Jonker et al, 1993b

Guidelines/Criteria	
Reference:	Jonker D, Jones MA, van Bladeren PJ, Woutersen RA, Til HP, Feron VJ. 1993b. Subacute (4-wk) oral toxicity of a combination of four nephrotoxins in rats: Comparison with the toxicity of the individual compounds. Food Chem Toxicol 31(2):125-136.
***In vivo* Study Type** Route of Administration Species & age of animals	 diet male and female wistar rats, 4week and 10 week old (range finder), 10 week old (main study)
Study Duration	4 week
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 four compounds dissimilar, partially not fully understood (mercuric chloride, lysinoalanine) nephrotoxicity via glutathione conjugation pathway/ß-lyase (HCBD) and α2µ-glubulin accumulation/hyaline droplets (d-Limonene). Not fully understood for mercuric chloride and lysinoalanine
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	 kidney, haematology and clinical chemistry, urinalysis, pathology and histology 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%, HgCl2 ≥ 99.5%, d-limonene, lysinoalanine, 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 yes, one quarter of the NNEL
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*)	 2 (NNEL and MNEL) for groups that received single compounds, and 3 (NNEL/4, NNEL and MNEL) for groups that received the four nephrotoxicants simultaneously mixtures contained compounds either at their individual NNEL, MNEL or NNEL/4 10 (controls and groups given the combination) and 5 (groups given the individual nephrotoxins)
Observations/Findings	* MNEL: combined exposure resulted in increased general toxicity (growth depression) and increased renal toxicity (impaired concentration ability of kidneys, increased absolute and relative kidney weight and histopathological changes) in males compared to individual exposure to the compounds. The sex difference was attributed to the facts that d-limonene is nephrotoxic in males only, and female rats are less susceptible to mercuric chloride * NNEL: only weak indications of increased toxicity, again only in males (slightly retarded growth and (less convincing) increased kidney weight) * NNEL/4: no signs of increased toxicity
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	 Good study. Concentrations below NOAEL were investigated. Same group has conducted more studies with similar results. Increased toxicity at combined exposure was observed (at effect levels of the individual compounds), however, no assumption of additivity can be done since this was not addressed in the study (and no increased toxicity was observed at the lower doses). Study was followed up by a similar study using a combination of nephrotoxicants with similar MoAs (see Jonker 1996).