Male reproductive toxicity in animal studies of diisobutyl phthalate (DIBP): a case study application of systematic review approaches

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Introduction

Diisobutyl phthalate (DIBP) is used as a plasticizer in a variety of industrial and consumer products. Although DIBP has been less widely studied compared to other phthalates, there is evidence that DIBP and its primary metabolite, monoisobutyl phthalate (MIBP), cause male reproductive toxicity. A recent systematic review of endocrine-related low-dose toxicity by the National Academies of Sciences (NAS) evaluated the effects of DIBP on three anti-androgenic outcomes [testosterone, anogenital distance (AGD), and hypospadias], and concluded that DIBP is a presumed human hazard based on decreased fetal testosterone in rodents exposed during gestation. The Integrated Risk Information System (IRIS) performed a systematic review of male reproductive effects of DIBP exposure that considered all outcomes and all life stages of exposure, following recommendations in the 2014 NAS review of the IRIS program. Here, we use studies that evaluated testosterone in male rodents exposed to DIBP or MIBP as a case study of the IRIS systematic review process. We also summarize the overall conclusions for male reproductive effects identified in the IRIS systematic review of DIBP, and compare these results to the findings of NAS.

Methods

Animal studies for DIBP or MIBP were identified by searching four online databases (PubMed, Web of Science, Toxline, and TSCATS2), using search terms designed to capture all potentially pertinent studies. The last update was in July 2017. Title/abstract screening was used to identify primary health effect studies that exposed non-human mammalian animals to any administered dose of DIBP or MIBP via oral, dermal, or inhalation routes. These studies were evaluated by at least two reviewers using the approach in Figure 1.

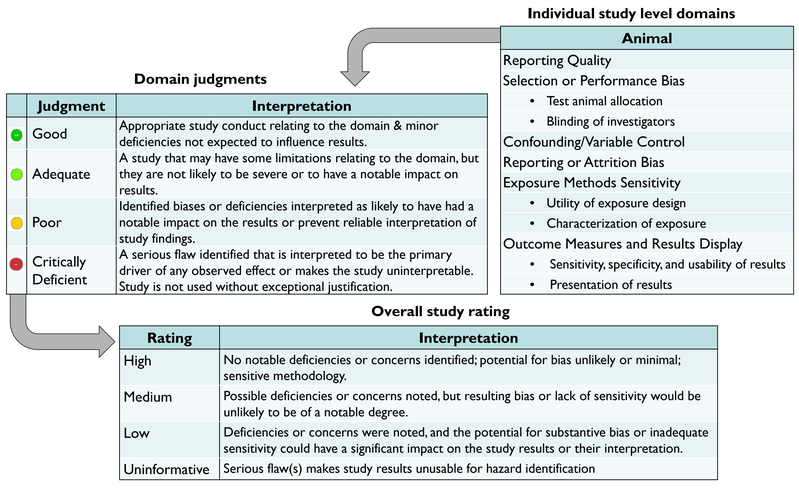

Figure 1. Study evaluation process

After study evaluation, the evidence for each health effect outcome was synthesized according to the developmental stage of exposure. Based on this synthesis, the evidence was assigned a conclusion of *robust*, *moderate*, *slight*, *indeterminate*, or *compelling evidence of no effect*. The ratings for individual outcomes were summarized into an overall conclusion for male reproductive effects using a structured framework (see Poster by Yost et al.).

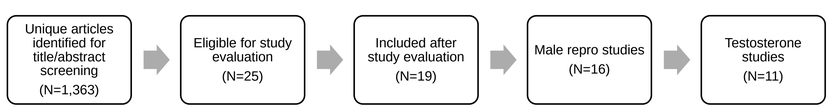

Figure 2. Abbreviated literature flow diagram

Results

Table 1. Animal studies of testosterone and DIBP or MIBP exposure. Of the 11 studies that evaluated testosterone in male rats or mice, 7 exposed animals during gestation and/or until weaning, and 4 were postnatal exposures of males near the time of puberty. The postnatal exposure studies had higher risk of bias because of reporting limitations, including uncertainty about the pubertal status of the test animals at the time of exposure.

Reference	Study description			Study evaluation										
	Population	Exposure	Outcome	Reporting quality	Test animal allocation	Blinding of investigators	Confounding / variable control	Reporting or attrition bias	Characterization of exposure	Utility of exposure design	Sensitivity, specificity, and usability of results	Presentation of results	Overall confidence
Borch et al. 2006	Rat (Wistar)	Diet GD 7-19	Fetal T prod/conc	G	G	A	G	G	A	A	G	G	High
Howdeshell et al. 2008	Rat (Sprague-Dawley)	Gavage GD 8-18	Fetal T prod	G	A	A	G	G	A	G	G	G	High
Saillenfait et al. 2017	Rat (Sprague-Dawley)	Gavage GD 13-19	Fetal T prod	G	G	A	G	G	G	G	G	G	High
Furr et al. 2014	Rat (Sprague-Dawley)	Gavage GD 14-18	Fetal T prod	G	G	A	G	A	A	G	G	G	High
Hannas et al. 2012	Rat (Sprague-Dawley)	Gavage GD 14-18	Fetal T prod	G	A	A	G	G	G	G	G	A	High
Hannas et al. 2011	Rat (Sprague-Dawley)	Gavage GD 14-18	Fetal T prod	G	A	A	G	G	A	G	G	G	High
Wang et al. 2017	Mouse (ICR)	Diet GD 0-21; GD 0-PND 21	Postnatal and Adult T conc	G	G	G	A	A	A	G	A	P	Medium
Oishi and Hiraga 1980a	Mouse (JCL:ICR)	Diet PND 35-42	Postnatal T conc	A	NR	NR	A	G	A	P	A	A	Medium
Oishi and Hiraga 1980b	Mouse (JCL:ICR)	Diet PND 35-42	Postnatal T conc	A	NR	NR	A	P	A	P	A	A	Medium
Oishi and Hiraga 1980c	Rat (JCL:Wistar)	Diet PND 35-42	Postnatal T conc	A	NR	NR	A	P	A	P	A	P	Low
Oishi and Hiraga 1980d	Rat (JCL:Wistar)	Diet PND 35-42	Postnatal T conc	A	NR	NR	A	P	A	A	A	A	Medium

Abbreviations: Gestation day (GD); Postnatal day (PND); Testosterone (T) production (prod) or concentration (conc)

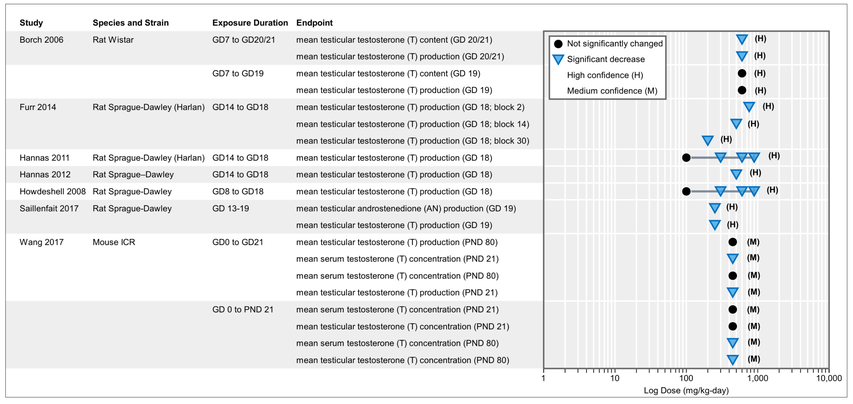

Figure 3. Summary of exposure-response for testosterone from gestational exposure studies.

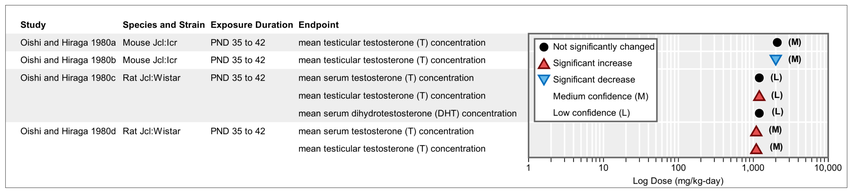

Figure 4. Summary of exposure-response for testosterone from postnatal exposure studies.

The synthesis of results for testosterone is summarized in an evidence profile table (Table 2). Gestational exposure studies provided *robust* evidence for effects on testosterone, whereas evidence from postnatal exposure studies was found to be *indeterminate*. Evidence judgments for other male reproductive endpoints identified in this systematic review are summarized in Table 3.

Table 2. Evidence profile table for animal studies of testosterone and DIBP

	Studies and interpretation	Factors that increase strength	Factors that decrease strength	Summary of findings
Gestational exposure	**High confidence** Borch et al. 2006; Furr et al. 2014; Hannas et al. 2011; Hannas et al. 2012; Howdeshell et al. 2008; Saillenfait et al. 2017 **Medium confidence** Wang et al. 2017	• Consistency • Exposure-response gradient • Effect size • Biological plausibility (support from mechanistic evidence) • Minimal concern for bias		⊕⊕⊕ ROBUST A dose-related decrease in testicular androgen levels or production (up to -96% compared to control) was observed in all studies in rats and mice that evaluated this endpoint. Several of these studies also demonstrate decreased testicular expression of genes and proteins in the steroidogenesis pathway, which provides support for biological plausibility.
Postnatal exposure	**Medium confidence** Oishi and Hiraga 1980a; Oishi and Hiraga 1980b; Oishi and Hiraga 1980d **Low confidence** Oishi and Hiraga 1980c	• Biological plausibility	• High risk of bias • Unexplained inconsistency	◯◯◯ INDETERMINATE A dose-related increase in androgen levels was observed in two rat studies (Oishi and Hiraga 1980c-d), whereas androgen levels were decreased or not changed in mice (Oishi and Hiraga 1980a-b).

Table 3. Within stream evidence judgments for animal evidence of male reproductive toxicity following DIBP exposure

Outcome	Includes these endpoints	Evidence following gestational exposure	Evidence following postnatal exposure
Testosterone	Androgen levels	Robust	Indeterminate
Male morphological development	AGD, nipple retention, preputial separation, hypospadias, cleft prepuce, exposed os penis, cryptorchidism	Robust	N/A
Sperm evaluation and histopathological effects in testis or epididymis	Sperm concentration and motility, oligospermia, azoospermia, granulomatous inflammation, tubular degeneration, tubular necrosis, interstitial hyperplasia	Robust	Moderate
Reproductive organ weight	Testis, epididymis, seminal vesicle weights	Moderate	Moderate
Male reproductive overall		Robust	

Discussion

Overall, the results from animal studies of male reproductive effects provide robust evidence of a hazard from DIBP exposure. Conclusions for testosterone are consistent with those of NAS (2017). The NAS review was limited to gestational exposure studies and excluded studies that exposed animals to a single high dose (≥500 mg/kg-day); therefore, NAS only considered two fetal testosterone studies, and had inadequate evidence to evaluate the effects of DIBP on AGD or hypospadias. The IRIS systematic review included all dose levels and life stages of exposure, and was able to evaluate a wider range of androgen-dependent and -independent male reproductive outcomes. *Disclaimer: The views expressed in this poster are those of the author(s) and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.*

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