Kunz and Fent, 2006

Guidelines/Criteria	
Reference:	Kunz PY, Fent K. 2006. Estrogenic activity of UV filter mixtures. Toxicol Appl Pharmacol 217:86–99.
***In vitro* Study Type** Route of Administration Species & age of animals	Yeast oestrogen assay expressing human ER alpha
Study Duration	72 h
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 Yes Yes Both Pure and partial hER alpha agonism
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects *In vitro*	 Synthesis of beta-galactosidase upon ligand binding to the receptor
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?	 UV filters 3-benzylidine camphor (3BC, CAS 15087-24-8); Benzophenone-1 (BP1, CAS 131-56-6); Benzophenone-2 (BP2, CAS 131-55-5); Benzophenone-3 (BP3, CAS 131-57-7); 4,4'-Dihydroxybenzophenone (4DHB, CAS 611-99-4); Ethyl-4-aminobenzoate (Et-PABA, CAS 94-09-7); Benzyl salicylate (BS, CAS 118-58-1); Phenyl salicylate (PS, CAS 118-55-8) Yes, in a previous investigation Yes, NOECs were defined as an increase of 0.3% of basal hER alpha activity No
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*)	 Binary mixtures: EC25, EC50 and EC75 Multiple mixtures (4 & 8 compounds): BC10 (10% increase of basal hER alpha activity), NOEC Equipotent mixtures on the basis of non-linear regression analysis Experiments were repeated twice; each plate had estradiol as positive control in triplicates).
Observations/Findings	Experiments were done against predicted effects using the concentration addition and independent action models. Most binary mixtures showed synergistic effects at all concentrations. Combinations of 4 or 8 pure and partial agonists showed synergistic effects at BC10 and NOEC levels.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	The number of replicates is low, but overall the study appears well conducted and comprehensive. Binary experiments tended to support a concentration addition effect, whereas quaternary and multi-component mixtures showed higher activities than predicted by the CA model, more so at NOEC compared to BC10 levels. Unfortunately, there was no testing below NOEC, the study is therefore of limited relevance for this report.