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STUDY OUTLINE
IN VITRO MAMMALIAN CELL MICRONUCLEUS TEST
(using V79 Cells /Chinese hamster lung, male)

Guideline:	9th Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 487, " In Vitro Mammalian Cell Micronucleus Test ", adopted 26th September, 2014.
Objective:	The objective of this study is to evaluate the clastogenic and aneugenic potential of test item by its effects on the frequency of micronuclei in cultured Chinese hamster lung cell lines treated in the absence and presence of a rat liver metabolizing system.
Tester strains:	The V79 cell line is well established in toxicology studies. Stability of karyotype and morphology makes it suitable for gene toxicity assays with low background aberrations.
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:	In the absence of S9: at least 3 concentrations/4-hour treatment, harvest 24 hours from the beginning of treatment. In the absence of S9: at least 3 concentrations/24-hour treatment, harvest 24 hours from the beginning of treatment. In the presence of S9: at least 3 concentrations/4-hour treatment, harvest 24 hours from the beginning of treatment.
Procedure:	In order to determine the treatment concentrations of test item in the main test a dose selection (cytotoxicity assay) will be performed. The results obtained will be used for dose selection of the test item used in the In Vitro Mammalian Cell Micronucleus Test. The In Vitro Mammalian Cell Micronucleus Test will be conducted in one experiment in the presence and in the absence of S9 mix. All slides will be independently coded before microscopic analysis and scored blind. One thousand cells from each culture (2000 per concentration) will be analyzed for micronuclei per test item concentration as well as the negative and positive controls.
Draft Report:	Approximately 4 weeks from the arrival of the test item