Guidelines/Criteria	
Reference:	Ito N, Hasegawa R, Imaida K, Kurata Y, Hagiwara A, Shirai T. 1995. Effect of ingestion of 20 pesticides in combination at acceptable daily intake levels on rat liver carcinogenesis. Food Chem Toxicol 33(2):159-163.
***In vivo* Study Type**	
Route of Administration	in the diet
Species & age of animals	male F344 rats, 6 week old
Study Duration	eight weeks (six weeks of treatment with test compounds)
Type of Mixture	
Binary	
>2 components	20 compounds
Similar acting or dissimilar	not known, most likely dissimilar, although all but one (endosufan) compounds were organophosphates
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 non-initiated rats (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.	Acephate (30560-19-1, 99.3%), butamifos (36335-67-8, 97.9%), chlorfenvinphos (470-90-6, 93.3%), chlorpyrifos (2921-88-2, 99.3%), dichlorvos (62-73-7, 98.9%), dimethoate (60-51-5, 99.0%), edifenphos (17109-49-8, 95.0%), endosulfan (115-29-7, 98.0%), etrimfos (38260-54-7, 94.0%), fenitrothion (112-14-5, 96.7%), iprobenfos (26087-47-8, 94.9%), isoxathion (18854-01-8, 95.2%), malathion (121-75-595.4%), methidathion (950-37-8, 92.0%), pirimiphos-methy (29232-93-7, 99.7%), prothiophos (34643-46-4, 94.7%), pyraclofos (77458-01-6, 98.4%), tolclofos-methyl (57018-04-9, 99.5%), trichlorfon 52-68-9, 99.0%), vamidothion (2275-23-2, 99.0)
Were dose responses established for individual components?	no
Were no effect levels established?	no, the work is based on previously established ADI levels
Were doses below the NO(A)ELs investigated?	yes - with respect to conventional NOAELs, no - with respect to the animal models used
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 the respective ADI or at 100 x ADI
No. of technical replicates per exposure condition (*in vitro*)	
No. of animals per dose group (*in vivo*)	18-19 for DEN initiated rats, 9-10 for non-initiated rats
Observations/Findings	Bodyweight of DEN-initiated animals was lower than that of non-induced animals. No differences were seen within the initiated groups or within the non-initiated groups. in DEN-induced animals the ADI mixture did not increase number or area of GST-P-positive liver foci compared to the corresponding control, however, in the 100 x ADI group these parameters were statistically significantly increased by 34% and 52%, respectively. In non-initiated rats neither the ADI nor the 100 x ADI mixture induced GST-P-positive liver foci. (A no initiation/no treatment group was not run)
Overall opinion	
(e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Comparably low animal numbers for non-initiated animals. No dose responses established for individual compounds in the model used, work is based on previously established ADIs (not established in the liver foci model). Assuming that 100 x ADI is the NOAEL for all / most compounds, the paper might indicate a moderate combination effect at the NOAEL, but not at the ADI = (roughly) 1/100 NOAEL for the sensitive initiated model only, but conclusive judgement is not possible, as single compounds were not tested in the model. Not in line with the Task Force's criteria (no testing of single compounds in the models used, no NOAEL established in the model), but potentially useful, since a large number of compounds was tested in a sensitive model at their respective ADIs.