Guidelines/Criteria			
Reference:	Akay MT, Özmen G, Elcüman EA. 1999. Effects of combinations of endosulfan, dimethoate and carbaryl on immune and hematological parameters of rats. Vet Hum Toxicol 41(5):296-299.	Selmanoğlu GÖ, Akay MT. 2000. Histopathological effects of the pesticide combinations on liver, kidney and testis of male albino rats. Pesticides 15:253-262.	Selmanoğlu GÖ, Akay MT. 2001. Biochemical study of the combined effects of endosulfan, dimethoate and carbaryl on albino rats. Pesticides 16:77-84.
***In vivo* Study Type** Route of Administration Species & age of animals	Subchronic rat study oral Swiss male albino rats 140-160g	Subchronic rat study oral Swiss male albino rats 140-160g	Subchronic rat study oral Swiss male albino rats 140-160g
Study Duration	3.5 months	3.5 months	3.5 months
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 Yes Yes Similar and dissimilar General effects on immunological and haematology parameters	 Yes Yes Similar and dissimilar Nothing specific	 Yes Yes Similar and dissimilar Nothing specific
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	 IgG and IgM and haematology Depends	 Liver, kidney and testes weights and histopathology Depends	 Serum liver enzymes, pseudocholinesterase activity and some clinical chemistry. Depends
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?	 Endosulfan, dimethoate and carbaryl Each was tested singly at 100x and 1000x the ADI. All singly had no effect at 100x ADI but effects were seen at 1000x ADI. Yes, 100x the ADI, i.e. 0.612mg/kg/d for endosulfan, 2.04mg/kg/d for dimethoate and 1.01mg/kg/d for carbaryl No	 Endosulfan, dimethoate and carbaryl No testing of single components was done, only binary and tertiary mixtures. No N/A	 Endosulfan, dimethoate and carbaryl Each was tested singly at 1000x the ADI, and each resulted in an effect. Other results from the rest of the lead author's thesis are mentioned, indicating that each chemical was also tested singly at 1x, 10x and 100x, and had no effect on these same endpoints in the same experimental model. Yes, 100x the ADI, i.e. 0.612mg/kg/d for endosulfan, 2.04mg/kg/d for dimethoate and 1.01mg/kg/d for carbaryl 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 (*in vitro*) No. of animals per dose group (*in vivo*)	 100x and 1000x the ADI for each pairwise mixture and for the three-way mixture. As effects were seen for single chemicals at 1000x ADI, only the 100x data are relevant. All tested at multiples of their ADIs. The relevant mixtures all contained either 2 or 3 of the components at 1xNOEL (i.e. 100x ADI) 10	 10x, 100x and 1000x the ADI for each pairwise mixture and for the three-way mixture. All tested at multiples of their ADIs. This is a study testing environmental mixtures. 10	 1x, 10x, 100x and 1000x the ADI for each pairwise mixture and for the three-way mixture. As effects were seen for single chemicals at 1000x ADI, the mixtures tested at this level are not relevant. All tested at multiples of their ADIs. The relevant mixtures all contained either 2 or 3 of the components at 0.01, 0.1 or 1xNOEL (i.e. 1x, 10x and 100x ADI). 10
Observations/Findings	Results for the 100x two- and three-way mixtures. The endosulfan + dimethoate mixture increased white blood cells and monocytes. The dimethoate + carbaryl mixture increased red blood cells. The other two way mixture and the three-way mixture caused no changes. Of the 48 parameters measured overall for the mixtures at this dose, only these three were statistically significant, so results appear to be chance rather than real.	There were various statistically significant effects on organ weights at 10x ADI, but none were replicated at 100x ADI. Dose-responses were not supportive of there being treatment-related effects on organ weights at 10x or 100x ADI. Some effects at 1000x are more convincingly treatment-related, but the authors had already shown that these chemicals singly had effects at 1000x ADI in this model. Histopathological changes in all three tissues for all 2- and 3-way mixtures, with a good dose-response and at least some effects at all doses tested. Inconsistent reporting of what effects were claimed to have been found. No mention of severity.	Paper focusses on tests at 1000x the ADI, but other results from the rest of the lead author's thesis are mentioned, indicating that each chemical was also tested singly and in mixtures at 1x, 10x and 100x using the same endpoints and experimental model. No effects of any single, two-way or three-way mixtures at 1x, 10x and 100x ADI. Effects of single chemicals and mixtures at 1000x ADI.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Narrow range of endpoints. An unusual study in that single chemicals and mixtures were tested at multiple of environmental exposure. Single chemical results confirm what is expected, i.e. no effects in animals at 100x ADI. Mixtures at 100x produce no effects that appear to be treatment related. Reviewing all papers by these authors together. The papers represent a single body of work, and apparently three studies, though they might in fact all be the same study, and they certainly use the same experimental model. Nowhere are the results interpreted as a whole, which is what is needed to make full and appropriate use of the data. Only the histopathology indicates any effects of mixtures at 10x or 100x - there were no effects on liver enzymes or other endpoints at these doses, yet the authors say these data are consistent, suggesting that they do not give weight to the histopathology findings (n.b. single chemicals results on histopathology are not reported). Inadequate quality of reporting and interpretation.	As there was no single-chemical testing, this is reviewed as a study testing environmental mixtures. The organ weight changes are unconvincing at 10x and 100x ADI, but the reported histopathology changes seem convincing as reported.	Taking the fully reported and other mentioned results together, this study alone shows no effects at 100x ADI for single chemicals and mixtures, but effects at 1000x ADI for single chemical and mixtures. Seems to be inconsistent with reported histopathology at 10x and 100x ADI in their previous study (Akay et al 2000), though the authors say the two datasets are consistent.