Micronucleus test

Background: Micronuclei are formed during metaphase/anaphase transition of mitosis and represent broken fragments of daughter chromosomes outside the nucleus. Micronucleus test is an important step in toxicology screening for new drug candidates. Analysis of micronuclei formation can be used as a tool for chromosomal DNA damage detection as it is a key marker of genotoxicity of a compound. Using combination of metabolic systems, 9000g supernatant fraction of rat liver homogenate (S9 fraction) and micronucleus genotoxicity test, allows identifying genotoxic agents which are biologically inactive unless they are metabolized. The in vitro micronucleus assay is conducted in CHO-K1 cells treated with test articles. Compounds with known mutagenic activity are used as positive controls in the assay: 4 nitroquinoline-N-oxide – clastogen, active without metabolic activation; cyclophosphamide – clastogen which requires metabolic activation; colchicine – compound with aneugenic activity. Dimethyl sulfoxide (DMSO) is used as a vehicle control in micronucleus assay both with and without metabolic activation. Cytochalasin B inhibits actin polymerization and blocks cytokinesis which results in formation of binucleated cells. This allows detection and analysis of micronuclei only in those cells that have completed mitosis. For evaluation and analysis of the results, 2000 binucleated cells per each concentration of the tested compound are scored. Statistical analysis is performed using Chi-square test comparing the number of micronucleated cells in the experimental and negative control samples.

Service Details: The CHO-K1 cell line was derived as a subclone from the parental CHO cell line initiated from a biopsy of an ovary of an adult Chinese hamster. CHO-K1 cells (ATCC CCL-61) are cultured according to the supplier's recommendations. The cells are seeded in 6 cm diameter Petri dishes and treated with the tested or reference compounds either for 24h in the assay without metabolic activation or for 4h in the assay with metabolic activation. Cytochalasin B is added after 24h and the cells are incubated for additional 24h. After this, the cells are harvested, fixed and scored for micronuclei. The highest test concentration of the compounds should correspond to 10 mM if no solubility or cytotoxicity limitations are observed. Three test concentrations with no more than a 3-fold difference between the concentrations are used. The experiments with the tested compounds and positive and negative controls are conducted in duplicate. The reference compounds with the known cytotoxicity are run with every experimental batch to verify assay validity.

Deliverable: Based on the statistically significant increase of frequencies of binucleated cells with micronuclei (in the assay with or without metabolic activation) compound can be classified as genotoxic. Full study report is provided.

Sample Submission: Up to 280 μmoles (~150 mg) of compound is required for this assay.