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In vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

Guideline:	OECD 476 (adopted 21st July, 1997);
Objective:	The purpose of this study is to evaluate whether the test item induces gene mutation (point mutations and/or gross chromosomal changes) at the thymidine kinase (*tk*) locus in L5178Y 3.7.2 C mouse lymphoma cells cultured *in vitro* in the absence and presence of a rat liver metabolic activation system (liver extract, S9 fraction).
Cell Line:	L5178Y 3.7.2 C mouse lymphoma ((ATCC) ; Manassas,Virginia)
Metabolic Activation:	The experiments are performed in the presence and absence of a post mitochondrial supernatant (S9) prepared from livers of phenobarbital/β-naphthoflavone-induced rats.
Dose levels:	Maximum dose level: for relatively non-cytotoxic, soluble compounds the maximum concentration is 5 mg/ml, 5 µl/ml, or 0.01M, whichever is the lowest. Depending on the solubility and cytotoxicity (the results of the preliminary experiments), in the main test at least four dose levels are tested with approximately half log or smaller intervals.
Preliminary Experiments:	A preliminary solubility test is performed for selection of the appropriate vehicle and a preliminary toxicity test for selection of the treatment concentrations. A 3-hour treatment in the presence and absence of S9 and a 24-hour treatment in the absence of S9 is performed to determine the test item toxicity. The treatment of cell cultures is the same as described below for the main mutation experiments (but single cultures are used and positive controls are not included).
Main Experiments:	Assay 1: 3-hour treatment in the presence and absence of S9. Assay 2: 24-hour treatment in absence of S9, 3-hour treatment in presence of S9. Phases of each assay: treatment, survival experiment (on 96-well plates cultures incubated for 10-14 days), 2-day expression period, viability experiment (on 96-well plates cultures incubated for 10-14 days), mutagenicity experiment (on 96-well plates cultures incubated for 10-14 days).
Parallels:	Duplicate cultures are used for each treatment, For survival and viability: each culture is placed into each well of two, 96-well microtiter plates (192 wells) in summary 384 wells; For mutagenicity: each culture is placed into each well of four, 96-well microtiter plates (384 wells) in summary 768 wells;
Amount of Test Item Required:	At least 3 g
Draft Report:	Approximately 10-12 weeks from the arrival of the test item