Cometto-Muñiz et al, 2004

Guidelines/Criteria	
Reference:	Cometto-Muñiz JE, Cain WS, Abraham MH. 2004. Chemosensory additivity in trigeminal chemoreception as reflected by detection of mixtures. Exp Brain Res 158(2):196-206.
***In vivo* Study Type** Route of Administration Species & age of animals	 Airborne Human, various ages, osmic and anosmic
Study Duration	Series of single tests
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 Yes None Not defined; one ester and one cyclic aromatic tested Trigeminal nerve stimulation (nasal pungency, eye irritation)
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	 Nose (pungency), eye (irritation) Pharmacological (odour), adverse (nasal pungency and eye irritation)
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?	 Ethyl propanoate (97+%), ethyl heptanoate (98+%) Yes Yes, stimulus-response (psychometric) functions were established using two-fold dilution steps. Yes, in terms of varying probabilities of detection by individual subjects
Mixtures Investigated Number of dose levels How does the mixture make-up compare to individual components? (eg low dose) equivalents used?) No. of technical replicates per exposure condition No. of subjects per dose group (*in vivo*)	 2 detection probability levels for both eye irritation and nasal pungency. Each level consisted of 5 tests (2 for the single substances, 3 mixtures) Two levels of probability (p) of detection for specific concentrations were calculated: for both eye irritation and nasal pungency (0.40 and 0.80). Binary mixtures included 1/4, 1/2 and 3/4 of a p of 0.80 or 0.40, respectively, of one chemical combined with each level of the other (i.e. 3 mixtures). Each subject participated in varying numbers of sessions of varying length and replicates. Twenty normosmic subjects, age 18 - 54 y Five anosmic subjects, 20 - 64 y For eye irritation , only normosmic subjects participated; for nasal pungency, only anosmic subjects were entered.
Observations/Findings	For both endpoints at the lower level of detection for single compounds (0.40) complete agonism was seen for the mixtures. At the higher level (p of detection 0.80) the mixtures showed complete additivity for nasal pungency, but only partial agonism for eye irritation.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	The same strengths and weaknesses as for Cometto-Muñiz 1999 & 2001 apply: reasonable number of subjects and replicates, use of detectability (psychometric functions), but only binary mixtures were tested. However, overall the series of papers is supportive of partial or complete dose-addition, but not synergism, of various chemical mixtures.