

Guidelines/Criteria	
Reference:	Benjamin SA, Yang RSH, Tessari JD, Chubb LW, Brown MD, Dean CE, Keefe TJ. 1999. Lack of promotional effects of groundwater contaminant mixtures on the induction of preneoplastic foci in rat liver. Toxicology 137(3):137-149.
<b>In vivo Study Type</b> Route of Administration Species & age of animals	Drinking water Fischer-344 rats, 60 days
<b>Study Duration</b>	Initiation promotion study (Study design described by Ito et al, 1989)
<b>Type of Mixture</b> Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	No Yes Dissimilar action assumed Liver toxicity
<b>Parameters/End points Measured</b> Target organs/Critical effects  Pharmacological changes or adverse effects	Hepatocarcinogenesis (placental glutathione-S-transferase (GST-P) preneoplastic liver cell foci after diethylnitrosamine initiation and partial hepatectomy) Adverse and non-adverse effects
<b>Individual Components</b> 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?	No Arsenic (arsenic trioxide), benzene, chloroform, chromium (chromium chloride hexahydrate), lead (lead acetate trihydrate), phenol, Trichloroethylene No No Unknown
<b>Mixtures Investigated</b> 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 ( <i>in vitro</i> ) No. of animals per dose group ( <i>in vivo</i> )	1x and 10x concentrations of the mixture were administered as ppm: Arsenic (arsenic trioxide) 3.1, 31, benzene 5, 50, chloroform 1.5, 50, chromium (chromium chloride hexahydrate) 0.7, 7, lead (lead acetate trihydrate) 3.7, 37, phenol 3.4, 34, Trichloroethylene 3.8, 38; --> 7 Chemical-1x, 7 Chemical-10x Submixtures of organic and inorganic chemicals (each 10x) --> Organic-10x, Inorganic-10x Trichloroethylene, lead, chloroform (10x, 100x) --> TLC-10x, TLC-100x 1x and 10x complete mixtures, 10x inorganic submixture and 10x organic submixture; 10x and 100x of trichloroethylene, lead and chloroform mixture Not applicable 6-18 rats/treated groups, 11-40/ control group
<b>Observations/Findings</b>	≥ 7 Chemical-1x Decreased body weight gain with and without DEN; increased liver weight only seen at 7 chemical-1x with DEN and 7 chemical-10x without DEN; Increased foci area/liver area only at 7 chemical-1x with DEN, decreased BrdU index; Organic-10x: increased body weight gain; decreased BrdU index with DEN Inorganic-10x: decreased body weight gain and decreased relative liver weights with and without DEN, decreased foci area/liver area ≥ TLC-10x: decreased number of foci/liver area and decreased foci area/liver area, decreased BrdU index.
<b>Overall opinion</b> (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Increased total areas of GST-P positive foci are only seen at the 7 chemical-1x not in the 7 chemical-10x mixture. This lack of dose-response raises the question whether this result is toxicologically relevant. In the other mixture groups, decreased total foci areas were detected, which makes the initial observation even more doubtful. Results of the study are considered to be of low relevance.