

Guidelines/Criteria	
	Reference: Ohlsson A, Ullerås E, Cedergreen N, Oskarsson A. 2010b. Mixture effects of dietary flavonoids on steroid hormone synthesis in the human adrenocortical H295R cell line. Food Chem Toxicol 48:3194-3200.
In vitro Study Type Route of Administration Species & age of animals	Hormone secretion in H295R cells
Study Duration	24h exposure to compounds
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	Binary and ternary (3 component) Presumed to be similar acting as were flavonoids Effects on corticosteroid and sex steroid hormone synthesis and secretion
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects <i>In vitro</i>	Cortisol, aldosterone, testosterone and estradiol secretion
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?	Three dietary flavonoids Daidzein Genistein Apigenin Yes Yes No
Mixtures Investigated Mixture components (if not all compounds used in mixture) 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>)	1 All three components 7 concentrations for individual components; 6 concentrations for ternary mixture More or less the same concentrations evaluated individually and in ternary mixture. Two independent experiments and 3 replicates per concentration
Observations/Findings	All three flavonoids inhibited hormone secretion, which with the exception of estradiol could be predicted by both CA and IA. The inhibition of estradiol secretion was underestimated by CA and especially IA.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Well conducted assays with clear expectations concerning predictivity cited at the beginning of the studies. Drawback was that concentrations below the NOECs did not seem to have been investigated.