

Male reproductive toxicity in animal studies of diisobutyl phthalate (DIBP): a case study application of systematic review approaches Erin E. Yost¹, Susan Y. Euling², James A. Weaver¹, Brandiese E. J. Beverly¹, Nagalakshmi Keshava¹, Anuradha Mudipalli¹, Xabier Arzuaga², Todd Blessinger², Laura Dishaw¹, Andrew

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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 antiandrogenic 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.

				Animal				
				Reporting Quality				
		Domain judg	gments	Selection or Performance Bias				
	Judgment		Interpretation	Test animal allocation				
		Appropriate study	conduct relating to the domain & minor	 Blinding of investigators 				
•	Good	deficiencies not ex	spected to influence results.	Confounding/Variable Control				
	Adequate	A study that may h	nave some limitations relating to the domain, but	Reporting or Attrition Bias				
•		they are not likely	to be severe or to have a notable impact on	Exposure Methods Sensitivity				
		Identified biases of	r deficiencies interpreted as likely to have had a	Utility of exposure design				
•	Poor	notable impact on	the results or prevent reliable interpretation of	Characterization of exposure				
		study findings.		 Outcome Measures and Results Display Sensitivity, specificity, and usability of results 				
•	Critically	A serious flaw ide	rved effect or makes the study uninterpretable					
	Deficient	Study is not used	without exceptional justification.	Presentation of results				
			Overall study rati	ng				
		Rating	Interpretat	tion				
		High	No notable deficiencies or concerns identified; potential for bias unlikely or minimal; sensitive methodology.					
Medium			Possible deficiencies or concerns noted, but resulting bias or lack of sensitivity would be unlikely to be of a notable degree.					
Low Deficiencies or concerns were noted, and the po sensitivity could have a significant impact on the				ential for substantive bias or inadequate cudy results or their interpretation.				
		Uninformative	Serious flaw(s) makes study results unusable for h	azard identification				

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.).

Office of Research and Development



Individual study level domains

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	Stu	udy descriptio	n					Study e	valuatio	n			
	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	А	G	G	А	А	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	Р	Medium
Oishi and Hiraga 1980a	Mouse (JCL:ICR)	Diet PND 35-42	Postnatal T conc	А	NR	NR	А	G	А	Р	А	А	Medium
Oishi and Hiraga 1980b	Mouse (JCL:ICR)	Diet PND 35-42	Postnatal T conc	А	NR	NR	А	Р	А	Р	А	А	Medium
Oishi and Hiraga 1980c	Rat (JCL:Wistar)	Diet PND 35-42	Postnatal T conc	А	NR	NR	А	Р	А	Р	A	Р	Low
Oishi and Hiraga 1980d	Rat (JCL:Wistar)	Diet PND 35-42	Postnatal T conc	А	NR	NR	А	Р	А	А	А	А	Medium
Abbreviations: Gestation day (GD); Postnatal day (PND); Testosterone (T) production (prod) or concentration (conc)													

Study	Species and Strain	Exposure Duration	Endpoint		
Borch 2006	Rat Wistar	GD7 to GD20/21	mean testicular testosterone (T) content (GD 20/21)	Not significantly changed	💙 (Н)
			mean testicular testosterone (T) production (GD 20/21)	Significant decrease	💙 (Н)
		GD7 to GD19	mean testicular testosterone (T) content (GD 19)	High confidence (H)	• (H)
			mean testicular testosterone (T) production (GD 19)	Medium confidence (M)	• (H)
Furr 2014	Rat Sprague-Dawley (Harlan)	GD14 to GD18	mean testicular testosterone (T) production (GD 18; block 2)		V (H)
			mean testicular testosterone (T) production (GD 18; block 14)		💙 (H)
			mean testicular testosterone (T) production (GD 18; block 30)		V (H)
Hannas 2011	Rat Sprague-Dawley (Harlan)	GD14 to GD18	mean testicular testosterone (T) production (GD 18)		• · · · · · · · · · · · · · · · · · · ·
Hannas 2012	Rat Sprague–Dawley	GD14 to GD18	mean testicular testosterone (T) production (GD 18)		💙 (Н)
Howdeshell 2008	Rat Sprague-Dawley	GD8 to GD18	mean testicular testosterone (T) production (GD 18)		• · · · · · · · · · · · · · · · · · · ·
Saillenfait 2017	Rat Sprague-Dawley	GD 13-19	mean testicular androstenedione (AN) production (GD 19)		V (H)
			mean testicular testosterone (T) production (GD 19)		V (H)
Wang 2017	Mouse ICR	GD0 to GD21	mean testicular testosterone (T) production (PND 80)		• (M)
			mean serum testosterone (T) concentration (PND 21)		(M)
			mean serum testosterone (T) concentration (PND 80)		• (M)
			mean testicular testosterone (T) production (PND 21)		V (M)
		GD 0 to PND 21	mean serum testosterone (T) concentration (PND 21)		• (M)
			mean testicular testosterone (T) concentration (PND 21)		• (M)
			mean serum testosterone (T) concentration (PND 80)		V (M)
			mean testicular testosterone (T) concentration (PND 80)		(M)
				1 10	100 1.000 10
				Log Do	ose (mg/kg-day)

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

Study	Species and Strain	Exposure Duration	Endpoint
Oishi and Hiraga 1980a	Mouse Jcl:Icr	PND 35 to 42	mean testicular testosterone (T) co
Oishi and Hiraga 1980b	Mouse Jcl:Icr	PND 35 to 42	mean testicular testosterone (T) co
Oishi and Hiraga 1980c	Rat Jcl:Wistar	PND 35 to 42	mean serum testosterone (T) conc
			mean testicular testosterone (T) co
			mean serum dihydrotestosterone (
Oishi and Hiraga 1980d	Rat Jcl:Wistar	PND 35 to 42	mean serum testosterone (T) conc
			mean testicular testosterone (T) co



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 		Definition of these studies also demonstrate decreases in the sticular and rogen 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		Rob	ust

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 (e500 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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