Rudzok et al, 2010

Guidelines/Criteria	
Reference:	Rudzok S, Schlink U, Herbarth O, Bauer M. 2010. Measuring and modeling of binary mixture effects of pharmaceuticals and nickel on cell viability/cytotoxicity in the human hepatoma derived cell line HepG2. Toxicol Appl Pharmacol 244(3):336-343.
***In vitro* Study Type** Route of Administration Species & age of animals	Cell viability/cytotoxicity in HepG2 liver cells
Study Duration	24h exposure to compounds
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 Binary Dissimilar Cell viability
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects *In vitro*	 Cell viability using three different measurements: MTT-, AlamarBlue® and NRU assay
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?	 One metal; one antibiotic; one anti-inflammatory Nickel Irgasan D Diclofenac Yes Yes No
Mixtures Investigated Mixture components (if not all compounds used in mixture) 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*)	 Binary Up to 9 concentrations for individual components Two designs were used : fixed ratio design (100:0, 75:25, 50:50, 25:75, 0:100 or 100:0, 33:66, 66:33, 0:100) using 9, or 5 single doses per chemical and full factorial design (7×7 concentrations per substance) to cover the whole dose–response curve Three independent experiments were conducted for each evaluation. No indication of number of replicates per experiment.
Observations/Findings	For each combination, antagonism was recorded at concentrations below the individual EC50s and synergism above the EC50s. However, in some cases the synergism was recorded only when one of the components was at toxic concentrations.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Experiments appear to be well conducted with clear objectives for each study given. No indication of number of replicates per experiment. Paper quite complicated to follow and therefore interpretation of data was difficult.