

Guidelines/Criteria	
	Reference: Howdeshell KL, Wilson VS, Furr J, Lambright CR, Rider CV, Blystone CR, Hotchkiss AK, Gray LE Jr. 2008. A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. Toxicol Sci 105(1):153-165.
In vivo Study Type Route of Administration Species & age of animals	Gavage Adult female Sprague-Dawley rats
Study Duration	Gestation day 8 - 18
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	None Five phthalate esters Similar Suppression of foetal testosterone production
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	Maternal body weight gain, whole litter loss, foetal mortality, foetal testicular testosterone production, testicular testosterone levels Adverse effects
Individual Components Characterisation of individual compounds Name, exact chemical name, CAS no. Were dose responses established for individual components? Were no effect levels established? Were doses below the NO(A)ELs investigated?	Benzyl butyl phthalate BBP (CAS 85-68-7, purity 98%); dibutyl phthalate DBP (CAS 84-74-2, purity 99%); diethylhexyl phthalate DEHP (CAS 117-81-7, purity 99%); diethyl phthalate DEP (CAS 84-66-2, purity 99%); diisobutyl phthalate DiBP (CAS 84-69-5, purity 99%), dipentyl phthalate DPP (CAS 131-18-0, purity 99%). Yes, at 4 - 7 dose levels per compound Yes Yes
Mixtures Investigated Number of dose levels How does the mixture make-up compare to individual components? (e.g. low dose) equivalents used? No. of technical replicates per exposure condition (<i>in vitro</i>) No. of animals per dose group (<i>in vivo</i>)	Seven Doses of individual phthalates were selected for estimated equipotency in reduction of testosterone production based on single compound experiments Between 2 and 7 (higher numbers in the steep part of the dose-response curve)
Observations/Findings	Maternal body weight gain was reduced at 520 mg/kg/d of the mixture (40% of top dose), foetal testosterone production was the most sensitive endpoint (reduction at 260 mg/kg/d; 20% of top dose) and was reduced at 1/2 to 1/3 of dose levels that increased foetal mortality. The effects seen closely matched a dose-addition model.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	A well designed and conducted study which is highly relevant for our project. Sufficient number of dose levels and the relevant MoA were investigated. In some dose groups the number of animals was low (n=2). Overall, this study lends credible support to a dose-addition model for chemical mixtures with a common mode of action.