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Collaborative Study of Liver Micronucleus Assays in Young Rats - Investigation of Twice dosing design by JEMS MMS Collaborative Study Group -

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To evaluate the suitability and utility of the young rat liver micronucleus assay, we have been investigating the assay in the collaborative study for several years. This year, to make the assay system simple and convenient furthermore, twice dosing-once sampling method was investigated using 7 compounds: diethylaminoazobenzene, dimethylaminoazobenzene, 1,4-dimethylaminozate, 2,4-dinitrobenzene, 2,6-dinitrotoluene, mitomycin C, and cyclophosphamide. These compounds are known to induce positive responses in the single dose liver micronucleus assay. The test compounds were administered twice in a 24-h interval at least 3 dose levels to male rats (F344/1CrUnr) at 26 or 27 days of age at the first administration. Hepatocyte suspensions were prepared 4 days after the second administration by liver perfusion with a collagenase solution and stained with AO-DAPI double fluorescent staining method. To calculate the incidence of micronucleated hepatocytes and the number of mictotic cells, 2000 cells per animal were examined under a fluorescent microscope. The results indicated that the twice dosing-once sampling method was more simple and convenient than conventional single dose-three time sampling method.

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Cyclophosphamide and Etoposide Canine Studies Demonstrate the Cross-Species Potential of the Peripheral Blood Micronucleated Reticulocyte Endpoint

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Accumulating data suggests that peripheral blood micronucleated reticulocytes (MN-RETs) represent an endpoint of cytogenetic damage, even in species with efficient splenic filtration function. Studies performed evaluated both dose response and time-course of micronucleus (MN) induction in the bone marrow and blood of beagle dogs after dosing with cyclophosphamide (CP) or etoposide (Eto).

CP was administered daily via intravenous injection for 5 days at 0, 6.25, 12.5 or 25 mg/m²/day. Eto was administered iv injection for 2 days at 0, 1.56, 6.25, and 12.5 mg/m²/day. Blood specimens were collected for analysis before dosing as well as at several intervals during treatment, and bone marrow was prepared at necropsy. Blood was prepared using the In Vivo MicroFlow® method and analyzed at Litton, while bone marrow was analyzed at Covance via microscopy (May-Grunwald and also acridine orange staining).

Robust MN-RET induction was observed in the blood of all CP-treated dogs by Day 4, with dose-related increases evident by Day 3. Comparable dose-related increases were observed in the bone marrow with microscopy-based scoring. While significant MN induction was not observed in the blood or bone marrow of dogs treated with Eto at 1.56 mg/m²/day, marked dose-related increases were noted in both compartments for the 6.25 and 12.5 mg/m²/day groups. Collectively, these results demonstrate the utility and sensitivity of blood-based automated MN-RET measurements in canines. These data have important implications in regard to the reduction and refinement of animal usage in genetic toxicology investigations.