Guidelines/Criteria	
Reference:	Tagliaferri S, Caglieri A, Goldoni M, Pinelli S, Alinovi R, Poli D, Pellacani C, Giordano G, Mutti A, Costa LG. 2010. Low concentrations of the brominated flame retardants BDE-47 and BDE-99 induce synergistic oxidative stress-mediated neurotoxicity in human neuroblastoma cells. Toxicol In Vitro 24:116-122.
In vitro **Study Type** Route of Administration Species & age of animals	In SK-N-MC cells: MTT assay, ROS formation via Carboxy-H2-DCFDA, lipid peroxidation via TBARS
Study Duration	
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 BDE-47 and BDE-99 similar cytotoxicity
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects *In vitro*	 In SK-N-MC cells: MTT assay, ROS formation via Carboxy-H2-DCFDA, lipid peroxidation via TBARS
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?	 none 2, 2', 4, 4'-Tetrabromodiphenylether (BDE-47) and 2, 2', 4, 4', 5-Pentabromodiphenylether (BDE-99) yes for MTT assay, but not for ROS and lipid peroxidation assays yes for MTT assay, but not described for ROS and lipid peroxidation assays Yes, NOEL (of individual substances) was lowest dose of BDE 99 in MTT assay, BDE 47 was applied at NOEL and 2.5 fold below
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*)	 7 for single substance dose response in MTT, in combination experiments 4 dose levels of BDE 47 and 6 dose levels of BDE 99 different ratios used MTT assay: 2 experiments with 4 replicates each
Observations/Findings	The authors compared the experimental cell viability with theoretical values calculated from single substance dose-response data for dose additivity (Loewe) and response additivity (Bliss). At some mixture doses, they observed dose additivity but at most data points, the statistics applied indicated less than additive or more than additive effects. Less than additive effects were more prominent at higher BDE47 concentrations (interestingly regardless of BDE99 conc) and more than additive effects occurred mostly at BDE47 concentrations below its single component IC50. No dose response data were established for the other endpoints, ROS and lipid perox so that these cannot be evaluated.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	The work does not really describe neurotoxicity but rather cytotoxicity in human neuroblastoma cells. No comparison to other cell lines was presented. The MTT work was carefully designed and shows some interesting results. It is difficult to judge on how the statistical analyses were performed in detail, especially how variation was taken into account. Even if those analyses and the findings of more than additive effects at dose levels around the BDE47 NOEL and less than additive effects at higher doses were robust, those departures from additivity are pretty marginal and would probably not be of biological significance: The relevance of a decreased cell viability in an *in vitro* assay of e.g. around 60% versus predicted 70% may be questionable. No dose response data were established for the other endpoints, ROS and lipid perox, so that these cannot be evaluated.