

Guidelines/Criteria	
Reference:	Jonker D, Woutersen RA, van Bladeren PJ, Til HP, Feron VJ. 1990. 4-week oral toxicity study of a combination of eight chemicals in rats: Comparison with the toxicity of the individual compounds. Food Chem Toxicol 28(9):623-631.
In vivo Study Type Route of Administration Species & age of animals	oral 4 week old male and female Wistar rats
Study Duration	4 week
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	8 compounds dissimilar; chemicals were arbitrary choses wrt MoA
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	Choice of compounds was fully arbitrary wrt target organs, MoA and (un)expected interaction. Standard observations and analyses were preformed Adverse effects
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?	Sodium metabisulphite, Mirex, Loperamide, Metaldehyde, di-n-octyltin dichloride, stannous chloride, lysinoalanine, potassium nitrite No Cas numbers were given, only supplier and (when applicable) purity Yes Yes NOAEL/10, NOAEL/3
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>)	four: NOAEL/10, NOAEL/3, NOAEL, MOAEL mixture contained compounds either at their individual MOAEL, their individual NOAEL or at 1/10 or 1/3 of their NOAEL 20: 10 males/10 females
Observations/Findings	* wide range of effects were observed in the MOAEL group, some more severe (growth retardation, reduced food intake, liver damage) and some less pronounced (changes in weight and morphology of the thymus) compared to the effects observed with the individual compounds * only a few minor changes were observed in the NOAEL group. These were considered minor either because they were considered chance findings unrelated to treatment (decreased ALAT and ALP activities in blood plasma in females) or they were of slight degree and occurred only in one sex (decreased haemoglobin content and increased relative kidney weights in males). * only three of the many parameters differed statistically significant from control values, but were considered isolated findings of no toxicological relevance. At dose levels below the NOAEL, there was no indication of increased toxicity of the combination of chemicals compared to the toxicity of the individual compounds.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	* doses below NOAEL were indeed tested * did not provide evidence of increased toxicity after exposure to a mixture of 8 chemicals, provided the exposure level of each individual compound is equal to or below its NOAEL * might be different for different mixtures - therefore, the same group tested mixtures comprising compounds with the same target organ, i.e. nephrotoxics with dissimilar (Jonker 1993) and similar MoAs (Jonker 1996) in subsequent experiments