Guidelines/Criteria	
Reference:	Ito N, Hasegawa R, Shirai T, Fukushima S, Hakoi K, Takaba K, Iwasaki S, Wakabayashi K, Nagao M, Sugimura T. 1991. Enhancement of GST-P positive liver cell foci development by combined treatment of rats with five heterocyclic amines at low doses. Carcinogenesis 12(5):767-772.
In vivo **Study Type**	
Route of Administration	in the diet
Species & age of animals	male F344 rats, six week old
Study Duration	eight weeks (six weeks of treatment with test compounds)
Type of Mixture	
Binary	
>2 components	five
Similar acting or dissimilar	probably similar acting
What Mode of Action was investigated?	carcinogenicity
Parameters/End points Measured	
Target organs/Critical effects	liver, GST-P-positive liver foci in diethylnitrosamine- (200 mg/kg i.p.) and uninduced rats (both models with partial hepatectomy after 3 weeks)
Pharmacological changes or adverse effects	early marker of tumour development
Individual Components	
Characterisation of individual compounds	
Name, exact chemical name, CAS no.	Trp-P-1, Glu-P-2, IQ, MeIQ, MeIQx, no purity or CAS numbers given
Were dose responses established for individual components?	yes, 1/1, 1/5, and 1/25 of the individual carcinogenic dose (without corresponding control group for uninduced rats). Carcinogenic doses: Trp-P-1 150 ppm, Glu-P-2 500 ppm, IQ 300 ppm, MeIQ 300 ppm, MeIQx 400 ppm)
Were no effect levels established?	yes for DEN-initiated rats, no for non-initiated rats
Were doses below the NO(A)ELs investigated?	only in part
Mixtures Investigated	
Number of dose levels	two
How does the mixture make-up compare to individual components? (e.g. low dose) equivalents used?)	each compound either at 1/5 or 1/25 of the individual carcinogenic dose
No. of technical replicates per exposure condition (*in vitro*)	
No. of animals per dose group (*in vivo*)	13-18, 9-12 for the non-initiated model
Observations/Findings	In DEN-induced animals reduced final body weight in most intermediate and high dose groups, less pronounced effects in uninduced rats. In DEN-induced animals in the 1/5 and 1/25 combinations the (net) number and area of foci is higher than those in the corresponding single compound groups. The sum of the net increase of no./area of foci from single compound groups is slightly lower (1/5) or clearly lower (1/25) compared to the corresponding mixtures. In the non-initiated group low numbers of foci and even lower areas were observed even at the 1/1 dose. There was not always a clear dose dependency for the number of foci, and areas in the 1/5 and 1/25 single and combination groups were more or less "0". Number of foci in the 1/5 mixture was higher than in the corresponding single compound groups. In the 1/25 mixture, number of foci was lower than that of the 1/25 MeIQx group.
Overall opinion	
(e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Initiated model: Dose selection for dose response not ideal, group size adequate. Proposed additivity for 1/5 combination in induced rats may be plausible, proposed strong synergy for 1/25 combination needs re-examination, as for calculation in part negative net no./areas of foci were used for calculation. Testing at 1/25 corresponds to testing at the NOAEL for the model. Non-initiated model: Problematic, as there is no concurrent control. Other paper suggest that control values for number/area of foci in non-initiated animals is "0". Under this assumption, and taking into account the variability of the parameters, the 1/25 and possible 1/5 combination may fulfil our criteria. Proposed additivity in the 1/5 combination needs careful evaluation in the light of the observed variability.