The Queen's Medical Research Institute  Medical School  Main Hospital

# Human relevance of testicular xenograft studies

Comments prepared at request of ACC High Phthalates Panel through a contract with ToxStrategies


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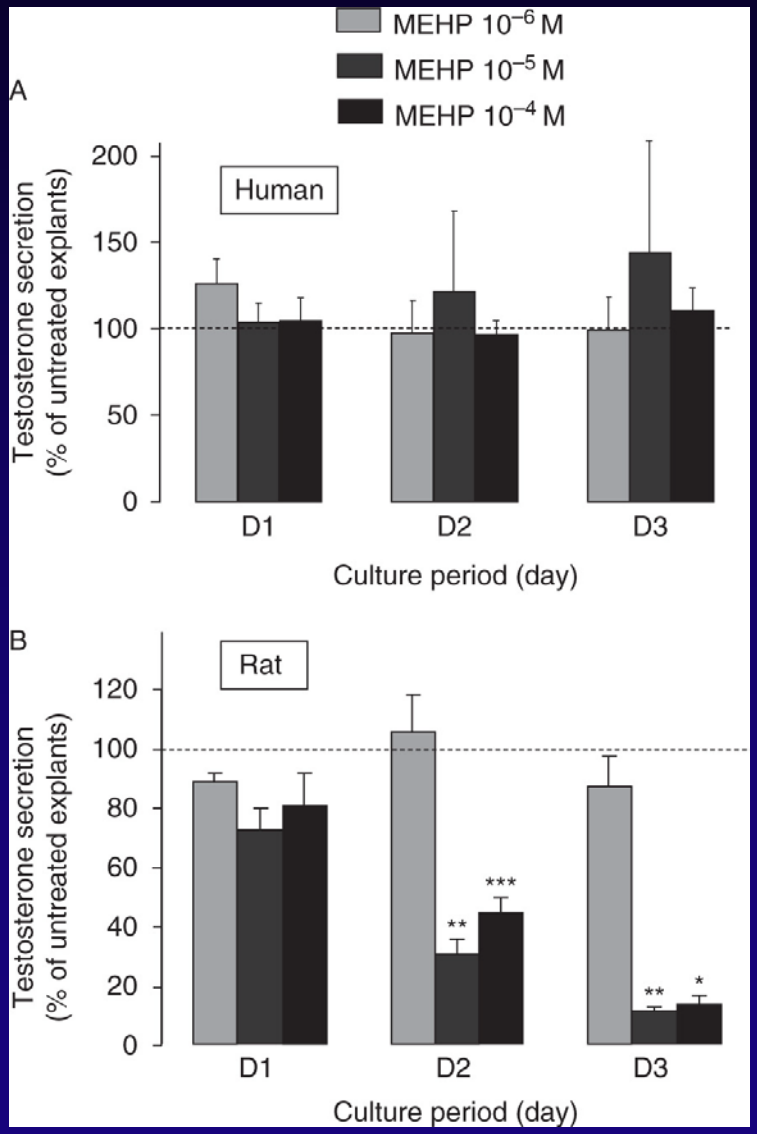
# Differential effects of MEHP in vitro on fetal rat and human testis explants

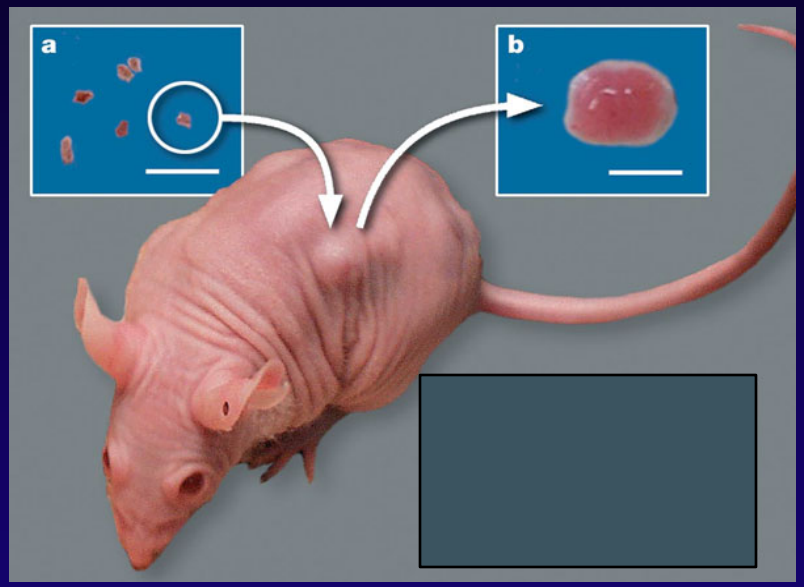



Explants for both species cover the period defined as the masculinisation programming window

Cultured under basal conditions

Taken from:
Habert et al (2014)
Reproduction 147: R119-R129

# Fetal human testis xenografting into (castrate male) nude mice



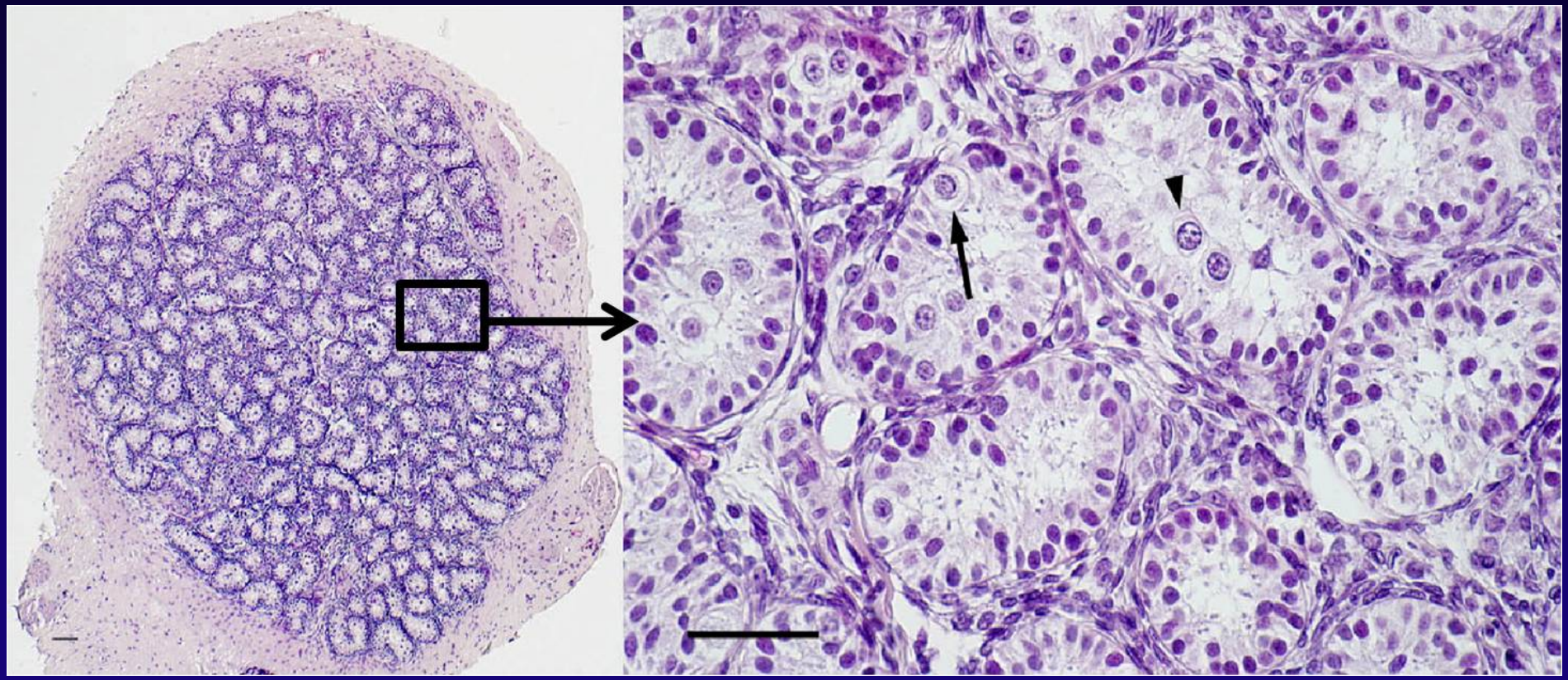

- Grafts grow normally for 6+ weeks

- Treating the host with DBP is like experimentally exposing the real human fetal testis

- Can measure testosterone production by the grafts by (i) serum T , and (ii) Seminal vesicle weight in the hosts

- Have to treat hosts with hCG to ensure T production

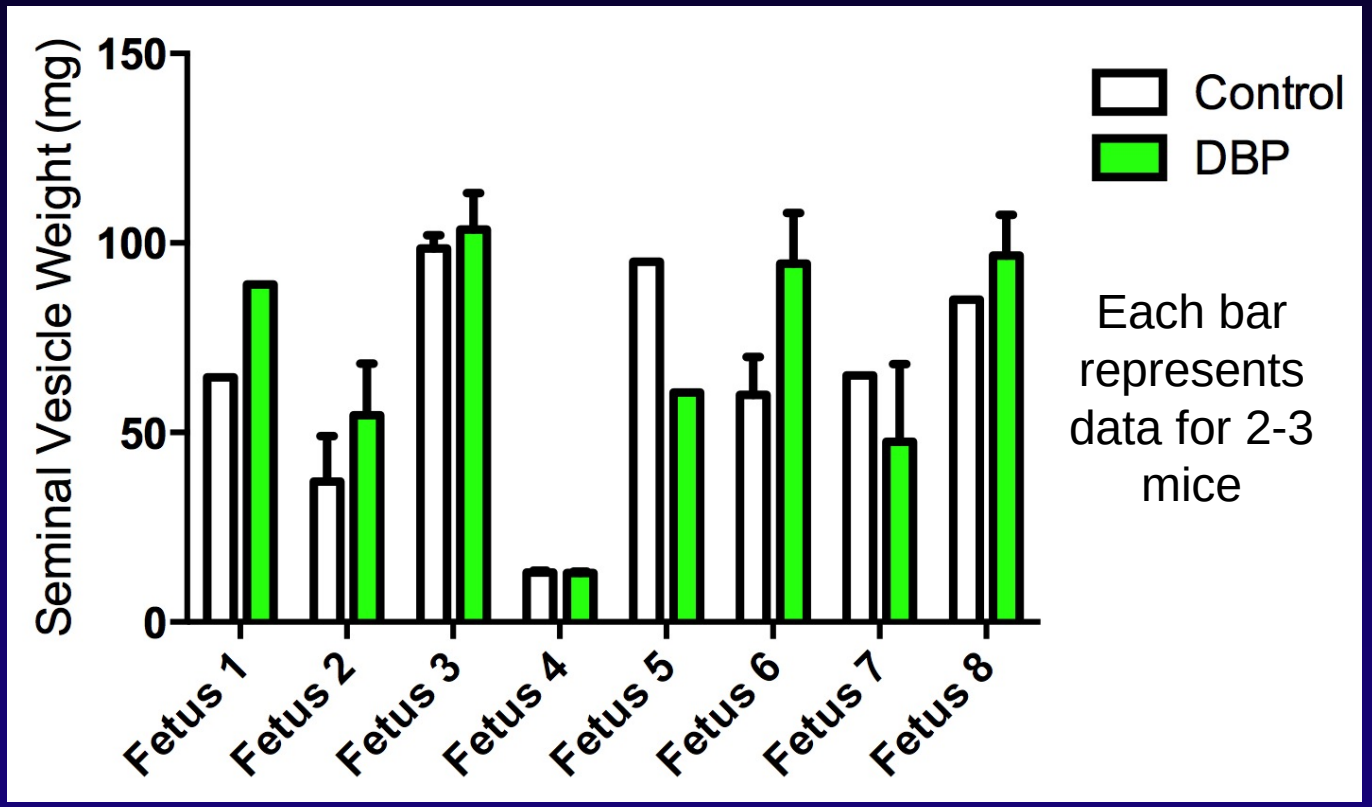
# Human fetal testis xenograft 6 weeks after grafting



Mitchell *et al* Hum Reprod 2010

# Exposure of human fetal testis xenografts to 500mg/kg/day DBP has no steroidogenic effects

Xenografts recovered + 6 weeks; hCG treatment from 1-6 weeks




Data show Means ± SEM for N=8 fetuses (14-20 weeks' gestation)
Statistical analysis was by 2-factor ANOVA



Adapted from: Mitchell et al 2012 J Clin Endocrinol Metab 97: E341-E348

**Human Reproduction, Vol.24, No.9 pp. 2244–2254, 2009**
Advanced Access publication on June 2, 2009 doi:10.1093/humrep/dep200

human reproduction

ORIGINAL ARTICLE *Reproductive biology*

# Effect of fetal or neonatal exposure to monobutyl phthalate (MBP) on testicular development and function in the marmoset

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**BACKGROUND:** Fetal exposure of male rats to some phthalates induces reproductive abnormalities, raising concerns for similar effects in humans. In order to address this in a more appropriate animal model, the aim of the present studies was to investigate the effect of fetal/neonatal exposure to monobutyl phthalate (MBP) in a non-human primate, the marmoset. In particular, to determine if exposure resulted in effects at birth, or in adulthood, similar to those in male rats, and whether there was evidence for induction of carcinoma-in-situ (*CIS*) or testicular germ cell tumours (TGCT).

**METHODS:** Pregnant female marmosets were dosed from ~7–15 weeks gestation with 500 mg/kg/day MBP and male offspring studied at birth (1–5 days; $n = 6$) or in adulthood ($n = 5$). In another study, newborn males ($n = 5$ co-twins) were dosed with 500 mg/kg/day MBP for 14 days, commencing at ~4 days of age.

**RESULTS:** Fetal exposure of marmosets to MBP did not affect gross testicular morphology, reproductive tract development or testosterone levels at birth, nor were germ cell number and proliferation, Sertoli cell number or germ:Sertoli cell ratio affected. In two of six MBP-exposed animals, unusual clusters of undifferentiated germ cells were found, but their significance is unclear. Neonatal MBP treatment did not affect germ cell numbers or differentiation. Fetal exposure to MBP did not affect testis size/morphology, germ cell numbers or fertility in adulthood. There was no evidence for *CIS* or TGCT.

**CONCLUSIONS:** Fetal exposure of marmosets to MBP does not measurably affect testis development/function or cause testicular dysgenesis, and no effects emerge by adulthood. Some effects on germ cell development were found, but these were inconsistent and of uncertain significance.

# MBP treatment of pregnant marmosets

- Administered the main ‘active’ metabolite of dibutyl phthalate, namely monobutyl phthalate (MBP), at 500mg/kg/day by oral gavage

- Treatment for 7 weeks starting from 6.5-8 weeks’ gestation (N=9) - will encompass the MPW

- Killed male offspring at either 2-4 days of age (N=6) or as adults (N=5)

- Assessed male reproductive phenotype

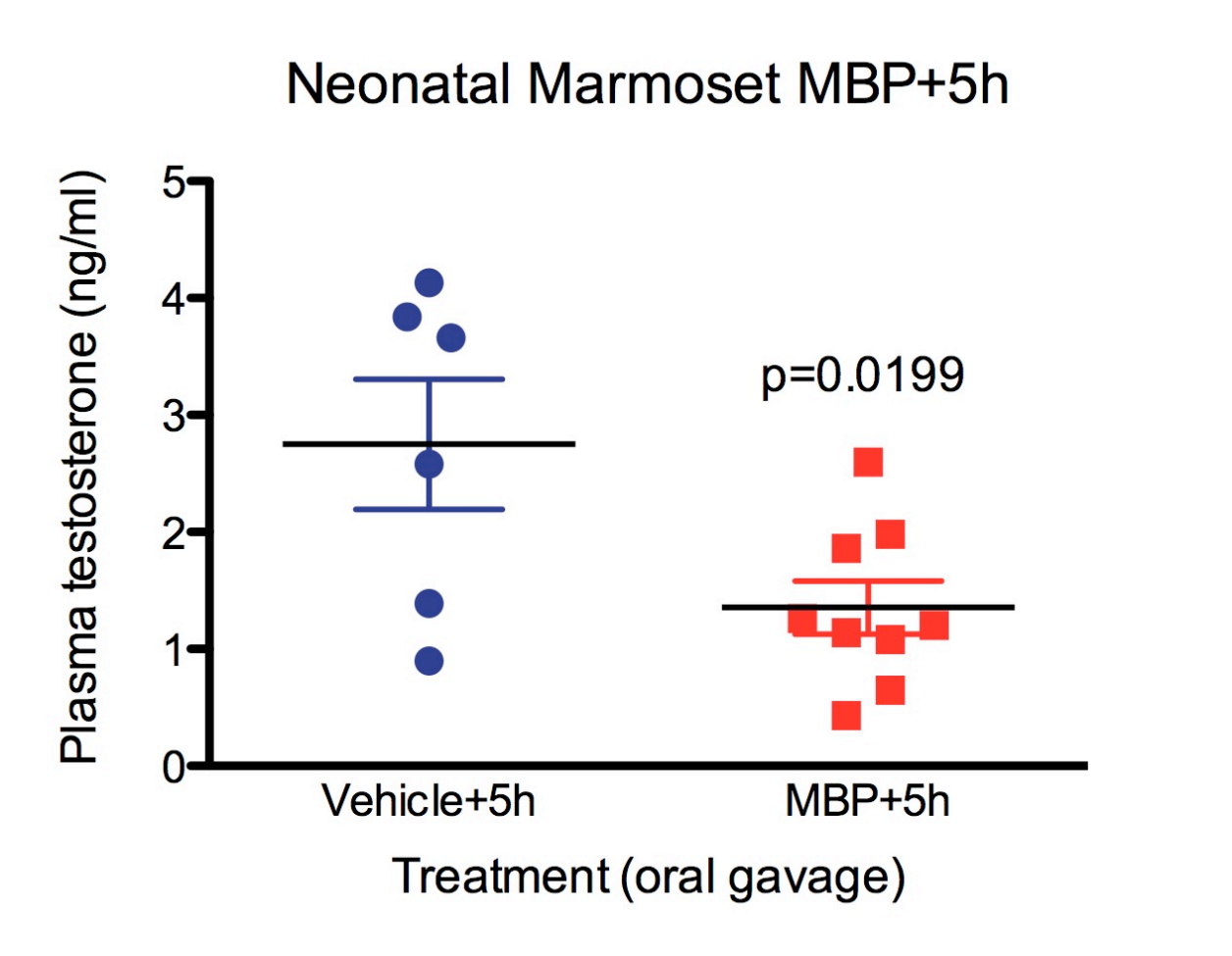
# Effect of in utero exposure of marmosets to 500mg/kg MBP (7-15 weeks' gestation)

| Abnormality | Incidence in rat studies | Number of affected marmosets out of N=11 | |
|---|---|---|---|
| | | Expected | Observed |
| Hypospadias | 17% | 2 | 0 |
| Cryptorchidism | ≥ 70% | ≥7 | 0 |
| Small testes/impaired spermatogenesis | ≥ 70% | ≥7 | 0 |
| Focal testicular dysgenesis | ≥ 50% | ≥5 | 0 |
| | | | |

Number of expected cases in the marmoset was based on the incidence in rat studies

From: McKinnell et al (2010) Hum Reprod 24: 2244-2254

# Effect of a single oral dose of 500mg/kg MBP on T levels +5h in neonatal marmosets




From N Hallmark et al 2007 Environ Health Perspect 115: 390-396

# Conclusion

DBP/MBP has no effect on steroidogenesis by the human/marmoset fetal testis but does impair steroidogenesis by the neonatal (marmoset) testis