

Guidelines/Criteria									
	Reference: Ohlsson A, Cedergreen N, Oskarsson A, Ullerås E. 2010a. Mixture effects of imidazole fungicides on cortisol and aldosterone secretion in human adrenocortical H295R cells. Toxicology 275:21-28.								
<b>In vitro Study Type</b> Route of Administration Species & age of animals	Hormone secretion in H295R cells								
<b>Study Duration</b>	24h exposure to compounds								
<b>Type of Mixture</b> 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 imidazole fungicides Effects on corticosteroid synthesis and secretion								
<b>Parameters/End points Measured</b> Target organs/Critical effects Pharmacological changes or adverse effects <i>In vitro</i>	Cortisol and aldosterone secretion								
<b>Individual Components</b> 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 imidazole fungicides Ketoconazole Prochloraz Imazilil Yes Yes for cortisol secretion but not for aldosterone secretion No								
<b>Mixtures Investigated</b> 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> )	<table border="0"> <thead> <tr> <th>1</th><th>2</th></tr> </thead> <tbody> <tr> <td>Binary combinations</td><td>All three components</td></tr> <tr> <td>9 concentrations for individual components; 8 concentrations</td><td>More or less the same concentrations evaluated individually and in ternary mixture. Binary concentrations were chosen where divergent effects were observed for cortisol and aldosterone secretion based on single component and ternary mixture concentration response curves.</td></tr> <tr> <td>Two independent experiments and between 2 and 6 replicates per concentration</td><td></td></tr> </tbody> </table>	1	2	Binary combinations	All three components	9 concentrations for individual components; 8 concentrations	More or less the same concentrations evaluated individually and in ternary mixture. Binary concentrations were chosen where divergent effects were observed for cortisol and aldosterone secretion based on single component and ternary mixture concentration response curves.	Two independent experiments and between 2 and 6 replicates per concentration	
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<b>Observations/Findings</b>	Cortisol secretion: inhibition observed by each of the components and EC50 values identical for the three molecules and the ternary mixture. Additivity was observed over the tested concentration range. Both CA and IA predictions described the effects. Aldosterone secretion: Biphasic dose responses were recorded for prochloraz and ketoconazole but not for imazilil. Consequently neither the CA or IA model could predict the effects of the mixture. Authors hypothesize that the inhibitory effect of the mixture was indicative of synergism as the effect was more potent than that observed for the individual components. However, they indicated that further studies were required to clarify this.								
<b>Overall opinion</b> (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 were not investigated. Claim of synergism needs further investigation.								