

## Appendix 2: Empirical evidence for KER 3488 “Decrease, intratesticular testosterone leads to hypospadias “

The table details the data sets considered reliable with or without restriction in the evaluation of methodological reliability.

For the effects on testosterone and hypospadias, ↓\* denotes a statistically significant reduction in the parameter at the given dose. In column 5, intratesticular testosterone refers to measurements in whole testes extracts, as compared to *ex vivo* incubation of testes and measurement of testosterone in culture media.

Abbreviations: GD=gestational day; Ns= not significant; PND=postnatal day

Study design				Upstream event (testosterone)						Downstream event (hypospadias)				Reference
Substance	Species	Exposure window	Doses (mg/kg bw/day)	Intratesticular/ <i>ex vivo</i>	Method	Timepoints	Effect (ng/testis)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Timepoint	Effect (% males)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	
Dibutyl phthalate	Rat	GD16-19	0 750	Intratesticular	ELISA	GD18 + GD22	↓*	-	750	PND90	0 23*	-	750	(van den Driesche et al., 2020)
Dibutyl phthalate	Rat	GD14-22 <sup>1</sup>	0 500	Intratesticular	Radioimmunoassay	GD18	↓* <sup>2</sup>	-	500	Adult	0 31*	-	500	(Drake et al., 2009)
Diisooctyl phthalate	Rat	GD12-21	0 0.01 0.1 0.5 1	<i>Ex vivo</i>	TFC-MS/MS	GD19	6.46 ±1.12 6.60 ±1.44 4.26 ±0.41* 1.8 ±0.5* 1.03 ±0.22*	0.01	0.1	10 or 12 weeks	0 N/A <sup>3</sup> 0 0 36 <sup>4</sup>	0.5	1	(Saillenfait et al., 2013)

\*statistically significant from control group

<sup>1</sup>For intratesticular testosterone, exposure period was GD14-16

<sup>2</sup>Not significant for litter means

<sup>3</sup>N/A: Not available, no measurement for this dose

<sup>4</sup>No statistics were reported in this study, but given that hypospadias is very rarely observed in control animals it is considered highly significant