

**Ashby and Styles, 1980**

| Guidelines/Criteria   |   |   |
|---|---|---|
|   | Reference: Ashby J, Styles JA. 1980. Carcinogenic synergism and its reflection <i>in vitro</i> . Br Med Bull 36(1):63-70.   |   |
| <b>In vitro Study Type</b><br>Route of Administration<br>Species & age of animals   | BHK cell transformation assay<br><br>Protocol details are given in Ashby J, Styles JA, Paton D. 1978. <i>In vitro</i> evaluation of some derivatives of the carcinogen butter yellow: Implications for environmental screening. Br J Cancer 38(1):34-50, and in Styles JA. 1977. A method for detecting carcinogenic organic chemicals using mammalian cells in culture. Br J Cancer 36(5):558-563. | BHK cell transformation assay   |
| <b>Study Duration</b>   | 14-21 days  |   |
| <b>Type of Mixture</b><br>Binary<br>>2 components<br>Similar acting or dissimilar<br>What Mode of Action was investigated?  | Yes<br>No<br>Unknown<br>Pre-neoplastic transformation   | Yes<br>No<br>Unknown<br>Pre-neoplastic transformation   |
| <b>Parameters/End points Measured</b><br>Target organs/Critical effects<br>Pharmacological changes or adverse effects<br><i>In vitro</i>  | Number of transformations   | Number of transformations   |
| <b>Individual Components</b><br>Characterisation of individual compounds<br>Name, exact chemical name, CAS no.<br><br>Were dose responses established for individual components?<br><br>Were no effect levels established?<br><br>Were doses below the NO(A)ELs investigated?           | S(+) and R(-) isomers of ethoxymethoxymorpholinophosphine<br><br>Yes<br><br>Yes, no effect at top dose of each component (25 ug/ml)<br><br>Yes, three   | 2-acetylaminofluorine and acetanilide<br><br>Yes<br><br>Yes, 0.025 ug/ml for 2-acetylaminofluorine (lowest dose tested) and 250 ug/ml for acetanilide (highest dose tested)<br>Only for acetanilide |
| <b>Mixtures Investigated</b><br>Number of dose levels<br>How does the mixture make-up compare to individual components? (e.g. low dose) equivalents used?<br>No. of technical replicates per exposure condition ( <i>in vitro</i> )<br>No. of animals per dose group ( <i>in vivo</i> ) | Four<br>Equimolar. Mixtures contained 0.0005x, 0.005x, 0.05x and 0.5x the NOEL of each component<br>Cannot find this information in any of the three papers   | Only one was at a NOEL for each component<br>Equimolar. Mixture contained 0.5x the NOEL for 2-acetylaminofluorine and 0.0001x the NOEL for acetanilide.   |
| <b>Observations/Findings</b>  | Increase in cell transformation seen at the top three doses, i.e. those containing 0.005x, 0.05x and 0.5x of each component. Author proposes that one of the isomers is metabolically activated, and that the presence of the other isomer competed with enzymes negating the effect of the first isomer. However, supporting evidence is not presented.  | No effect on cell transformation seen for the one relevant mixture.   |
| <b>Overall opinion</b><br>(e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)  | Well conducted study. An interesting case, in that just by reforming a resolved mixture, an effect can be produced, at least <i>in vitro</i> .  | Well conducted study.   |