

Guidelines/Criteria	
	Reference: Charles GD, Gennings C, Tornesi B, Kan HL, Zacharewski TR, Gollapudi BB, Carney EW. 2007. Analysis of the interaction of phytoestrogens and synthetic chemicals: An in vitro/in vivo comparison. Toxicol Appl Pharmacol 218(3):280-288.
In vitro Study Type Route of Administration Species & age of animals	ER alpha reporter assay using MCF-7 cells
Study Duration	24h
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	Six synthetic chemicals plus two phyto-oestrogens Similar acting Determination of oestrogenic activity via ER alpha activation
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects <i>In vitro</i>	Chimeric receptor-reporter gene transactivation (ER alpha activation)
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?	4-tert-octylphenol Bisphenol A Methoxychlor o,p-DDT β-hexachlorocyclohexane DPN Genistein Diadzein Yes NOELS established For some components
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>)	5 - 6 dose levels of the synthetic compounds investigated +/- phyto-oestrogen mix (5-6 dose levels). Dose range spanned very low subthreshold levels to dose level at which each component was present at its individual oestrogenic threshold (1/100, 1/10, 1/2, 1, 1.5 x NOEL for each component). Not given
Observations/Findings	Low concentrations of synthetic chemical mixture did not increase oestrogenic response compared to those induced by phyto-oestrogens alone. At higher concentrations of synthetic chemicals, PE/synthetic chemical responses were significantly increased.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	ER alpha activation was observed with the two mixtures at the dose combinations investigated. Difficult to ascertain what exactly was tested and if effects were reproducible. Dose levels investigated included those below threshold for oestrogenic responses.

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In vivo Study Type Route of Administration Species & age of animals	Immature mouse uterotrophic assay Oral gavage Mouse, 17 - 18 days old
Study Duration	3 days
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	Six synthetic chemicals plus two phytoestrogens Similar acting Estrogenic activity
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects <i>In vitro</i>	Uterus Uterine weight 24h after last dose
Individual Components Characterisation of individual compounds Name, exact chemical name, CAS no.	4-tert-octylphenol Bisphenol A Methoxychlor o,p-DDT β -hexachlorocyclohexane DPN Genistein Diadzein
Were dose responses established for individual components?	Yes
Were no effect levels established?	LOELs (ie 20% increase in UT weight compared to controls)
Were doses below the NO(A)ELs investigated?	Unknown
Mixtures Investigated Number of dose levels	5 dose levels of synthetic chemical mix +/- 3 dose levels of phytoestrogen mix
How does the mixture make-up compare to individual components? (e.g. low dose) equivalents used?)	Synthetic mix: 1/100, 1/50, 1/10, 1/6, 1 of the individual LOELs
No. of technical replicates per exposure condition (<i>in vitro</i>)	
No. of animals per dose group (<i>in vivo</i>)	6 animals/group
Observations/Findings	Low concentrations of synthetic chemical mixture did not increase estrogenic response compared to those induced by phytoestrogens alone. At higher concentrations of synthetic chemicals uterotrophic response was increased +/- PE.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Sufficient dose levels investigated. Dose levels below LOEL investigated. Valid paper to include.