Hasegawa et al, 1994a

Guidelines/Criteria	
Reference:	Hasegawa R, Tanaka H, Tamano S, Shirai T, Nagao M, Sugimura T, Ito N. 1994a. Synergistic enhancement of small and large intestinal carcinogenesis by combined treatment of rats with five heterocyclic amines in a medium-term multi-organ bioassay. Carcinogenesis 15(11):2567-2573.
***In vivo* Study Type** Route of Administration Species & age of animals	 in the diet male F344 rats, six week old
Study Duration	28 weeks (4 weeks of initiation with five carcinogens - DMBDD schedule, then 24 weeks of treatment with test compound),
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 five similar acting carcinogenicity, induction of preneoplastic lesions
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	 Small and large intestines, less extensive analysis of thymus, thyroid, lung, tongue, oesophagus, forestomach, liver, kidney, bladder, zymbal preputial and mammary glands, soft tissue and other tissues Tumour formation, GST-P positive liver cells
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?	 PhIP, Glu-P-1, Glu-P-2, IQ, MeIQ, no purity given Yes (three dose levels), 1/1, 1/5, and 1/25 of a previously established carcinogenic dose yes Not for all compounds, as for two compounds only the lowest tested dose was a NO(A)EL
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*)	 Two 1/5 and 1/25 dose, i.e., intermediate and low doses of the individual dose levels Final no. 10 to 18, 6 to 16 for 1/1 dose of individual compounds
Observations/Findings	Carcinogenicity: Decreased final body weights (all individual compounds at 1/1, MeIQ at 1/5 and mixtures at 1/5 and 1/25), decreased relative liver weights (all compounds at 1/1, all individual compounds except Glu-P-2 at 1/5, and mixtures at both 1/5 and 1/25. In the small and large intestine increased number of tumour bearing rats and/or increased number of tumours per rat for all individual compounds at 1/1, for PhIP, Glu-P-1, and MeIQ at 1/5, and for mixtures at 1/5 and 1/25. In the cymbal gland the incidence of carcinomas was much higher in the mixture groups. GST-P positive liver cells: Increased numbers and areas of GST-P positive foci were observed for Glu-P-1, IQ, and MeIQ at 1/1, for Glu-P-1 and IQ at 1/5 and the mixture at 1/5.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Dose responses and NO(A)ELs were established. NOAELs for tumours in intestines are problematic due to high background (control) incidences. Statistics may be potentially be misleading. No testing below the NO(A)EL (?! see above). For the intestinal tumours, the authors claimed isoadditivity. For cymbal gland tumours, a strong trend (p=0.08) for synergy was reported for the 1/25 mixture. No evidence for a combination effect was observed for GST-P liver foci for the 1/25 mixture. For liver foci the 1/25 dose was a clear NOAEL for all compounds. Tumour incidence data need further discussion. Liver foci data fulfil our criteria.