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/ *ex vivo***	**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[1]	0 500	Intratesticular	Radioimmuoassay	GD18	↓*[2]	-	500	Adult	0 31*	-	500	(Drake et al., 2009)
Diisooctyl phthalate	Rat	GD12-21	0 g/kg bw/day) 0.01 0.1 0.5 1	*Ex vivo*	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[3] 0 0 36[4]	0.5	1	(Saillenfait et al., 2013)

*statistically significant from control group
[1]For intratesticular testosterone, exposure period was GD14-16
[2]Not significant for litter means
[3]N/A: Not available, no measurement for this dose
[4]No statistics were reported in this study, but given that hypospadias is very rarely observed in control animals it is considered highly significant