Evidence profile table for DIBP and male reproductive toxicity

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The evidence profile table is a tool that complements the evidence integration narrative for human and animal data. Explanations for factors that increase or decrease confidence are provided in summaries.

Outcome*		Studies and interpretation	Factors that increase strength	Factors that decrease strength	Summary of findings	Within stream evidence judgment	Inference across evidence streams	Overall conclusion
HUMAN STUDIES							**Relevance of animal data to humans** •Role of androgen-dependent and –independent pathways in male reproductive system development, maturation, and function is conserved across mammalian species. **Cross-stream coherence** •Testosterone is reduced with phthalate exposure in both humans and animals during different lifestages. **Susceptibility** •Developmental stages are particularly susceptible to perturbation by phthalates **Other relevant information** •Evidence from DBP, a structurally similar phthalate, provides robust evidence of male reproductive toxicity in humans, likely due to higher exposure levels and a larger number of studies	⊕⊕⊕ Overall conclusion that DIBP causes male reproductive toxicity, based on: 1) Robust evidence from oral exposure studies in rats and mice, with significant outcomes in gestational exposure studies at doses as low as 300 mg/kg-day; 2) Moderate evidence in human epidemiological studies of decreased testosterone in adult men with median metabolite concentrations in urine ranging from 7-48 ng/mL. Evidence for other outcomes was from populations with low urine metabolite concentrations, which reduced study sensitivity; and 3) Supporting mechanistic evidence demonstrating decreased testicular steroidogenesis and INSL-3. Evidence from animals is presumed relevant to humans. Lower level of evidence in humans can be explained by low sensitivity and few available studies.
Testosterone (adult)		(All cross sectional studies) **Medium confidence** Meeker and Ferguson (2014) Pan et al., 2015 **Low confidence** Chang et al. (2015) Den Hond et al. (2015)	• Consistency • Minimal risk of bias in medium confidence studies	• Few studies available	⊕⊕◯ MODERATE Inverse associations between DIBP exposure and testosterone levels in 3/4 studies (Meeker and Ferguson et al., 2014, Pan et al., 2015, Chang et al., 2015), 2 of which were statistically significant. No studies examined exposure-response gradient.	⊕⊕◯ MODERATE Based on data for testosterone in adults, supported by slight evidence in other outcomes with low sensitivity and few available studies explaining lack of clear associations.		
Anogenital distance (AGD), semen parameters, pubertal development, time to pregnancy, hypospadias/cryptorchidism					⊕◯◯ SLIGHT			
ANIMAL STUDIES								
Gestational exposure	Testosterone	**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 risk of 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.	⊕⊕⊕ ROBUST Supported by consistency and coherence across outcomes, with mechanistic evidence (e.g. decreased testicular expression of steroidogenic enzymes and INSL-3) providing support for biological plausibility. The greatest weight of evidence came from gestational exposure studies, whereas postnatal exposure studies were limited by risk of bias concerns.		
	Male morphological development	**High confidence** Borch et al. 2006 Saillenfait et al. 2006 Saillenfait et al. 2008 Saillenfait et al. 2017 **Medium confidence** Wang et al. 2017	• Consistency within rat studies • Exposure-response gradient • Effect size • Biological plausibility • Minimal risk of bias		⊕⊕⊕ ROBUST All rat studies observed a dose-related increase in effects consistent with decreased testosterone and INSL-3, including increased time to puberty, decreased AGD, nipple retention, cryptorchidism, hypospadias, exposed os penis, and cleft prepuce. No effects on AGD were observed in mice (Wang et al. 2017).			
	Sperm evaluation and histopathological effects in testis or epididymis	**High confidence** Saillenfait et al. 2008 **Medium confidence** Borch et al. 2006 Wang et al. 2017	• Consistency • Exposure-response gradient • Effect size • Biological plausibility		⊕⊕⊕ ROBUST Adverse effects on the testis and/or sperm were observed in rats and mice, including a dose-related increased incidence of pathological lesions of the testis (Borch et al. 2006, Saillenfait et al., 2008), epididymal oligo- or azoopermia (Saillenfait et al. 2008), and decreased sperm concentration and motility (Wang et al. 2017).			
	Reproductive organ weight	**High confidence** Saillenfait et al. 2008 **Medium confidence** Wang et al. 2017	• Exposure-response gradient • Biological plausibility • Minimal risk of bias	• Few studies	⊕⊕◯ MODERATE Decreased reproductive organ weights were observed in rats (Saillenfait et al. 2008), whereas a consistent trend in testis weight was not observed in mice (Wang et al. 2017).			
Postnatal exposure	Testosterone				◯◯◯ INDETERMINATE			
	Sperm evaluation and histopathological effects in testis or epididymis	**Low confidence** Oishi and Hiraga 1980c Foster et al. 1981	• Consistency • Biological plausibility • Coherence with gestational exposure studies	• High risk of bias • Few studies	⊕⊕◯ MODERATE Rats were found to have increase testicular atrophy (Foster et al. 1981) and decreased spermatocytes and spermatogonia (Oishi and Hiraga 1980c).			
	Reproductive organ weight	**Medium confidence** Oishi and Hiraga 1980a Oishi and Hiraga 1980b Oishi and Hiraga 1980c Oishi and Hiraga 1980d **Low confidence** Foster et al. 1981 U. Rochester 1954 Zhu et al. 2010	• Consistency within rat studies • Biological plausibility • Coherence with gestational exposure studies	• High risk of bias in some studies • Unexplained inconsistency	⊕⊕◯ MODERATE In rats, a dose-related decrease in absolute testis weight was consistently observed (Oishi and Hiraga 1980c-d, Foster at al. 1981, University of Rochester 1954). In mice, Zhu et al. (2010) observed decreased testis weight in the highest dose group, whereas Oishi and Hiraga (1980a-b) observed increased testis weight.			

*Outcomes with slight or indeterminate evidence received a full systematic review, but were not significant contributors to the overall conclusion, so the details of the evidence are not provided here.

Comparison with findings from the National Academy of Sciences (NAS) systematic review of the low-dose toxicity of phthalates (2017)

Table 1: Summary of conclusions for DIBP from NAS 2017.

Endpoint	Initial hazard evaluation
Testosterone	Presumed human hazard • Based on high level of evidence from animal studies and inadequate evidence from human studies
AGD	Not classifiable • Based on inadequate evidence from human and animal studies
Hypospadias	Not classifiable • Based on inadequate evidence from human and animal studies

Both IRIS and NAS (2017) concluded that DIBP is likely to cause male reproductive toxicity in humans. However:

- NAS was only able to draw this conclusion for testosterone, based on the high level of evidence from rodent studies. Other endpoints (AGD and hypospadias) were determined to have inadequate evidence available.
- The IRIS systematic review was broader in scope (see Table 2) and was able to draw conclusions for a range of androgen-dependent and –independent male reproductive outcomes.

Table 2: Summary of major scoping differences between the IRIS and NAS systematic reviews of DIBP

	IRIS	NAS
Exposure	All life stages and dose levels	In utero exposure; Animal studies using a single dose e500 mg/kg-day are excluded.
Outcomes	Any male reproductive outcome	Testosterone, AGD, hypospadias

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