# Mode of action and human relevance evaluation of Dibutyl Phthalate (DBP)-induced male reproductive system toxicity.

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## Introduction

Dibutyl phthalate (DBP) is used as a plasticizer in a variety of commercial and consumer products (US EPA, 2014; Kavlock et al. 2002). The largest source of DBP exposure in humans is food, with inhalation and dermal exposures considered minimal (Kavlock et al. 2002). Epidemiological studies provide evidence of human exposure and altered androgen levels during lifestages at which androgen production is critical for the normal development and function of the male reproductive system (WHO/UNEP, 2013), and experimental studies using rat models have reported that exposure to DBP is associated with adverse responses in the male reproductive system. Effects include decreased androgen production, agenesis of the male reproductive system and increased incidence of internal and external malformations after developmental exposures (e.g. degeneration of seminiferous tubules, hypospadias), and decreased fertility and sperm counts (CPSC, 2010; Makris et al 2013; US EPA, 2009). Evidence from post-natal exposure studies also suggests that young animals are more sensitive to phthalate-induced testicular injury than adults (Boekelheide et al 2004). However, recent studies using ex-vivo human tissue culture preparations, or rodent and human testicular tissue xenografts report that human fetal testes are resistant to phthalate induced disruption of testosterone production (Johnson et al., 2012; Albert and Jégou, 2014). Such findings raise questions about the human relevance of the androgen-related endpoints measured in experimental rodents exposed to phthalates.

A mode of action framework was used to evaluate the available evidence from experimental and in-vitro studies according to lifestage of exposure. Studies considered for this analysis include:

- Exposures during the masculinization programming window (MPW; gestational period during which development of the male reproductive system occurs).
- Exposures during early post-natal stages.

## Methods

The experimental and mechanistic studies considered in this analysis were obtained from the literature search performed by the US EPA Integrated Risk Information System (IRIS). Studies for DBP or MBP were identified from online databases (PubMed, Web of Science, Toxline, and TSCATS2) using search terms designed to capture pertinent studies. The last update was performed in July 2017. Title/abstract screening followed by a full text review was performed to identify relevant studies on male reproductive effects and related mechanisms/pathways (See Figure 1 below). The types of in-vivo and in-vitro studies considered most informative to our evaluation were:

- Gestational DBP exposure studies that use mammalian in-vivo and in-vitro models, and human xenograft and ex vivo models treated during the masculinization programming window.
- Additional ex-vivo studies that expose human fetal testis tissue cultures to DEHP or its metabolite MEHP.
- Studies aimed at characterizing the receptor for DBP at a molecular level.
- Post-natal DBP exposure studies that use mammalian model species, including in-vivo, xenograft, and cell culture models.

The available mechanistic and toxicological evidence was analyzed in concordance with the framework and levels of biological organization used for mode of action Action analysis for non-cancer effects and development of Adverse Outcome Pathways (Bobbis et al 2008; Edwards et al 2016). As recommended by US EPA's Framework for Assessing Health Risk of Environmental Exposures to Children and the World Health Organization International Programme on Chemical Safety, the available mechanistic and toxicological studies and endpoints that inform the mode of action for DBP-induced male reproductive effects were evaluated according to the lifestage of exposure

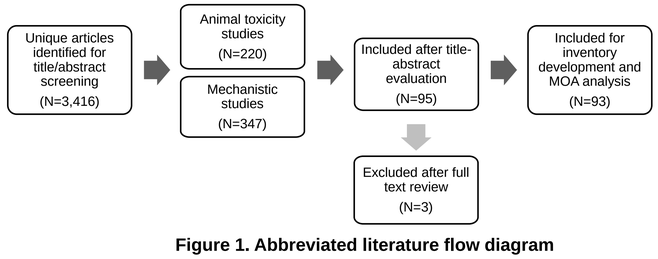
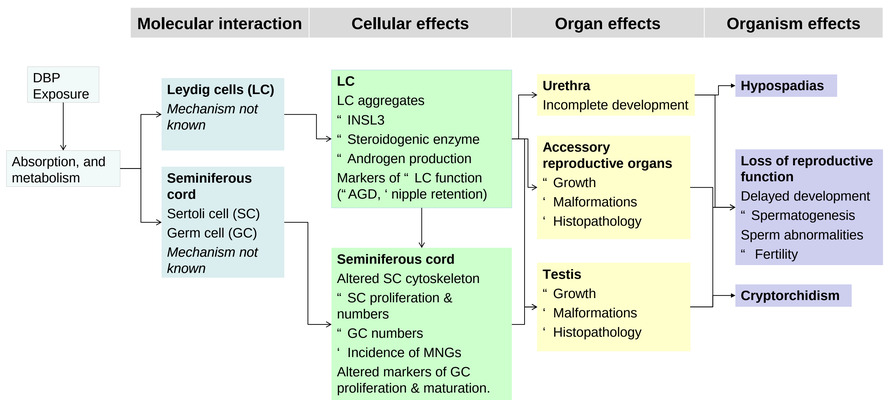



**Figure 1. Abbreviated literature flow diagram**

## Figure 2: Pathway for DBP-induced male reproductive effects after gestational exposure during MPW

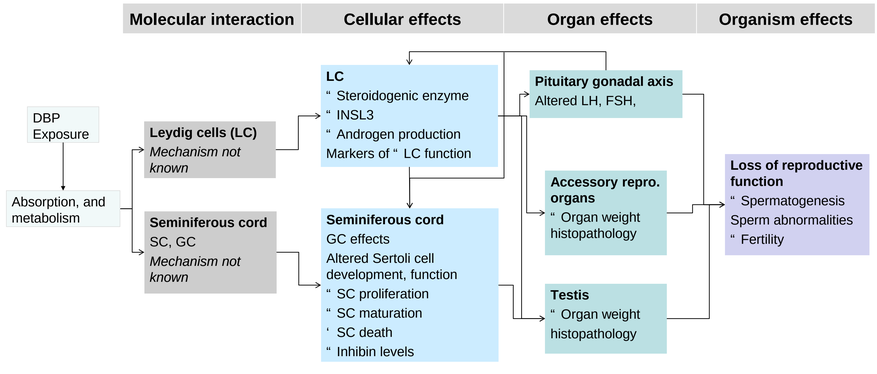



## Table 1: Preliminary cross-species coherence analysis for gestational effects

| Key event | Animal in-vivo evidence | Animal (ex-vivo, xenograft) | Humans evidence (ex-vivo, xenograft) |
|---|---|---|---|
| Leydig cells (LCs) | No evidence | | Not identified in studies |
| Sertoli cells (SCs), germ cells (GCs) | No evidence | | Not identified in studies |
| LCs | ■ Rat [*ms*] & rabbits [1]<br>■ Mice [1]<br>☐ Marmosets [1] & mice [3] | ■ Rat xenograft [2]<br>☐ Rat ex-vivo* [2]<br>☐ Mice xenograft [1]<br>☐ Mice ex-vivo [1] | ☐ Human xenografts [3]<br>☐ Human ex-vivo [2] |
| SCs, GCs | ■ SC and GC effects in rats [*ms*]<br>■ SC and/or GC effects in rabbits [1] mice [3]<br>☐ Marmoset [1] | ■ Mice ex-vivo [2]<br>■ Mice xenograft [1] | ■ Human xenografts [3]<br>■ Human ex-vivo [1] |
| Urethra | ■ Rats [*ms*] | ■ Rat xenograft [1] | Not evaluated |
| Accessory reproductive organs | ■ Rats [*ms*] & rabbits [1]<br>☐ Marmoset [1] | | ☐ Human xenograft [2] |
| Testis | ■ Rats [*ms*], rabbits [1] & mice [2]<br>☐ Marmoset [1] & mice [3] | | No evaluated |
| Organism effects: reproductive functions | ■ Rats [*ms*] & rabbits [1]<br>☐ Marmoset [1] | | Not evaluated |

■ – Evidence of response to exposure
☐ – Evidence of no response (or reduced sensitivity) to exposure
[ ] – number of studies identified in the literature
*ms* – Many studies
* Both rat and mouse ex-vivo studies report no effect on basal T production, but gonadotropin-stimulated T was inhibited by exposure.

## Figure 3: Pathway for DBP-induced male reproductive effects in post-natal lifestages




## Results and discussion

***Gestational exposure studies:***

- Fetal rats appear more sensitive to DBP-induced anti-androgenic effects than are mice and may be more sensitive than other rodent species, non-human primates, and human fetal testis xenografts and ex-vivo tissue cultures.
- DBP-induced androgen-independent effects in the seminiferous cord (SC & GC) are conserved among most mammalian models (rats, rabbits and mice) and human xenografts.

***Post-natal lifestage studies using peri-pubertal or sexually mature animals:***

- DBP-induced Leydig cell effects are conserved in different mammalian species: (rats, rabbits, mice, gerbils, and guinea pigs, non-human primates [in-vivo and xenografts]).
- DBP-induced effects in the seminiferous cord (SC & GC) are also conserved among most mammalian models (rats, mice, and non-human primate [xenograft]).

## Table 2: Preliminary cross-species coherence analysis for effects in early post-natal lifestages

| Key Event | Animal evidence (in-vivo) | Animal evidence (cell culture, xenograft) | Human evidence (ex-vivo, xenograft) |
|---|---|---|---|
| Leydig cells (LCs) | No evidence | | No studies available |
| Sertoli cells (SCs), Germ cells (GCs) | No evidence | | No studies available |
| LCs | ■ Rats [17], mice [3], rabbits [1], marmoset [1],<br>☐ Rats [1], mice [1] | ■ Cell culture models (rat [3], mouse [7] & dog [1]),<br>■ Rhesus monkey xenografts [1] | No studies available |
| SCs, GCs | ■ Rats [30], mice [5],<br>☐ Marmoset [1] | ■ Cell culture (rats [9]), mice [3], rhesus monkey xenografts [1] | No studies available |
| Pituitary gonadal axis | ■ Rats [6]<br>☐ Rabbits [1], mice [3], rats [1] | | No studies available |
| Accessory reproductive organs | ■ Rats [10], rabbits [1], gerbils [1], mice [2]<br>☐ Mice [3], rats [8] | ■ Rhesus monkey xenografts [1] | No studies available |
| Testis | ■ rats [34], rabbits [1], mice [5], & guinea pigs [1]<br>☐ Hamsters [1], rats [5] mice [3], marmosets [2] | ■ Rhesus monkey xenografts [1] | No studies available |
| Reproductive functions | ■ Rats [12], rabbits [1], mice [5], & guinea pigs [1]<br>☐ mouse [2], rats [1] | | No studies available |

■ – Evidence of response to exposure
☐ – Evidence of no response (or reduced sensitivity) to exposure
[ ] – number of studies identified in the literature

## Selected references

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