

Guidelines/Criteria	
	Reference: Kakko I, Toimela T, Tähti H. 2000. Piperonyl butoxide potentiates the synaptosome ATPase inhibiting effect of pyrethrin. <i>Chemosphere</i> 40:301-305.
<b>In vitro Study Type</b> Route of Administration Species & age of animals	Rat synaptosomes
<b>Study Duration</b>	1 hour
<b>Type of Mixture</b> Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	Yes No Dissimilar Ion-channel disruption
<b>Parameters/End points Measured</b> Target organs/Critical effects Pharmacological changes or adverse effects <i>In vitro</i>	Total ATPase and Mg-ATPase activity
<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?	Pyrethrin and piperonyl butoxide (PBO) Yes Yes, 100um for pyrethrin and 4000um for PBO according to the authors. But from the data presented it appears that the NOELs were 10um and 40um respectively. Yes, several
<b>Mixtures Investigated</b> 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, of which 4 were below that authors view of single component NOELs. From the data it appears that only 3 were below single component NOELs. Mixtures accordingly to the authors view were 0.001x & 0.0001x the NOELs for pyrethrin and PBO respectively at the lowest dose, 0.01x & 0.001x at the next dose, 0.1x & 0.01x at the next, and 1x & 0.1x at the next dose.  From the data it appears that mixtures tested were 0.01x & 0.01x the NOELs for pyrethrin and PBO respectively at the lowest dose, 0.1x & 0.1x at the next dose, 1x & 1x at the next dose. Triplicate
<b>Observations/Findings</b>	According to the authors, no effects at the three lowest mixture doses tested, and a significant reduction of total and Mg-ATPase activity at fourth dose (1x and 0.1x NOEL).  From the data it appears that there was no effect at the lowest mixture dose tested, a probably reduction in total ATPase activity at the second dose (0.1x and 0.1x the NOEL), and a reduction on total and Mg-ATPase activity at the third dose (1x & 1x the NOEL).
<b>Overall opinion</b> (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	The replication seems to have been inadequate, or inadequate stats, or both. This make it hard to interpret the results. Using the authors' view of effect levels, or using the apparent evidence presented leads to similar conclusions. Besides, the wrong endpoint has been examined - effects on ion channels would be the most sensitive endpoint rather than this presumably secondary and less sensitive ATPase endpoint.