Guidelines/Criteria	
Reference:	Rider CV, Furr JR, Wilson VS, Gray LE Jr. 2008. A mixture of seven antiandrogens induces reproductive malformations in rat. Int J Androl 31:249-262.
In vivo **Study Type**	
Route of Administration	oral gavage
Species & age of animals	Pregnant Sprague-Dawley rats on gestation day 2
Study Duration	until PND175-195
Type of Mixture	
Binary	
>2 components	seven antiandrogens
Similar acting or dissimilar	dissimilar
What Mode of Action was investigated?	disrupt androgen signalling pathway via different mechanisms of toxicity: androgen receptor antagonism in the reproductive tract vs. inhibition of androgen synthesis in the foetal testis
Parameters/End points Measured	
Target organs/Critical effects	androgen signalling pathway disruption: anogenital distance, retained areolae/nipples, hypospadias, epidiidymal agenesis, gubernacular agenesis, ectopic undescended test
Pharmacological changes or adverse effects	adverse effects
Individual Components	
Characterisation of individual compounds	
Name, exact chemical name, CAS no.	vinclozolin, procymidone, linuron, prochloraz, benzyl butyl phthalate, dibutyl phthalate, diethylhexyl phthalate. CAS numbers were not given.
Were dose responses established for individual components?	yes
Were no effect levels established?	no
Were doses below the NO(A)ELs investigated?	yes (see mixture make-up)
Mixtures Investigated	
Number of dose levels	four (100, 75, 50 and 25% of top dose).
How does the mixture make-up compare to individual components? (e.g. low dose) equivalents used?)	The two lowest dose groups contained individual chemicals at or below their NOAELs (data from D-R curves) for inducing male reproductive tract malformations. At the high dose, each chemical was in the mixture at 1/7 of its ED100 for inducing reproductive tract malformations
No. of technical replicates per exposure condition (*in vitro*)	
No. of animals per dose group (*in vivo*)	
Observations/Findings	* at the time of necropsy on PND 175-195 all of the androgen sensitive endpoints examined displayed dose-dependent effects following exposure to the mixture of seven chemicals. Mixture exposure significantly decreased the weight of androgen sensitive organs (ventral prostate, seminal vesicles, testes, epididymis and LABC). Non-significant decrease glans penis weight. * these adverse reproductive tract responses appeared to conform best to toxic equivalency or dose-addition models of mixture toxicity (response addition and integrated addition models under-estimated mixture effects in the higher dose groups). * the authors suggest that cumulative risk assessments may need to move from limiting to chemicals that act via identical cellular and molecular mechanisms to a broader definition of 'mode of action' or 'mechanism of toxicity' based upon common effects.
Overall opinion	
(e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	* good study, although the experimental part was difficult to follow (group sizes, concentrations investigated, etc.) the mixture part and modelling was described very well. * concentrations below the individual NOAELs of the chemicals in the mixture were investigated. * One of the studies that describes dose-additive responses. In other words: how to address chemicals that disrupt a common pathway by disrupting different cellular or molecular targets? (at the tissue level, the chemicals do not act independently and thus act in a dose-additive manner). * belongs to a series of studies conducted by the same group (see the reviews by Rider 2009 and 2010).