Jacob et al, 2011

Guidelines/Criteria	
Reference:	Jacob CC, Reimschuessel R, Von Tungeln LS, Olson GR, Warbritton AR, Hattan DG, Beland FA, Gamboa da Costa G. 2011. Dose-response assessment of nephrotoxicity from a 7‐day combined exposure to melamine and cyanuric acid in F344 rats. Toxicol Sci 119(2):391‐397.
In vivo **Study Type** Route of Administration Species & age of animals	 diet F344 rat, 6 weeks
Study Duration	7 days
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 melamine and cyanuric acid act together via formation of less soluble complex. Melamine similar (bladder stones), would need to follow up on cyanuric acid. crystal formation
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	 Kidney histopath, urinary bladder lavage, blood urea nitrogen, creatinine, body weights, clinical symptoms
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?	 melamine (CAS 108-78-1), cyanuric acid not in this study yes yes, but not environmentally relevant levels if not adulteration of food
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*)	 single substances: only 1388 ppm binary mixture (1:1) tested @ 0 / 7 / 23 / 69 / 229 / 694 ppm (target doses 1 / 3.3 / 10 / 33 / 100 mg/kg /day, but not met in melamine group and high dose mixture group) highest mixture group dose level sum equals single substance control dose 6
Observations/Findings	Single substance groups: few crystals detected in melamine animals' renal tubules, but no other findings in those at 1388ppm Binary mixture groups: kidney effects detected at 694 (sacrificed moribund at day 3) and 229 ppm. NOEL 69 ppm (NOAEL 8 mg/kg bw)
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	 NOEL of mixture exposure is about 10 fold lower than would be expected from additivity. Dose levels are though not environmentally relevant. Melamine NOAEL/BMDL 13week study reported as 19-63mg/kg bw/day Interesting example of a special interaction type.