Kroes et al, 1974

Guidelines/Criteria	
Reference:	Kroes R, van Logten MJ, Berkvens JM, de Vries T, van Esch GJ. 1974. Study on the carcinogenicity of lead arsenate and sodium arsenate and the possible synergistic effect of diethylnitrosamine. Food Cosmet Toxicol 12:671-679.
In vivo **Study Type** Route of Administration Species & age of animals	 in the diet / oral intubation (diethylnitrosamine=DEN) male and female Wistar rats, new-born
Study Duration	120 weeks, new-born rats were initially exposed through their mothers' milk, later on through the diet
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 binary mixtures only dissimilar carcinogenicity
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	Food intake & body weight gain, mortality, haematology, histopathology of most organs and tissues Changes in body weight gain, mortality, haematological changes, histopathological changes including tumours Adverse effects
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?	 lead arsenate (appr. 60% Pb, 20.9% As), sodium arsenate (> 98.5%) DEN , no purity given, no CAS numbers were given only for lead arsenate (two doses) yes / for sodium arsenate NOEL for histopath/carcinogenicity is not a NOEL for general toxicity no
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*)	 one each compound at the respective NO(A)EL (Cave: sodium arsenate, see above) 29 males & 19 females for high dose lead arsenate, 40 to 60 animals for all other groups
Observations/Findings	DEN (5 µg/day): Slightly, but significantly increased food intake in both sexes and marginal decrease of mean corpuscular haemoglobin content in males (NO(A)EL); lead arsenate (1850 ppm): decreased food intake and body weight gain and increased mortality in both sexes, consistent and significant negative effects on red blood picture in males, histopathological changes in the liver of both sexes, lead arsenate (463 ppm): NO(A)EL, lead arsenate (463 ppm) plus DEN: NO(A)EL; sodium arsenate (416 ppm): decreased food intake and body weight gain; sodium arsenate (416 ppm) plus DEN: decreased food intake and body weight gain compared to DEN group, unfortunately no comparison with the control group was made. No specific tumours could be clearly associated with any one of the groups.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Well performed study. The combination lead arsenate (463 ppm) plus DEN fits to the Task Force's criteria, because no adverse effects were observed for the compounds when given alone. Likewise, no effects for the combination were observed. The combination including sodium arsenate does not fully fit, as the compound alone was generally toxic (decreased body weight gain). However, when focusing on histopathological changes and carcinogenicity only, also this mixture would fit: Both compounds at the respective dose level represented a NO(A)EL. Also the corresponding mixture represented a NO(A)EL. 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.