Guidelines/Criteria	
Reference:	Groten JP, Schoen ED, van Bladeren PJ, Kuper CF, van Zorge JA, Feron VJ. 1997. Subacute toxicity of a mixture of nine chemicals in rats: Detecting interactive effects with a fractionated two-level factorial design. Fundam Appl Toxicol 36:15-29.
***In vivo* Study Type**	subacute study
Route of Administration	oral (feed, all other substances) and inhalation (dichloromethane and formaldehyde)
Species & age of animals	Wistar rat male
Study Duration	4 weeks
Type of Mixture	
Binary	no
>2 components	Two studies: Main Groups: 9 substances @ 3 doses (same ratio) Satellite Groups: 16 different combinations of 5 substances out of the nine (fractionated factorial design to identify interactions) @ one effect dose => satellite groups not relevant for the review
Similar acting or dissimilar	Both; selection criterion was that TNO had run previous subacute studies with published results
What Mode of Action was investigated?	multiple
Parameters/End points Measured	
Target organs/Critical effects Pharmacological changes or adverse effects	Various endpoints included in repeated dose studies
Individual Components	
Characterisation of individual compounds	analytical grade'
Name, exact chemical name, CAS no.	aspirin, cadmium choride, stannous chloride, loperamide, spermine, BHA, DEHP, dichloromethane, formaldehyde
Were dose responses established for individual components?	not within this study. Dose levels selected based on previous subacute studies performed in same lab
Were no effect levels established?	yes (there was one parameter statistically significantly altered at 1/3 NOEL, but not dose related and most probably not treatment related)
Were doses below the NO(A)ELs investigated?	yes, assumed NOAELs and 1/3 thereof (main groups)
Mixtures Investigated	
Number of dose levels	Main group with 9 substances at same ratio: 3 dose levels: 1/3 NOAEL, NOAEL and MOAEL
How does the mixture make-up compare to individual components? (e.g. low dose) equivalents used?)	each substance was present at its 1/3 NOAEL, NOAEL and MOAEL identified in previous subacute studies at the lab
No. of technical replicates per exposure condition (*in vitro*)	
No. of animals per dose group (*in vivo*)	8 animals per main groups (5 per satellite group)
Observations/Findings	Conclusion by the authors: *"simultaneous exposure to the nine chemicals does not constitute an evidently increased hazard compared to exposure to each of the chemicals separately, provided the exposure levels of each chemical in the mixture is at most similar or lower than its own NOAEL."* With the high number of endpoints examined, and nine substances present, multiple effects were observed especially at the minimal observed adverse effect level (MOAEL). Many of the observations were in line with the expectations from the previous subacute studies in the individual substances, some were not. Interactions at effect levels were identified by the fractionated factorial design part of the study, but do not allow any conclusions on the type of interaction (more or less than additive) or the presence at non-effect-levels due to the use of only one (effect) dose group and the fact that the single compounds were not examined in the same study. Hardly any effects were observed at the NOAEL and 1/3 NOAEL dose groups with the nine-substance mixture.
Overall opinion	
(e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Well performed repeated-dose study with good discussion and interpretation of the data. Due to the 'restricted' design, especially that the dose-response information for individual compounds was not generated in the same study, and that the fractionated factorial study was performed on only one (effect) dose level, hardly any specific conclusions on the type of interaction or non-interaction are possible. However, the study demonstrated an absence of a relevant concern for mixture effects at doses at or below the individual NOAELs (mixture of 9 substances with partly similar and partly dissimilar MoA on the different endpoints). Nothing unexpected.