

Appendix 2: Empirical evidence for KER 3449 decreased intratesticular testosterone leading to short AGD.

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

For the effects on testosterone and AGD, ↓* 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 (AGD)				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 (AGDi)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	
α-cypermethrin	Rat	GD13-19	0 0.1 1 5 10	Ex vivo	Liquid chromatography mass spectrometry	GD19	Ns Ns ↓* ↓*	1	5	GD19	1.77 ±0.07 1.74 ±0.07 1.75 ±0.08 1.74 ±0.12 1.78 ±0.08	10	-	(Saillenfait AM et al., 2017)
Butyl benzyl phthalate	Rat	GD14-18	0 500	Ex vivo + Intratesticular	Radioimmunoassay	GD18	↓*	-	500	PND2	↓* ⁵	-	500	(Hotchkiss AK et al., 2004)
Butyl benzyl phthalate	Rat	GD14-18	0 500	Ex vivo + Intratesticular	Radioimmunoassay	GD18	↓*	-	500	Adult	Ns ⁵	500	-	(Hotchkiss AK et al., 2004)
Butyl benzyl phthalate + Linuron	Rat	GD14-18	0 500 + 75	Ex vivo + Intratesticular	Radioimmunoassay	GD18	↓*	-	500 + 75	PND2	↓* ⁵	-	500 + 75	(Hotchkiss AK et al., 2004)
Butyl benzyl phthalate + Linuron	Rat	GD14-18	0 500 + 75	Ex vivo + Intratesticular	Radioimmunoassay	GD18	↓*	-	500 + 75	Adult	↓* ⁵	-	500 + 75	(Hotchkiss AK et al., 2004)
Dibutyl phthalate	Rat	GD13-20	0 500	Intratesticular	Enzyme immunoassay	GD20	↓*	-	500	GD20	↓*	-	500	(Lourengo AC et al., 2014)
Dibutyl phthalate	Rat	GD13-21	0 100 500	Intratesticular	Enzyme immunoassay	GD21	Ns ↓*	100	500	GD21	2.04 ±0.03 1.88 ±0.04* 1.79 ±0.04*	-	100	(Martino-Andrade AJ et al., 2009)
Dibutyl phthalate	Rat	GD16-20	0 100 500	Intratesticular	Radioimmunoassay	GD20	Ns ↓*	100	500	PND5	4.73 ±0.29 ² 4.74 ±0.20 4.29 ±0.27*	100	500	(Pike et al., 2014)
Dibutyl phthalate	Rat	GD12-19	0 100	Intratesticular	Radioimmunoassay	GD19	Ns after 4 h, ↓* after 24 h	-	100	GD19	1.95 ±0.28 ⁵ 1.90 ±0.27	100	500	(Struve MF et al., 2009)

			500				(~70% reduction) ↓* (app. 90-95% reduction)				1.67 ±0.18*			
Dibutyl phthalate	Rat	GD15.5-18.5	0 750	Intratesticular	Enzyme immunoassay	GD17.5 + GD21.5	↓*	-	750	GD22	↓* ²	-	750	(van den Driesche et al., 2020)
Dibutyl phthalate	Rat	GD15.5-18.5	0 750	Intratesticular	Enzyme immunoassay	GD17.5 + GD21.5	↓*	-	750	PND90	6.04 ±0.09 4.91 ±0.19*	-	750	(van den Driesche et al., 2020)
Dibutyl phthalate + Diethylhexyl phthalate	Rat	GD13-21	0 100 + 150	Intratesticular	Enzyme immunoassay	GD21	↓*	-	100 + 150	GD21	2.04 ±0.03 1.90 ±0.06	100 + 150	-	(Martino-Andrade AJ et al., 2009)
Diethylhexyl phthalate	Rat	GD7-PND17	0 750	Ex vivo + intratesticular	Fluoroimmuno assay	GD21	↓*	-	750	PND3	↓* ⁴	-	750	(Borch J et al., 2004)
Diethylhexyl phthalate + Diethylhydroxylamine	Rat	GD7-PND17	0 750 + 400	Ex vivo + intratesticular	Fluoroimmuno assay	GD21	↓*	-	750 + 400	PND3	↓* ⁴	-	750 + 400	(Borch J et al., 2004)
Diisonyl phthalate	Rat	GD7-21	0 300 600 750 900	Intratesticular ¹	Fluoroimmuno assay	GD21	Ns ↓* ns ns	900	600	PND1	11.60 ±1.04 11.43 ±0.82 11.31 ±0.20 11.29 ±0.75 11.01 ±0.88*	750	900	(Boberg et al., 2011)
Diisobutyl phthalate	Rat	GD13-19	0 250	Ex vivo	Liquid chromatography mass spectrometry	GD19	↓*	-	250	GD19	1.77 ±0.07 1.68 ±0.07*	-	250	(Saillenfait AM et al., 2017)
Diisonyl phthalate	Rat	GD12-19	0 50 250 750	Intratesticular	Radioimmunoassay	GD19 ⁴	100 % of control 104 50* 35*	50	250	GD20	15.53 15.14 15.29 15.64	750	-	(Clewell et al., 2013)
Ketoconazole	Rat	GD7-21	0 50	Ex vivo + intratesticular	Fluoroimmuno assay	GD21	Ex vivo: 4.90 ±3.80 Intratesticular: 1.64 ±0.68 Ex vivo: 1.10 ±0.06 Intratesticular: 0.35 ±0.44 *	-	50	GD21	2.47 ±0.03 2.20 ±0.07*	-	50	(Taxvig et al 2008)

Linuron	Rat	GD14-18	0 75	Ex vivo + Intratesticular	Radioimmunoa ssay	GD18	↓*	-	75	PND2	↓* ⁵	-	75	(Hotchkiss AK et al., 2004)
Linuron	Rat	GD14-18	0 75	Ex vivo + Intratesticular	Radioimmunoa ssay	GD18	↓*	-	75	Adult	↓* ⁵	-	75	(Hotchkiss AK et al., 2004)
Prochloraz	Rat	GD7-PND16	0 50 150	Ex vivo + intratesticular	Fluoroimmuno assay	GD21	↓* ↓*	-	50	PND1	11.6 ±0.4 10.9 ±0.6* 10.2 ±0.8*	-	50	(Laier P et al., 2006)
Prochloraz	Rat	GD7-PND17	0 30	Ex vivo + intratesticular	Fluoroimmuno assay	GD21	Ex vivo ns; Intratesticular ↓*	-	30	PND0	4.34 ±0.32 ² 4.43 ±0.29	30	-	(Vinggaard AM et al., 2005)
Mixture (prochloraz, deltamethrin, methiocarb, simazine, tribenuron)	Rat	GD7-PND17	0 20	Ex vivo + intratesticular	Fluoroimmuno assay	GD21	↓*	-	20	PND0	4.34 ±0.32 ² 4.43 ±0.29	20	-	(Vinggaard AM et al., 2005)
Mixture (BBP, DBP, DCHP, DEHP, DHEP, DHP, DIBP, DIHEP, DPEP, linuron DDE, prochloraz, procymidone purifluquinaz on, vinclozolin)	Rat	GD14-18	0 0.05% ³ 0.5% 3.3% 6.25% 12.5% 25% 50% 100%	Ex vivo	Radioimmunoa ssay	GD18	10.0 ±0.5 9.6 ±0.6 10.2 ±0.8 9.6 ±.8 7.6 ±.5s* 7.4 ±0.9* 3.9 ±0.5* 1 ±0.2* 0.6 ±0.2*	3.3%	6.25%	PND2	1.83 ±0.03 1.85 ±0.05 1.85 ±0.5 1.82 ±0.02 1.88 ±0.03 1.73 ±0.05* 1.47 ±0.02* 1.23 ±0.02* 1.23 ±0.09*	6.25%	12.5%	(Conley JM et al., 2021)

*statistically significant from control group

¹Also measured *ex vivo* testosterone, but no effect was observed.

²AGD (mm), not corrected for body weight in statistics.

³% of full dose. 50% dose contains NOEL for each chemical.

⁴Testosterone also measured at GD20, but with no significant effect.

⁵AGD (mm), corrected for body weight in statistics.