Jonker et al, 1993a

Guidelines/Criteria	
Reference:	Jonker D, Woutersen RA, van Bladeren PJ, Til HP, Feron VJ. 1993a. Acute (24hr) toxicity of a combination of four nephrotoxicants in rats compared with the toxicity of the individual compounds. Food Chem Toxicol 31(1):45-52.
***In vivo* Study Type** Route of Administration Species & age of animals	 oral gavage (d-limonene, hexachlorobutadiene), s.c. (mercuric chloride, potassium dichromate) male Wistar rats, 10 week old (range finder), 12 week old (main study)
Study Duration	24 hr
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 four compounds dissimilar, in part not fully understood modes of action β-lyase-dependent nephrotoxicity, hyaline droplet formation, not well defined modes
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	 kidney; histopathology, related clinical chemistry and urinalysis 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?	 hexachlorobutadiene (HCBD) ≥ 98%, $HgCl_2$ ≥ 99.5%, d-limonene, $K_2Cr_2O_7$, no CAS numbers were given range finders were performed for each compounds to establish a no nephrotoxic effect level (NNEL) and a minimum nephrotoxic effect level (MNEL) yes no, but estimates of NO(AEL)s may have been conservative in part
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*)	 two mixtures contained compounds either at their individual NNEL or MNEL five
Observations/Findings	Range finder: for all compounds except d-limone dose-dependent tubular necrosis and associated changes in clinical chemistry and urinalysis, for d-limone dose dependent accumulation of hyaline droplets were observed. Main study: combination at the NNEL and individual compounds at their NNEL did not induce tubular necrosis/changes in clinical chemistry or urinalysis, however, the combination at the NNEL and d-limonene at its NNEL induced hyaline droplet accumulation with high incidence. Combination at the MNEL and individual compounds at their MNEL except d-limonene induced tubular necrosis and changes in clinical chemistry and urinalysis, however, the combination or d-limonene did not induce hyaline droplet accumulation or other changes.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Range finders do not represent comprehensive dose response studies, and hyaline droplet accumulation, in contrast to other findings, was not reproducible. Selection of the NNEL/MNEL is debatable in some cases. The numbers of groups is considered sufficient for the purpose, however, group size is too low to allow a detailed analysis of mixture effects. Proposed additivity, synergy or antagonism for certain parameters should be considered with caution: No algorithms or clear-cut explanations for these conclusion were provided. Hyaline droplet formation was not reproducible across the various experiment, no statements related to this parameter are possible.