl)
  - Limit evaporation of volatiles, increasing the duration over which the scent can be perceived

Phthalate ester metabolism and disposition



- Phthalate diesters are converted to monoesters in the gut
- The monoesters are the active form
- GI absorption is extensive
- Primary route of excretion is via urine
- Elimination of a dose is relatively rapid
  - Close to 100% of an oral dose in rats is accounted for in urine in 24-48 hours

Phthalate Ester SAR

- Significant DART effects are limited to esters with side groups with 4-8 carbons, with some activity with side groups of 9-10 carbons
  - N-butyl, isobutyl, 2-ethylhexyl, butylbenzyl, pentyl have high activity
- Effects are very limited for dimethyl and diethyl phthalate (and their monoester metabolites)

Dibutyl Phthalate Reproductive Toxicity

- Decreased sperm production and testicular atrophy in rats at 250 mg/kg/day in juvenile and adult rats by gavage (EPA), 720 mg/kg/day in feed (NTP).
  - Species differences exist, with mice and hamsters less sensitive than rats and guinea pigs
- Female reproductive effects are less marked: no effect on estrous cyclicity but pregnancy rate and pups/ litter decreased above 1250 mg/kg/day in rats

Diethyl Phthalate Reproductive Toxicity

- No effects on reproduction in a two-generation study in rats at up to 15000 ppm in feed (daily intake of 720-1900 mg/kg/day in males, 800-2200 mg/kg/day in females)
- No effects on sperm production in that study, some effect on spot measurements of serum testosterone, but deemed to be clinically irrelevant
- Fujii et al (2005) J. Toxicol. Sci. 30 (Special Issue): 97-116.

Dibutyl Phthalate Developmental Toxicity

- Increased embryonic death, including a marked increase in whole litter loss, in rodents at 250 mg/kg/day and higher in rats and mice.
- Some skeletal and soft tissue malformations at doses of 750-1000 mg/kg/day (and higher) in rats and mice

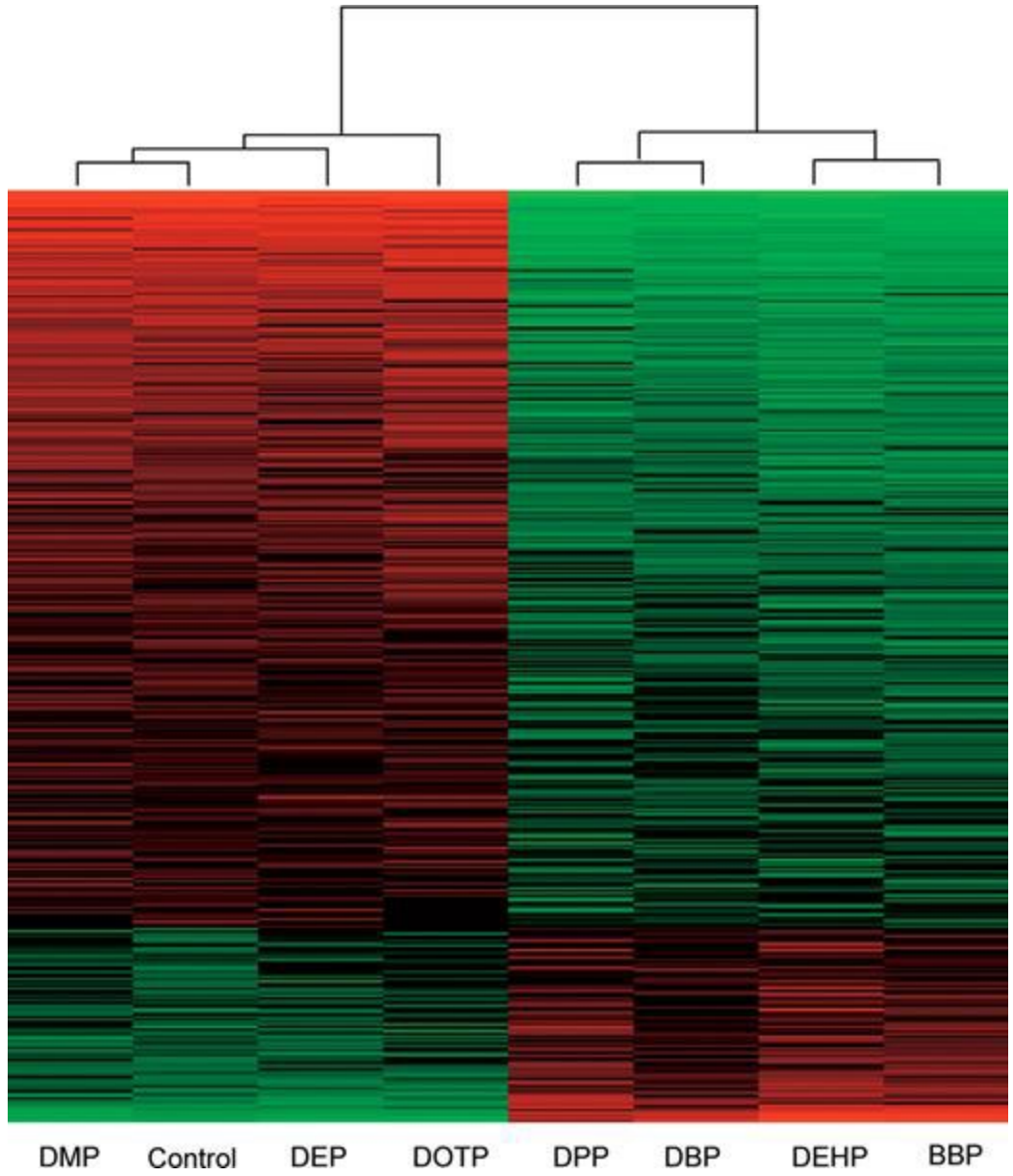
- The hallmark developmental effect, however, is on male reproductive development
  - Undescended testes, epididymal agenesis and malformed seminiferous tubules, hypospadias
  - Howdeshell et al. (2008) Environ. Res. 108:168-176.
  - Effect may occur at a dosage as low as 1.5 mg/kg/day (Lee et al (2004) Toxicology 203:221-238)

Diethyl and Dimethyl Phthalate Developmental Toxicity

- NTP study in rats at doses up to 5% in feed (approx. 4 g/kg/day), gestation days 6-15
- Effects on maternal body weight gain and increased relative liver weight at high dose for both compounds
- No adverse developmental effects at any dose level

Field et al (1993) Teratology 48:33-44

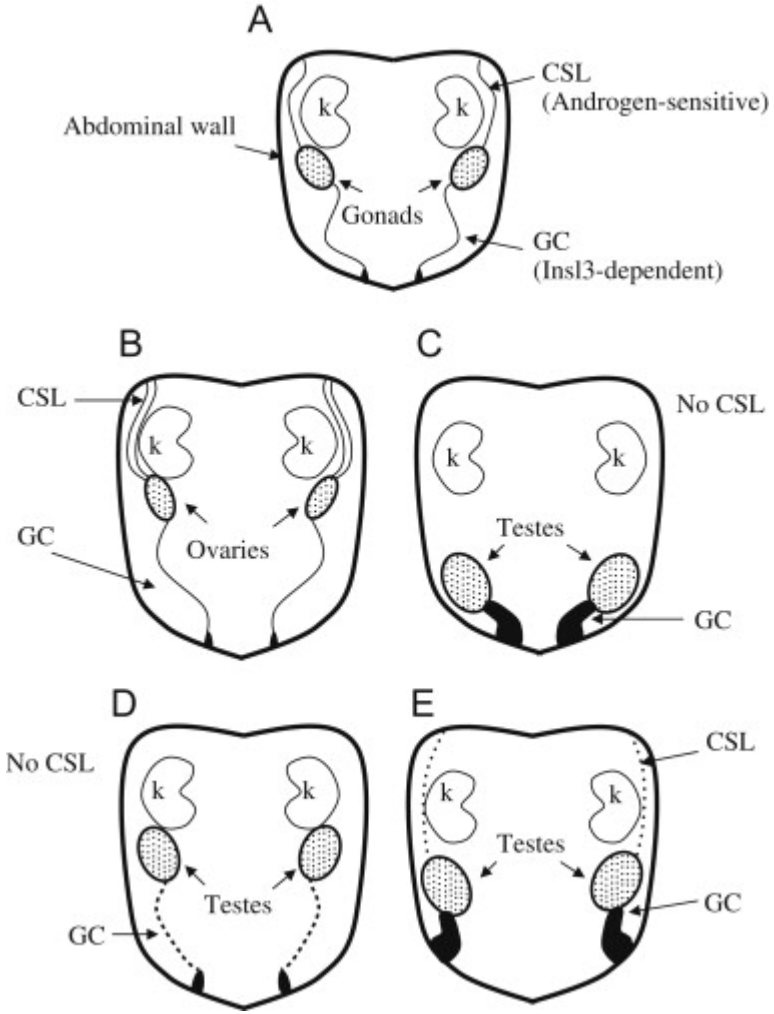
- Note: The male reproductive effects produced by dibutyl are not produced by dimethyl or diethyl phthalate


DMP
Control
DEP
DOTP
DPP
DBP
DEHP
BBP

Dibutyl Phthalate Mechanism of Action

- Anti-androgenic effects, but not through action on the androgen receptor
- Interference with steroid synthesis, especially in the fetal Leydig cell
  - Steroidogenic acute regulatory gene (StAR) and possibly cyp17a
- Interference with insulin-like hormone 3 (insl3) expression
- Decreased expression of genes that regulate cell-cell interactions in the testis

- NB: It is possible that synthesis of progesterone could be affected in adults, which could explain the whole litter loss in developmental studies but this has not been directly studied



Gonad placement and migration in the fetus

- During the indeterminate stage, the gonads are located just caudal to the kidneys and attached via the cranial suspensory ligaments and gubernacular cord
- Male fetuses normally produce testosterone, which causes regression of the CSL and expression of insl3, which promotes GC growth, pulling the testes into the inguinal region.
- In the absence of androgens, the CSL is retained and the GC regresses, leaving the ovaries more rostral
- Interference with androgen synthesis in males inhibits testicular descent

Other mechanisms to consider

- Estrogen receptor binding in vitro
  - DBP weakly interacts with the estrogen receptor in vitro, with a potency somewhere between 3000 and 100,000 fold less than 17-beta estradiol
  - MBP does not produce a response
  - No uterotrophic effects in vivo
- Zinc chelation
  - Early male reproductive tox and subchronic studies measured high levels of zinc in the urine of rats, correlated this with toxicity
  - May be chelation by the monoester or phthalic acid
  - Zinc deficiency may explain low level of malformations at high dose levels in rodent studies

Phthalates and human reproductive outcomes

- Some correlations between adverse outcomes and urinary phthalate levels:
  - Pubertal progression in boys
  - Pre-term delivery
  - Anogenital distance
  - Most robust associations are with DEHP and DBP (or MEHP and MBP)
- Phthalates are often lumped together
- Effect sizes are small
- Plausibility is often questionable
  - E.g., higher correlations for phthalic acid than for monoester metabolites, when measured

Conclusions

- Dibutyl phthalate is a significant developmental toxicant with effects on male reproductive development at dose levels around 1.5 mg/kg/day in rodents
- The mechanism of action is an inhibition of androgen synthesis in the fetal testis
- Diethyl and dimethyl phthalate do not act via this mechanism and do not have the same toxicity. NOAELs for reproductive toxicity are orders of magnitude higher.